Physiological, Hormonal and Histological Effects of Fennel seeds (*Foeniculum vulgare*) on Thyroid and Testes in Male Rats

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**Abstract**: In various parts of the world Fennel seeds *Foeniculum vulgare* is used as a herbal medicine. The present study aims to shed light on fennel’s side effects in male rats on the weights, hormonal, histological changes and some of the physiological parameters of thyroid and testes.

60 Spargue-Dawley albino adult male rats were daily fed with fennel pellet in three different doses (50, 100, 200) gm/kg bw in three different periods of time (10, 20, 30) days. They were divided into 12 groups each of five rats, as following: 

- (Group 1, 2, 3) normal control rats that were fed with chow pellet only for (10, 20, 30) days subsequently,
- (Group 4, 5, 6) experimental treated groups that respectively received fennel pellet in three doses of (50, 100, 200) gm/kg for 10 days,
- (Group 7, 8, 9) experimental treated groups that respectively received fennel pellet in three doses of (50, 100, 200) gm/kg for 20 days,
- (Group 10, 11, 12) experimental treated groups that respectively received fennel pellet in three doses of (50, 100, 200) gm/kg for 30 days.

The end of each experiment was followed by weighing the animals, blood sample of each animal was collected by heart puncture then directly centrifuged and the serum was kept at -80°C for hormonal, biochemical analysis and some histological standards, the animals were dissected, then thyroid and testes were excised and fixed in neutral buffered 10% formalin for histological preparation.

Increased doses of fennel consumption and treatment duration statistically caused:

- Highly significant increase (p<0.01) in thyroid weights in experimental treated groups (7, 8, 9, 10, 11, 12) while group (5 and 6) showed significant increase (p<0.05) compared to the control group.
- No changes illustrated in values of Thyroid stimulating hormone (TSH) in all periods of time and in all concentrations of fennel in comparison with the control group.
- Significant (p<0.05) decrease in Triiodothyronine (T3) and Thyroxine (T4) hormone serum levels in treated groups (7, 8, 9, 10, 11, 12) compared to the control group.
- Significant (p<0.05) decrease in both left and right testes weights of fennel treated group (12) in comparison with the control group.
- Significant (p<0.05) decrease in Testosterone serum levels of fennel treated groups (8, 9, 10, 11, 12) compared to the control groups with the increase of fennel doses and treatment duration.
- Histological study of the organs demonstrated histological changes after an exposure to fennel for short and long periods of time and in all concentrations.
- Thyroid gland sections showed certain follicles empty from colloid, degenerated follicles and necrosis.
Testes sections showed seminiferous tubules with certain degeneration and necrosis of spermatogonia cells besides necrotic debris inside the lumen, no sperms appear inside the lumen.

**Key words**: Foeniculum vulgare, TSH, Triiodothyronine, Thyroxine, Testosterone, necrosis.

**Introduction**

F. vulgare is known as a culinary herb also useful for pharmaceutical industry for its high content in 1,8-cineole, linalool, fenchone and estragol. Foeniculum vulgare Mill contains 6.3% of moisture, 9.5% protein, 10% fat, 13.4% minerals, 18.5% fibre and 42.3% carbohydrates and the rest of its components are minerals and vitamins Calcium, potassium, sodium, iron, phosphorus, thiamine, riboflavin, niacin and vitamin C. Its calorific value is 370kcal. Healthy importance of fennel comes from its numerous chemical compounds, such as volatile compounds, flavonoids, phenolic compounds, amino acids, and fatty acids, hence it has been used for abundant types of disorders. Fennel shows antispasmodic activities. As a curative in infantile colic. As it improves the milk supply of a breast-feeding mother, so it has been used as a galactagogue that occurs due to the presence of phytoestrogens present in fennel which promote growth of breast tissue.

The thyroid is one of the larger endocrine glands of the body, it is a highly vascular structure that has a butterfly shape it consists of two soft lateral large lobes connected by a stretch of tissue known as "isthmus". The first primary function of the thyroid gland is synthesize and secretion of thyroid hormones T3 and T4, which control essential functions, such as regulation of energy metabolism and basal metabolic rate (BMR) and the promotion of protein synthesis and growth. Thyroid gland also produce a third hormone "calcitonin" which is not considered a thyroid hormone.

