Plant Archives Vol. 18 No. 1, 2018 pp. 341-353 *ISSN 0972-5210*

PHYSIOLOGICAL, HORMONAL AND HISTOLOGICAL EFFECTS OF FENNEL SEEDS (*FOENICULUM VULGARE*) IN THYROID AND TESTES OF MALE RATS

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Abstract

In various parts of the world Fennel seeds *Foeniculum vulgare* has been used in a herbal medicine. The present study aims to shed light on fennel's side effects in male rats in the weights , hormonal, histological changes and some of the physiological parameters of thyroid and testes. About 60 Spargue-Dawley albino adult male rats were daily fed with fennel pellet in three different doses (50, 100, 200)gm/kg bw for three different periods of time (10, 20, 30) days. After end of each experiment animals were weighed then it scarified for blood and tissue collection , blood collected by heart puncture then it centrifuged for serum separation and kept at -80^{o}C to hormonal, biochemical analysis and some histological standards , then thyroid and testes were excised and fixed in neutral buffered 10% formalin for histological preparation. The results showed that 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 Thyroxin(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, thyroxin, testosterone, necrosis.

Introduction

Foeniculum vulgare is known as a culinary herb also useful for pharmaceutical industry for its high content in 1,8-cineole, linalool, fenchone and estragol. *F. vulgare* Mill contains 6.3% of moisture, 9.5% protein, 10% fat, 13.4% minerals, 18.5% fiber 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 (Díaz *et al*., 2006; Özcan and Chalchat, 2010). 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 (Badgujar, 2014). Fennel shows antispasmodic activities. As curative in infantile colic .also 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 (Ostad *et al*., 2001; Alexandrovich *et al*., 2003; Agarwal *et al*., 2008).

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" (Wilson, 2002). 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 (Thies, 2012). Thyroid gland also produce a third hormone "calcitonin" which is not considered a thyroid hormone (Vander *et al*., 2001).

Testes are primary male sex organs that form sperm cells (spermatogenesis) and male sex hormones (testosterone) (Costanzo, 2014).

Materials and Methods

Plant preparation

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 about (2100 kg) were powdered in a seed grinder (15,900 kg) 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.

Animals lab

Sixty male rats were randomly divided into twelve groups of five rats in each groups as the following :

Group 1, 2 and 3 : (control) animals without any treatment,

Group 4, 5 and 6 : (experimental groups) that respectively received 18- 20 g fennel pellet in three doses of (50, 100 and 200g/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 200g/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.

Collection of blood

After end of each experiment animals were weighed then 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.

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.

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 Gharib *et al.* (1971), T4 according to Baker (1948) and TSH according to Fisher (1996). Also, the reproductive hormone testosterone was measured.

Histopathological preparation

The preparation for histological sections was performed according to the method of Humason (1979).

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.

Results

Thyroid weight and functions

Results in fig. 1 shows the effects of fennel in thyroid weights as following : Fennel consumption for 10 days demonstrated significant increase ($p<0.05$) in thyroid weights of the experimental treated groups in concentrations (100, 200gm/kg) fennel (0.026±0.01) ,(0.027±0.001gm), 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, 200gm/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, 200gm/kg) (0.031±0.002), (0.035±0.002), (0.039±0.002gm), 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.

The statistical analysis of the present study for fennel effects in thyroid hormones that included TSH, T3 and T4 in the figs. 2, 3, 4 reveals that the Fennel effects illustrated no significant changes in values of TSH(µlU/ml) in all periods of time (10, 20, 30days) and in all concentrations of fennel (50, 100, 200gm/kg) when compared with control groups as shown in fig. 2.

Also non-significant differences due to fennel consumption in 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, 200gm/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 fig. 3.

The present study shows findings about fennel consumption effect in thyroxin 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 in (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), but 20 days duration of fennel consumption illustrated significant decrease ($p<0.05$) in T3 level at experimental groups with concentration (100, 200 gm/kg) (3.00±0.09), (2.87±0.26 µg/dl), respectively, when compared with control group (4.33±0.55 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 fig. 4.