An adult human male has two testes as apart of the male reproductive system, they are ovoid structures both testes are suspended outside the abdomen in a saclike scrotum. Testes are primary male sex organs that form sperm cells (spermatogenesis) and male sex hormones (testosterone).

**2. Material and Method**

**2.1 The plant**

Fennel seeds were purchased from the local markets in the Ishrin Street of Al-Baya'a/ Baghdad. They were obtained as a fennel herb for culinary use, then they were prepared to be used for the experiment.

**2.2 Fennel pellet preparation**

Fennel seeds about (2100 kg) were powdered in a seed grinder, (15,900kg) of pellet which contained (20% soya, 10% protein of fish powder, 20% American protein, 40% corn, 10% wheat flour, and additives such as di calcium, prigmy, and antioxidants) was powdered as well by the grinder, then the components were mixed and kneaded by addition of tap water and distributed into three groups as followed:

1. group of 50 grams fennel powder + 950 gram pellet powder.
2. group of 100 grams fennel powder + 900 gram pellet powder
3. group of 200 grams fennel powder + 800 gram pellet powder.

Small cylinder blocks were made from this dough similar to the normal rodent pellet. mg/kg of body weight.

**2.3 Animals**:

In this experimental study, after an adaptation period of one week, sixty male rats were randomly divided into twelve groups of five rats each, described as following:

**Group 1, 2, and 3**: (control) did not receive any dose they fed with rat chow pellet only for 10, 20, and 30 days subsequently.
Group 4, 5, and 6: (the experimental groups) that respectively received 18-20 g fennel pellet in three doses of (50, 100, and 200)g/kg every 24 hours for 10 days.

Group 7, 8, and 9: (the experimental groups) that respectively received 18-20 g fennel pellet in three doses of (50, 100, and 200)g/kg every 24 hours for 20 days.

Group 10, 11, and 12: (the experimental groups) that respectively received 18-20 g fennel pellet in three doses of (50, 100, and 200)g/kg every 24 hours for 30 days.

2.4 Collection of Blood

The end of each experiment was followed by weighing the animals, they were fully anaesthetized by diethyl ether for several minutes and blood samples were obtained by heart puncture. 4 ml of the blood was used to obtain sera (0.5-1.0) ml separated by centrifugation.

2.5 Collection of Organs

The animals were dissected and their left and right kidneys were excised, washed with normal physiological saline 0.9% (NaCl), blotted with filter paper, weighed and kept in the fixative solution (neutral buffered 10% formalin) for histological study.

2.6 Measurement of the Levels of Hormones Concentration

It was represented by the enzyme immunoassay tests (ELIZA) for the quantitative determination of concentrations of thyroid gland hormones T3 according to 13, T4 according to 14 and TSH according to 15. Also, the reproductive hormone testosterone was measured according to 16.

2.7. Histopathological Preparation

The preparation for histological sections was performed according to the method of 17.

2.8 Statistical Analysis

The Statistical Analysis System-SAS(2012) program was used to effect of difference factors in study parameters (ANOVA). Least significant difference-LSD test was used to significant compare between means in this study.

3. Results

3.1 Thyroid weight and Functions

Results in Figure(1) shows the effects of fennel on thyroid weights as follows: Fennel consumption for 10 days demonstrated significant increase (p<0.05) in thyroid weights of the experimental treated groups in concentrations (100, 200)gm/kg fennel (0.026±0.01) (gm), respectively, compared with the control (0.020±0.001) (gm). As well, there was highly significant increase (p<0.01) in thyroid weights of experimental groups that fed fennel for 20 days in concentrations (50,100, 200)gm/kg (0.025±0.001) (gm), (0.030±0.002), (0.032±0.002) (gm) respectively, compared with the control (0.019±0.00) and between the experimental groups themselves. The results also revealed a highly significant increase (p<0.01) in thyroid weights at 30 days feeding fennel in all experimental treated groups with concentrations (50,100, 200)gm/kg (0.031±0.002), (0.035±0.002), (0.039±0.002) (gm), respectively, compared with the control group (0.019±0.00) (gm) and between groups of 50gm/kg and 200gm/kg. Significant increase (p<0.05) was observed in all concentrations of experimental treated groups with the increment of duration comparing with the control.

Statistical analysis of the present study for fennel effects on thyroid hormones that included TSH, T3 and T4 in the Figures (2),(3),(4) reveals that:

- Fennel effects illustrated no significant changes in values of TSH(µlU/ml) in all periods of time (10,20,30)days and in all concentrations of fennel (50, 100, 200)gm/kg when compared with control groups as shown in Figure(2).

 
Non-significant differences due to fennel consumption on serum level of (T3) (ng/ml) related with treatment duration when concentration was a fixed factor in all treated groups compared to the control groups with one exception, which was in the treated group of 200gm/kg concentration (0.04±0.08) (ng/ml) in 30 days feeding fennel, it showed significant decrease (p<0.05) compared with control group(0.780±0.08) (ng/ml) while at 20 days duration of fennel consumption showed highly significant reduction (p<0.001) in T3 level.
in all experimental groups with concentration (50,100,200) gm/kg (0.653±0.05), (0.573±0.04), (0.563±0.04) (ng/ml), respectively. At 30 days period of fennel consumption, significant decrease(p<0.05) in T3 level was observed in experimental treated groups with concentrations (100, 200)gm/kg (0.458±0.10),(0.404±0.08) (ng/ml), respectively, compared to the control groups (0.780±0.06) (ng/ml) as shown in Figure(3).

- The present study shows findings about fennel consumption effect on thyroxine hormone (T4) level(µg/dl) in treated rats in comparison with control groups similar to the results of T3 hormone. There was non-significant differences due to fennel consumption on (T4)level related with treatment duration when concentration was a fixed factor in all treated groups compared to the control groups, except in the treated group of 200gm/kg concentration (2.01±0.34) (µg/dl) in 30 days fennel administration as it showed significant decrease (p<0.05) compared with control group (4.29±0.69) (µg/dl). At 30 days period of fennel consumption, significant decrease (p<0.05) in T4 level was observed in experimental treated groups with concentrations (100, 200)gm/kg (2.74±0.49),(2.01±0.34) (µg/dl), respectively, compared to the control group (4.29±0.34) (µg/dl) as shown in Figure(4).

The results of the present study which showed reduction in thyroid hormones T3 and T4 agreed with results of another previous study by 18 after adminstration with alcoholic extract of celery (member in Apiaecea family) with doses (50,100,200)mg/kg in male rats, but it caused significant increase in TSH, which disagrees with the present study, as it illustrated no change in TSH values. These results disagreed with consequence of 19 whom exposed female rats to (100,400,800 and 1600)mg/kg hydroalcoholic extract of caraway for 45 days which raised T3 and T4 levels while TSH was decreased significantly at high doses compared to those in control groups. It also disagreed with 20 who fed male rats with a diet containing 10% seeds of anise, fennel and ajowan (all members of the same family), A significant increase in T3 level was observed only in ajowan seed-fed groups, while on the 7th day all 3 herbs led to significant increase in T3 level. It seems that the mentioned seeds promote the conversion of T4 to T3by working directly on the thyroid gland for generation of T4. Flavonoids are important contents of fennel and other plants of Apiaceae family. Flavonoids possess a variety of biological activities including anti-thyroid effects in experimental animals and humans. They are widely distributed in plant-derived foods.

Flavonoids also could affect the availability of thyroid hormones to target tissues, by inhibiting thyroperoxidase, deiodinase activity and decrease the expression of the thyrotropin receptor and thyroglobulin genes.

4.2.2.Histological Changes of Thyroid

The main histological changes in all treated rats with fennel seeds on thyroid tissues in different periods of time compared to control groups is shown as follows:
Thyroid sections showed different histological changes after treatment with fennel in concentration of 50gm/kg of body weight for 10 days, included scalloping vacuoles at the apical pole of the follicular epithelial cells Figure (6), while in a period of 20 days thyroid sections showed histological changes less than the changes that observed in 30 days. In a period of 30 days, scalloping of colloid material became clearer in thyroid follicles. Other certain follicles appeared without colloid material Figure(7) compared with the histological sections of thyroid from control groups of rats Figure (5).