The results of the present study which showed reduction in thyroid hormones T3 and T4 agreed with results of another previous study by Kooti *et al*. (2015) after administration with alcoholic extract of celery (member in Apiaecea family) with doses (50, 100, 200mg/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 Dehghani *et al.* (2010) 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 Parvinroo *et al.* (2014), 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 T3 by working directly on the thyroid gland for generation of T4 (Nilsson and Peterson, 1975). Flavonoids are important contents of fennel and other plants of Apiaceae family (Faudale *et al*., 2008). Flavonoids possess a variety of biological activities including anti-thyroid effects in experimental animals and humans, they are widely distributed in plant-derived foods (de Souza Dos Santos *et al*., 2011).

Flavonoids also could affect the availability of thyroid hormones to target tissues, by inhibiting thyroperoxidase, deiodinase activity (Committee on Toxicity of Chemicals in Food, 2014) and decreases the expression of the thyrotropin receptor and thyroglobulin genes (Giuliani *et al*., 2014).

Histological changes of thyroid

The main histological changes in all treated rats with fennel seeds in 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 (fig. 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 (fig. 7) compared with the histological sections of thyroid from control groups of rats (fig. 5).

Histological changes after treatment with fennel in concentration of 100gm/kg of body weight for 10 days

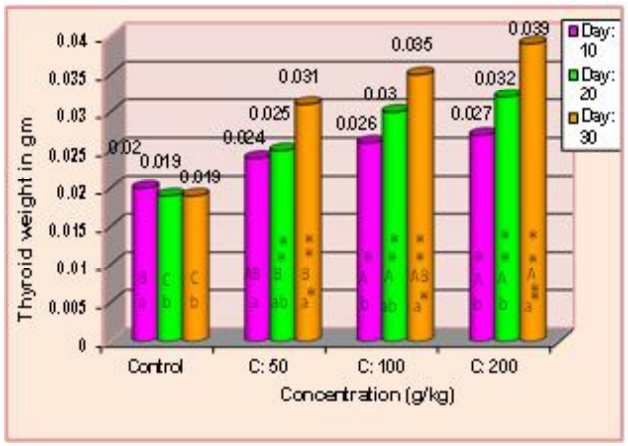

Fig. 1 : Effect of different concentration of femel (50, 100, 200g/kg on the thyroid weights of rats with different period of time (10, 20, 30 days) in comparision with control groups.

(*) significant increase (P=0.05). (**) highly significant increase (P=0.01)

(A, B, C) represent the significant difference between groups with days as a fixed factor and concentration as a variable factor.

(a, b, c) represent the significant difference between groups with concentration as a fixed factor and days as a variable factor.

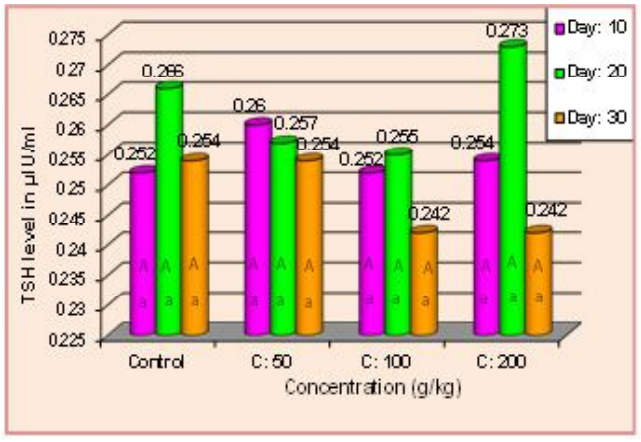

Fig. 2 : Effect of different concentration of femel (50, 100, 200g/kg on the TSH levels of rats with different periods of time (10, 20, 30 days) in comparision with control groups.

(A, B, C) represent the significant difference between groups with days as a fixed factor and concentration as a variable factor.

(a, b, c) represent the significant difference between groups with concentration as a fixed factor and days as a variable factor.

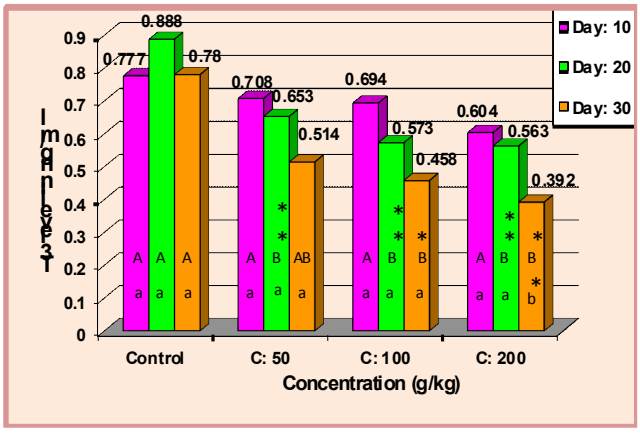

Fig. 3 : Effect of different concentration of fennel (50, 100, 200 g/kg) on the T3 levels of rats with different periods of time (10, 20, 30 days) in comparison with control groups.

(*) significant decrease (P=0.05). (**) highly significant increase (P=0.01)

(A, B, C) represent the significant difference between groups with days as a fixed factor and concentration as a variable factor.

(a, b, c) represent the significant difference between groups with concentration as a fixed factor and days as a variable factor.

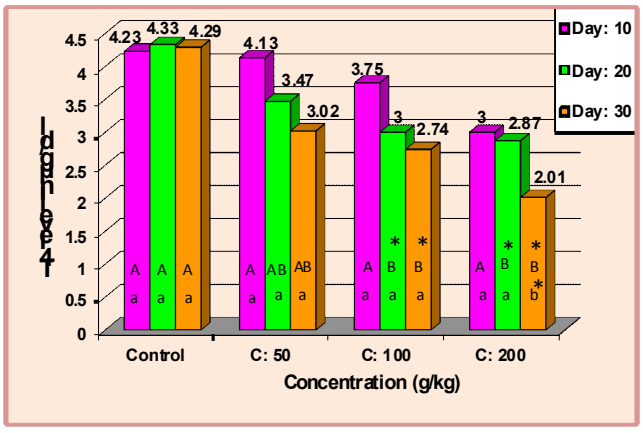

Fig. 3 : Effect of different concentration of fennel (50, 100, 200 g/kg) on the T4 levels of rats with different periods of time (10, 20, 30 days) in comparison with control groups.

(*) significant decrease (P=0.05).

(A, B, C) represent the significant difference between groups with days as a fixed factor and concentration as a variable factor.

(a, b, c) represent the significant difference between groups with concentration as a fixed factor and days as a variable factor.

on thyroid sections showed that certain follicles are empty from colloid while others contain colloid with small scalloping vacuoles (fig. 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 (fig. 9), compared with the histological sections of thyroid from control groups of rats (fig. 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 didn't contained colloid. Follicular epithelial cells have converted from columnar to cuboidal form with a vacuolar

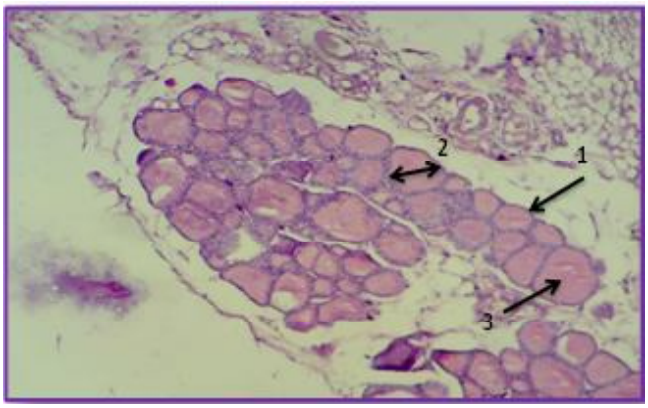

Fig. 5 : Section of thyroid gland from control groups showing Follides lined by cuboidal cells and most of follides contain pink colloid material : 1. Follicular epithelium, 2. Follicular lumen, 3. Colloid material (H & E) 200x.

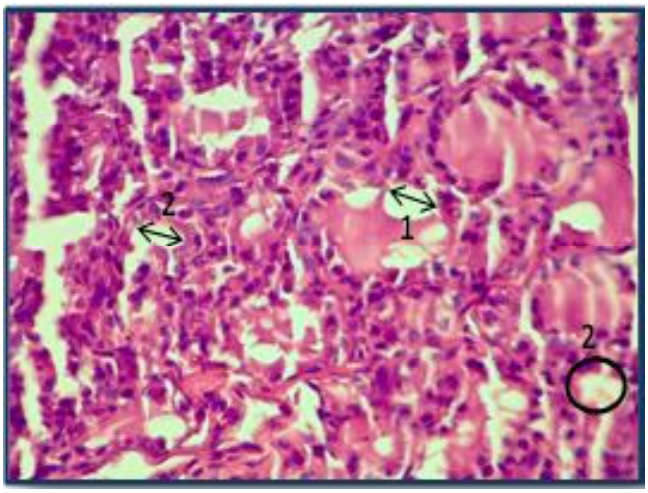

Fig. 7 : Section of thyroid gland from rat groups treated with 50 g/kg of bw fennel for 30 days, showing 1. thyroid follides with scalloping, 2. Follides without coll oid material (hypothyroid). (H&E) 400 k.

degeneration of follicular epithelial cells (fig. 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 (fig. 11) compared with the histological sections of thyroid from control groups of rats (fig. 5).