Histological changes after treatment with fennel in concentration of 100gm/kg of body weight for 10 days on thyroid sections showed that certain follicles are empty from colloid while others contain colloid with small scalloping vacuoles Figure (8), whilst 20 days experimental group showed further effects that are less than 30 days treatment with fennel that illustrated the majority of the follicles containing no colloid but few of them contain colloid material with scalloping vacuoles, a sign of hypothyroidism Figure(9), compared with the histological sections of thyroid from control groups of rats Figure (5).

Histological sections of thyroid gland from treated groups with 200mg/kg of bw fennel consumption for 10 days demonstrated that the majority of the follicles contained no colloid. Follicular epithelial cells have converted from columnar to cuboidal form with a vacuolar degeneration of follicular epithelial cells Figure (10), but it seems to show worse effects in experimental group of 20 days period of time that are comparable to the results from groups administrated fennel for 30 days of time, as sections of thyroid showing follicles became degenerated and necrosis occurred to the follicular epithelial cells and nonfunctional necrotic debris inside the lumen, that indicates advanced hypothyroidism Figure (11) compared with the histological sections of thyroid from control groups of rats Figure (5).

Consumption with fennel in high concentrations and a long period of time showed advanced hypothyroidism. The current study results agree with^26 who studied the effect of large number of environmental agents on thyroid function, he observed their interference with thyroid gland morphology and function, posing
the danger of thyroid disease, the most prominent effect of these agents was thyroid enlargement or goiter by acting directly on thyroid gland or indirectly by altering the regulatory mechanisms of thyroid gland, excretion of thyroid hormones and the peripheral metabolism. Several studies have shown goitrogenic and anti-thyroid effects of flavonoids that differ in terms of the mechanisms and potencies between each individual flavonoid. Phytoestrogens fall into the class of flavonoids found in soy and other plant sources known to have effects on the thyroid. Endocrine disruption of the hypothalamus-pituitary-thyroid hormone axis occurs after exposure to flavonoids as they inhibit thyroid peroxidase enzyme that is involved in thyroid hormone synthesis. Experimental studies in vitro illustrated interference with iodide organification in thyroid cells and follicles by several flavonoids and to cause goiter. Experimental studies suggest that flavonoids consumption could play a role in the etiology of thyroid cancer. The current study also agreed with the results of after treatment with 10mg/kg flavonoids of either genistein or daidzein for three weeks to male rats, they revealed that the height of thyrocytes and index of activation rate increased, while decrement occurred in thyroid hormones and the luminal colloid. In another study by on 60 rats treated with caraway extract (a member in Apiaceae family) in concentrations (100, 400, 800 and 1600)mg/kg for 45 days, results showed increment in T3 and T4 while TSH significantly decreased and caused hypothyroidism. While a study by on male rats treated with hydroalcoholic extract of Dorema aucheri (100, 200 and 400)mg/kg for three weeks, results showed significant increase in TSH level in the lowest dose with no change in T3 and T4 levels, the study of Dorema aucheri disagreed with the present study. Many minerals alter the synthesis of T3 and T4 through the interference with concentration of iodide and binding by thyroid gland. In Iranian traditional medicine, caraway is usually prescribed for weight loss, ingesting caraway over a long time develop symptoms resembling hyperthyroidism. Other reports showed that over use of D-limonene which is a component in Apiaceae herbs, in rat and mouse causes weight loss. Triiodothyronine has an influence on myxedematous patients, itis manifested by an increased basal metabolic rate, decreased cholesterol of the blood and body weight.