Consumption with fennel in high concentrations and a long period of time showed advanced hypothyroidism. The current study results agree with (Hall, 2016), who studied the effect of large number of environmental agents in thyroid function, he observed their interference with thyroid gland morphology and function, posing the

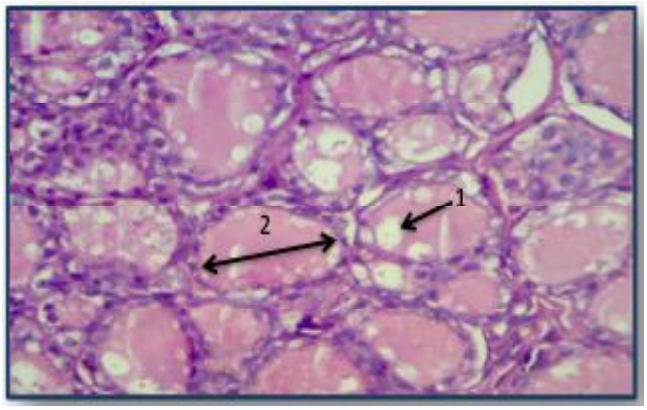

Fig. 6 : Section of thyroid gland from rat groups with 50g/kg of bw fennel for 10 days showing 1. Thyroid follicles with scallping vacoules of colloid material at the apical pole of the follicular epithelial cells, 2. Colloid. (H & W) 400x.

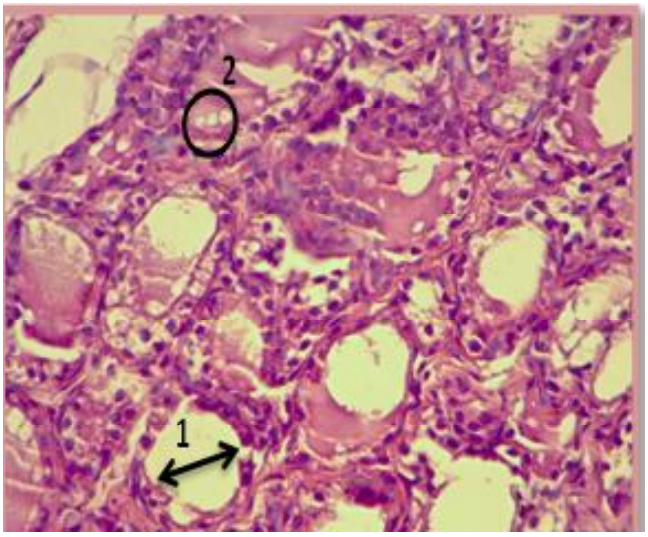

Fig. 8 : Section of thyroid gland from rat groups treated with 100 g/k of bw fenned for 10 days, showing 1 Emptyfollide 2 Follicles containing colloid with small scalloping vauoles (H&E) 400 K.

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 (Chandra and De, 2013). Phytoestrogens fall into the class of flavonoids found in soy and other plant sources known to have effects on the thyroid (Saljoughian, 2007). Endocrine disruption of the hypothalamus-pituitary-thyroid hormone axis occurs after exposure to flavonoids (Hamann, 2006) as they inhibit thyroid peroxidase enzyme that is involved in thyroid hormone synthesis (Ferreira *et al*., 2002).

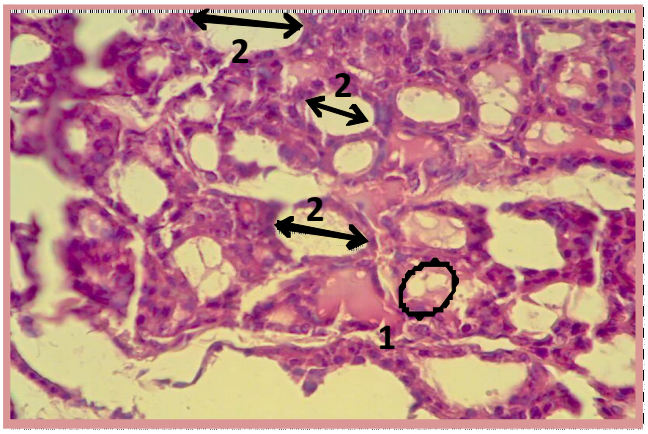

Fig. 9 : Section of thyroid gland from at groups treated with 100g/kg of bw fennel for 30 days showing : 1. Follicles containing colloid material with rare scalloping vacuoles (Hypothyroidism), 2. Major of follices containing no colloid. (H&E) 400 x.

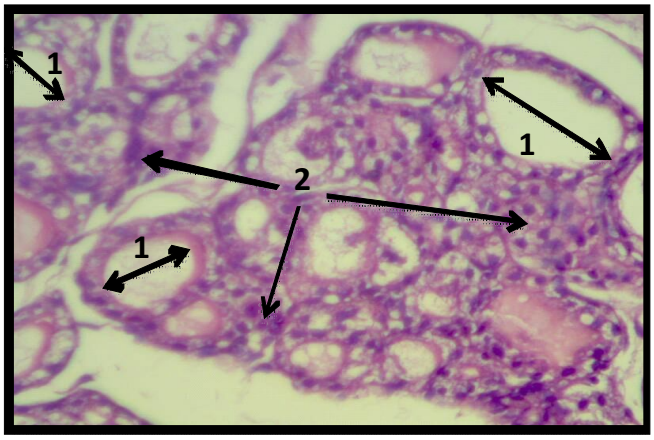

Fig. 10 : Section of thyroid gland from at groups treated with 200g/kg of bw fennel for 10 days showing : 1. Majority of thyroid follicle containing no colloid with vacuolar degeneration of follicular epithelial cells, 2. Hypertrophy. (H&E) 400 x.

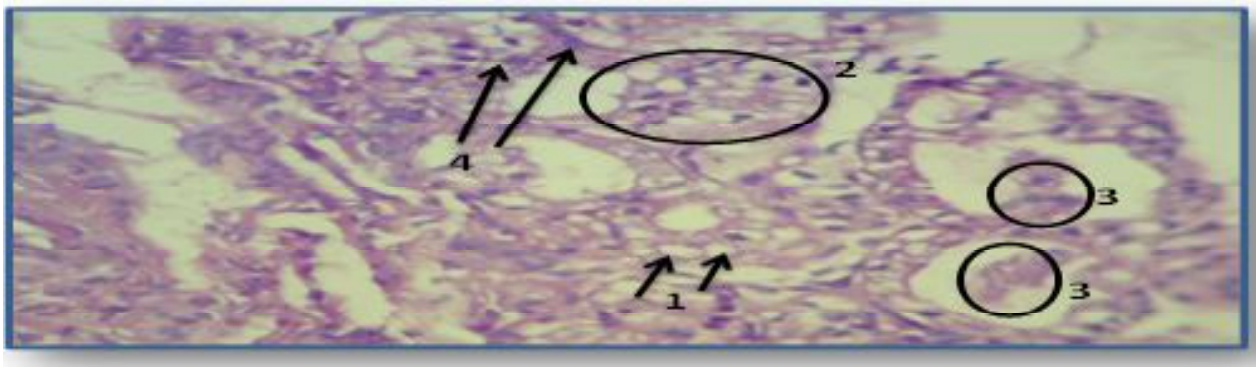

Fig. 11 : Section of thyroid gland from rat groups treated with 200 g/kg of bw fennel for 30 days, showing : 1. Degenerated follicle. 2. Necrosis of the follicular apithelial cells, 3. Non functional necrosis debris inside the lumen, 4. Hypertrophy (Advanced hypothyroidism) (H & E) 400x.