3.2 Testis Weight and Testis Functions

The statistical analysis of the present study of fennel effects on left and right Testis weights(gm) in Figure (12), (13) shows that:

Consumption of fennel for 10 and 20 days showed non-significant decrease in left and right testis weights of the experimental treated groups with concentrations (50, 100, 200)gm/kg fennel in comparison with control groups. While results revealed a significant decrease (p<0.05) in the left testis weights at 30 days experimental treated group of (200) gm/kg fennel (0.371±0.11) in comparison with the control group.
As well there was significant decrease (p<0.05) in right testis weights at 30 days feeding with fennel in experimental treated group of (200)gm/kg fennel (0.404±0.11) (gm) in comparison with control group (0.760±0.12) (gm). Both testes weight showed non-significant decrement between experimental treated groups when concentrations were fixed factors with the increment of the experimental duration in concentrations (50,100, 200)gm/kg fennel with one exception at 30 days fennel consumption in concentration (200)gm/kg (0.371±0.11) (gm) which showed significant decrement (p<0.05) in left testis weights when comparing between treated groups. Right testis weights results as well observed one exception at 30 days fennel consumption in concentration (200)gm/kg (0.404±0.11) which showed significant decrement (p<0.05) when comparing between treated groups.

Statistical analysis of the present study of fennel effects on testes functions that included Testosterone in the Figure (14) reveals that:

Fennel consumption for 10 days duration showed non-significant decrement in Testosterone level(ng/ml) in experimental treated groups with concentration (50)gm/kg in comparison with control group, but in the 20 days duration treatment with fennel illustrated significant decrement (p<0.05) in Testosterone level in experimental groups with concentrations (100,200)gm/kg (0.421±0.06), (0.422±0.11) (ng/ml), respectively, comparing with the control group (0.672±0.09) (ng/ml). Even a 30 days treatment with fennel showed a significant decrement (p<0.05) in Testosterone level in experimental groups with concentrations (50,100,200)gm/kg (0.372±0.07), (0.332.29±0.07), (0.303±0.05) (ng/ml), respectively, in comparison with the control group (0.672±0.07) (ng/ml). There was non-significant decrease due to fennel consumption on Testosterone level related with treatment duration when concentration was a fixed factor in all treated groups when comparing the treated groups at the same concentrations.
The results of the present study are consistent with previous findings about the decrement of testes weight and Testosterone level. In a study conducted in male mice that received 3mg/kg of aqueous extract of *Ferula hormonis* for 6 weeks, results demonstrated significant clinical signs of toxicity on body weight gain and weight of testis (p<0.001) as compared to the control group, that shows the fact that prolonged exposure to *Ferula hormonis* leads to fertility disturbances, which suggests possible alterations in secretion of a selective testicular regulator of testosterone secretion. Similar results were reported by on male rats after administration with (6, 30 and 60)mg/kg/day acetonic extract of *Ferula hormonis* for 10 days which showed a reduction in body weight gain, weight of testes and other accessory organs, but Testosterone serum level significantly increased (p<0.001) at high doses of 60 mg/kg/day. However administration with 6 mg/kg/day resulted in a significant decrease (p<0.05) in Testosterone serum level in comparison with the control group which suggests an antiandronic action of the plant extract. *Ferula hormonis* herb has antifertility effects that have been recently confirmed by in male and female mice after the administration with 3mg/kg/day *Ferula hormonis* extract for six weeks which resulted in significant reduction (p<0.001) in male and female mice fertility. In agreement with the present findings, reported that oral administration of the leave extract of *Centella asiatica* for a period of 60 days which equals the period of spermatogenic process in rats causes male reproductive toxicity in rats in addition to significant decrease (p<0.05) in testes weight in experimental groups compared to the control group, in the process body weights showed significant increase of treated males when compared with the control group, testes weight decrement was further confirmed concurrently with the decrease in androgen levels. It has been reported that physiological concentrations of testosterone, LH and FSH, play an important role in spermatogenesis. So the decrement in these hormones concentrations could reduce both the number and function of germinal and somatic cells of testis that leads to reduction in testes weight. Studied the effect of acetone extract of *Foeniculum vulgare* seeds administration in male rats for 15 days, results showed no significant change in either the organ weights nor in the final body weight. Phytoestrogens fundamentally fall into the class of flavonoids. They naturally occur as non-steroids plant chemicals that act like the female hormone estrogen. However, excessive consumption of phytoestrogens leads to some health problems.

### 4.5.2. Histological Changes of Testes

The main histological changes in all treated rats with fennel seeds on testes tissues in different periods of time compared to control groups is shown as follows:

Testes sections showed no histological changes after treatment with fennel in concentration of 50gm/kg of body weight for 10, 20 and 30 days. Sections look like normal structure appearance, development of seminiferous tubules with presence of abundant sperms inside the lumen Figure (16) for 10 days duration, Figure (17) for 30 days duration. compared with the histological sections of testes from control groups of rats Figure (15).

Histological changes after treatment with fennel in concentration of 100gm/kg of body weight for 10 days on testes sections showed seminiferous tubules with certain degeneration and necrosis of spermatogonia cells besides that less sperms become inside the lumen Figure (18). In 20 days duration testis sections showed worst effects, while experimental group of 30 days treatment with fennel illustrated seminiferous tubules with an improper development of spermatogonia cells and sperm formation Figure (19) in comparison with the histological sections of testes from control groups of rats Figure (15).
Histological sections of testes from treated groups with 200mg/kg of bw fennel consumption for 10 days demonstrated seminiferous tubules with necrosis of spermatogonia cells and rare sperm development, with necrotic debris inside the lumen as shown in Figure (20). After 20 days expose of fennel demonstrated significant histological changes but it seems to show worsen effects in experimental group of 30 days period of time, in which the testes showing seminiferous tubules with more prominent necrosis of spermatogonia cells and necrotic debris inside the lumen. No sperms appear inside the lumen which refers to chronic damage to testis tissue that could also effect testis functions as shown in Figure (21) in comparison with the histological sections of testes from control groups of rats Figure (15).

Figure (15) Section of normal structure of testis from rat control Groups shows normal seminiferous tubules consists of: 1.Normal nature of spermatogonia cells, 2. Presence of sperms inside the lumen. (200x)H&E.

Figure (17) Section of testis from rat groups treated with 50g/kg of bw fennel for 30 days, showing: normal structure appearance of the testes, 1.Presence of sperms inside the lumen, 2.Leydig cells. (400x)H&E.

Figure (18) Section of testis from rat groups treated with 100g/kg of bw fennel for 10 days, showing: 1. Leydig cells, 2. Seminiferous tubules with certain degenerative of spermatogonia cells, 3.Necrosis , 4.Less sperms inside the lumen. (400x)H&E.
indicated that the reproductive system is a target for fennel extracts action and its main component trans-anethole can cause changes in male and female organs and tissues that are associated directly or indirectly with the reproductive mechanisms. Anethole has been considered the active estrogenic agent in the essential oils of fennel and anise. In a study by they found that polyanethole compounds that exist in fennel oil are five times more active than pure anethole. Anethole possesses antispermatogenic effects and reduces sperm concentrations in epididymis in adult male albino rats. Results from showed that trans-anethole has anti-fertility activity. However, estrogenic compounds have the ability to inhibit gonads size. Estrogenic activity of fennel cause some side effects such as decrease in protein concentration in addition to decrement in acid and alkaline phosphatase in male genital organs. Similar results were demonstrated by a study of after 15 days administration of fennel acetone extract in rats. Administration of anethole at 10 and 50 mg doses caused significant reduction in seminal vesicle weight, also data appears that anethole may interfere with either action or biosynthesis of androgen on sex accessory tissues. In general, sex accessory organs weight is a crude bioassay of testosterone production or action. Plants containing phytoestrogen cause a decrease in blood testosterone level. Another study reported increasing prevalence of infertility due consumption of phytoestrogens from plants if consumed in high amounts, such compounds can affect the reproductive system as well as reducing fertility. However, further research to determine fennel effects on reproductive system in male rats that were divided in groups and administered with (100, 250 and 500) mg/kg of organic extract of fennel for 30 days showed that high dose of 500 mg/kg resulted in hyalinization of the seminiferous tubules.
with apoptosis in germ cells but Leydig and Sertoli cells seemed to be normal, toxic effects of fennel observed necrosis and karyolysis of spermatogenic cells, results also revealed that doses at (250 and 500) mg/kg of fennel significantly increased estradiol serum level, but decreased testosterone level leading to a decline in germ cells, stimulation of Sertoli cells could be in charge of these changes\textsuperscript{36}. Acute (24 hours) and chronic (90 days) toxicity studies on the ethanolic extract of fennel exhibited swollen testes in a male mice after 60 days of treatment with body weight gain but there was weight loss and no signs of toxicity in treated female mice\textsuperscript{60,61}. Reported that administration of (300 and 600) mg/kg bwestragole in corn oil to mice caused significant decrease in testis weights. A survey on male mice treated with 3mg/kg aqueous extract of F. hormonis for 6 weeks showed that the treatment produced lower sperm counts, reduction of activities and higher percentage of abnormalities which suggest possible organ damage, perhaps at the level of the germinal cells of testes\textsuperscript{38}. Opposite effects have been demonstrated from acute and chronic administration with F. hormonis on male rats, acute treatment improved the performance in sexual dysfunctions, while chronic treatment exerted toxic effects as decrease in total body weight, weight of testes and other accessory organs also exhibited atrophic testis, but subchronic treatment showed reduced testosterone levels\textsuperscript{40}. In agreement with previous studies, exposure of rats to leave extract of C. asiatica was followed by weight decrease of reproductive organs and a decrease in testosterone level with an increment inbody weight which suggests reproductive toxicity that includes degenerative changes in the seminiferous tubules, absence of spermatozoa in the testes, and germ cell apoptosis\textsuperscript{42}, showed that body weights in animal cases with leptin deficiency increased significantly, while the weights of testes are reduced in comparison with the control groups, considering some studies on leptin receptors and leptin expression on germ cells inside the testis\textsuperscript{62}. According to a study by Ibrahim ,2008 sections of testicular tissue from rats which were treated by fennel oil demonstrated no histopathological changes compared to the control groups, while anise-oil treated rats demonstrated several histopathological changes like necrotic spermatocyte cells and inhibition in Sertoli cell numbers. The benign effects of fennel oil extract are probably related to the antioxidant components in fennel\textsuperscript{63}. On the other hand, destructive effects of anise oil extract are probably related to its safrole component\textsuperscript{64}.