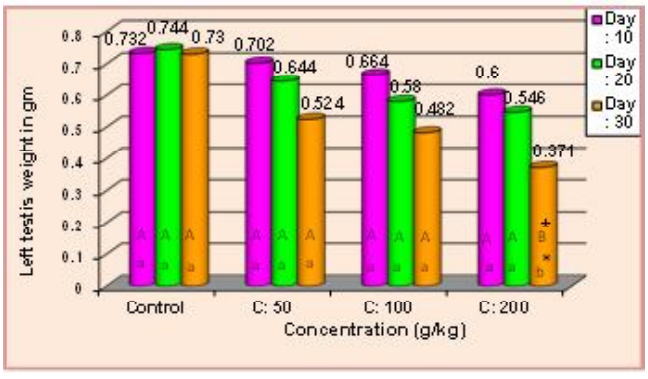

Fig. 12 : Effect of different concentration of fennel (50, 100, 200 gm/kg) on left testis weights of rats with different periods of time (10, 20, 30 days) in comparison with control groups.

(*) significant decrease (P≤0.05). (A, B, C) represent the significant difference between group with days as a fixed factor and concentration as a variable factor. (a, b, c) represent the significant difference between groups with concentration as a fixed factor and days as a variable factor.

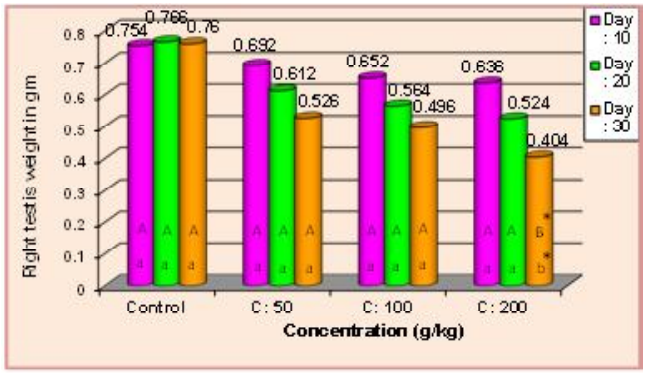

Fig. 13 : Effect of different concentration of fennel (50, 100, 200 gm/kg) on right testis weights of rats with different periods of time (10, 20, 30 days) in comparison with control groups.

(*) significant decrease (P≤0.05). (A, B, C) represent the significant difference between group with days as a fixed factor and concentration as a variable factor. (a, b, c) represent the significant difference between groups with concentration as a fixed factor and days as a variable factor.

Experimental studies *in vitro* illustrated interference with iodide organification in thyroid cells and follicles by several flavonoids (van der Heide *et al*., 2003) and to cause goiter (Committee on Toxicity of Chemicals in Food, 2014). Experimental studies suggested that flavonoids consumption could play a role in the etiology of thyroid cancer (Xiao *et al*., 2014). The current study also agreed with the results of Sosiæ-Jurjeviæ *et al*. (2010) 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 Dehghani *et al*. (2010) on 60 rats treated with caraway extract (a member in Apiacea 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 Neoushan *et al*. (2010) 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 (Sarne, 2010). In Iranian traditional medicine, caraway is usually prescribed for weight loss, ingesting caraway over a long time develop symptoms resembling hyperthyroidism (Jazayeri, 1981). Other reports showed that over use of *D*- limonene, which is a component in Apiaceae herbs, in rat and mouse causes weight loss (National Toxicology Program, 1990a).

Thus, depending on the dose and type of treatment and species, flavonoids seem to show different effects on pituitary-thyroid axis. The current study supports the idea that high consumption of flavonoid-rich plant products allied to the nutritional deficiency of iodine might participate in the development of hypothyroidism and goiter, in addition flavonoids have anti-oxidant effect that could contribute to the thyroid hormone synthesis inhibition, as they could scavenge H_2O_2, which is the cofactor of thyroperoxidase.

Testis weight and testis functions

- The statistical analysis of the present study of fennel effects in left and right Testis weights (gm) as showed in figs. 12, 13.

The 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 gm) in comparison with the control group (0.730±0.13 gm). 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 in testes functions that included Testosterone in the fig. 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 in 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 in 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 (Khleifat *et al*., 2001). Similar results were reported by Zanoli *et al*. (2004) in 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 Homady *et al.* (2002) 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 of Heidari *et al.* (2014) 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 (Choi and Lee, 2004). 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 (Heidari *et al*., 2014). Malini *et al.* (1985) 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 (Saljoughian, 2007). They naturally occur as non-steroids plant chemicals that act like the female hormone estrogen (Al-Yawer, 2011).