Considering the results of aforesaid studies and their different results and in comparison with the current study, these outcomes could be related to the excessive amounts of fennel ingested. The loss in testicular weight likely corresponded to a dose-dependent decrease of spermatogenic cells and degeneration of seminiferous tubules followed by significant decline in sperm density that are thought to be evidence of toxicity in male reproductive organs. The destructive effects of fennel including, histological changes in spermatogenetic cells may be related to safrole, as it has been associated with the presence of safrole-DNA adduct and finally causing genotoxicity. A Study by\textsuperscript{65} considered that safrole biotransformation is related with the outcome of safrole-DNA adduct. Spermatogenesis is stimulated by androgen but inhibited by estrogen and progestrone\textsuperscript{66}. Anethole is a phytoestrogen found in fennel and has an estrogen like effects, it can decrease blood testosterone level through Sertoli cells that can convert testosterone to estradiol and secrete inhibin which suppresses FSH synthesis leading to decline in Testosterone level. Sertoli cells are rich in thyroid hormone receptors in neonatal testes, therefore their important effects of hypothyroidism in this tissue\textsuperscript{67}. Thyroid gland tissue changes reflect its function by the hormonal production on the maturation of spermatozoa and the function of sex hormones\textsuperscript{68}, so deficiency of thyroid hormones retards maturation of Sertoli cells, thyroid hormones delay will prolong the period of proliferation.\textsuperscript{69} reported that thyroid hormones have significant effects on testis function in general, and on Leydig cells in particular. Other studies indicate that differentiation of Leydig cells in neonatal and adult testis may be arrested by hypothyroidism besides atrophic changes in size and organelle content of Leydig cells\textsuperscript{70}. The relation between fennel and testicular damage and hormonal disturbance was demonstrated after the decline in thyroid hormones that effect spermatogenesis directly via thyroid receptors on spermatocytes causing decrement in spermatogenesis, or indirectly via thyroid receptors on Leydig cells causing decline in testosterone followed by adverse effects on spermatogenesis resulting no sperms in the lumen of seminiferous tubules. The hormonal inhibition and the decrement in sperm count and sperm motility are perhaps of low androgen levels\textsuperscript{64}. So the impairment that occurred in thyroid gland after fennel consumption in the current study led to an impairment in the production and function of testis sexual hormone.

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