Histological changes of testes

The main histological changes in all treated rats with fennel seeds in 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 (fig. 16) for 10 days duration, fig. 17 for 30 days duration. compared with the histological sections of testes from control groups of rats (fig. 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 (fig. 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 (fig. 19) in comparison with the histological sections of testes from control groups of rats (fig. 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 fig. 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 effected testis functions as shown in fig. 21 in comparison with the histological sections of testes from control groups of rats (fig. 15).

Ostad *et al*. (2004) 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. Results from EMEA (2006) showed that *trans*-anethole has anti-fertility activity. However, estrogenic compounds have the ability to inhibit gonads size (Alemany-Costa *et al.,* 2012). 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 (Rahimi and Ardekani, 2013). 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 (Farook *et al*., 1991). In general, sex accessory organs weight is a crude bioassay of testosterone production or action (Dixon, 1986). Plants containing phytoestrogen cause a decrease in blood testosterone level (Weber *et*

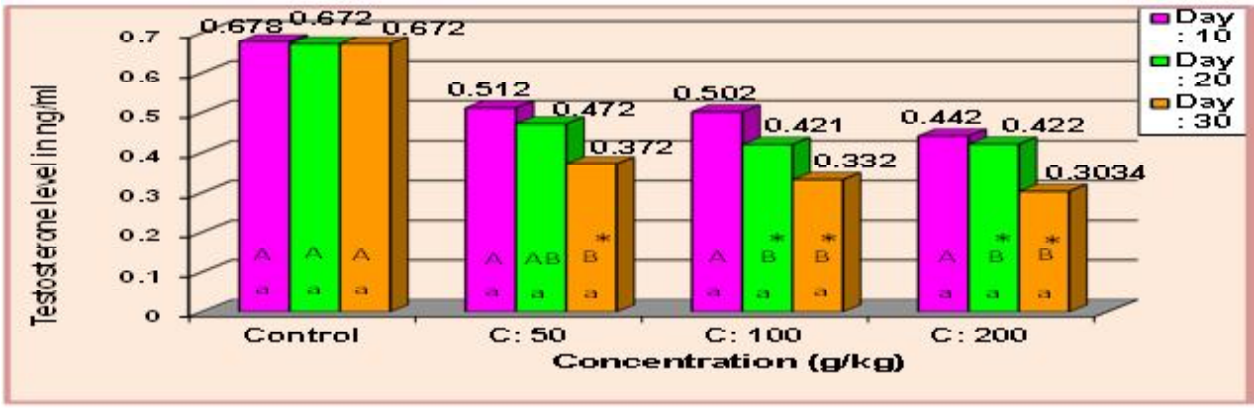

Fig. 14 : Effect of different concentration of fennel (50, 100, 200 gm/kg) on Testosterone levels of rats with different periods of time (10, 20, 30 days) in comparison with control groups.

(*) significant decrease (P≤0.05). (A, B, C) represent the significant difference between group with days as a fixed factor and concentration as a variable factor. (a, b, c) represent the significant difference between groups with concentration as a fixed factor and days as a variable factor.

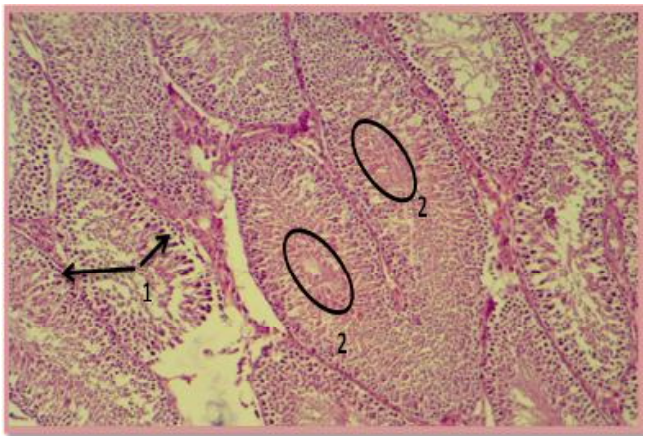

Fig. 15 : Section of normal structure from rat control Groups shows normal seminiferous tubules consists of : 1. Normal nature of spermatogonia cells, 2. Presence of sperms inside the Lumen. (200x) H & E.

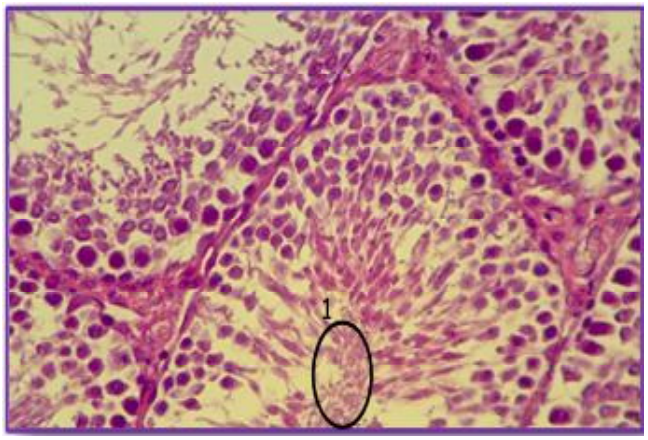

Fig. 16 : Section of testis from rat groups treated with 50g/kg of bw fennel for 10 days, showing normal structure appearance and development of seminiferous tubules, 1. Presence of abundant sperms inside the lumen. (400x) H & E.

al., 2001). 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 (Csupor-Loffler *et al*., 2009). However, further research to determine fennel effects in 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 (Dehghani *et al*., 2005). 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 (Shah *et al*., 2011). National Toxicology Program (2011) reported that administration of (300 and 600 mg/kg) bw estragole in corn oil to mice caused significant decrease in testis weights. A survey on male mice treated with 3mg/kg aqueous extract of *Ferula hormonis* for 6 weeks showed that the treatment produced lower sperm counts, reduction of activities and higher percentage of abnormalities which suggested possible organ damage, perhaps at the level of the germinal cells of testes (Khleifat *et al*., 2001). Opposite effects have been demonstrated from acute and chronic administration with *Ferula hormonis* in male rats, acute treatment improved the performance in sexual dysfunctions, while chronic treatment exerted toxic effects as decrease in total body

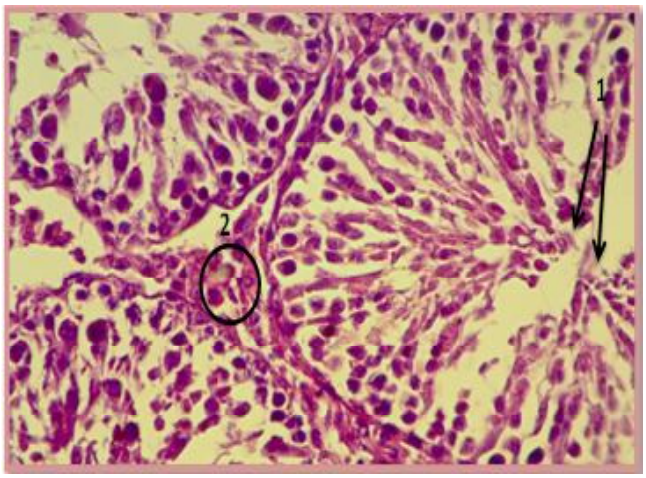

Fig. 17 : Section of testis from rat groups treated with 50g/kg of bw fennel for 30 days, showing normal structure appearance of the testis, 1. Presence of sperms inside the lumen. 2. Leydig cells (400x) H & E.

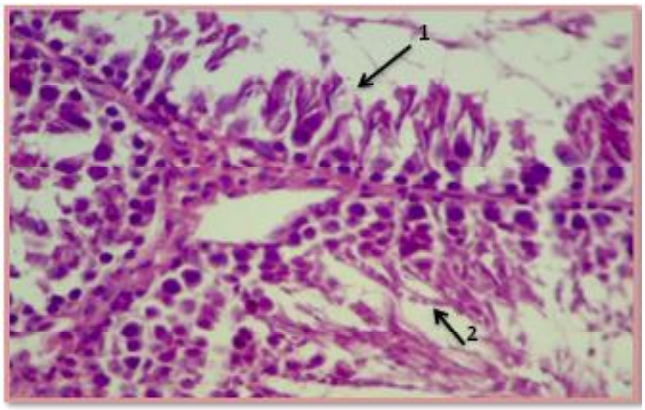

Fig. 19 : Section of testis from rat groups treated with 100g/kg of bw fennel for 30 days. The seminiferous tubules showing : improper development of spermatogonia cells and sperm formation, 1. No sperm, 2. Few sperms. (400x) H & E.

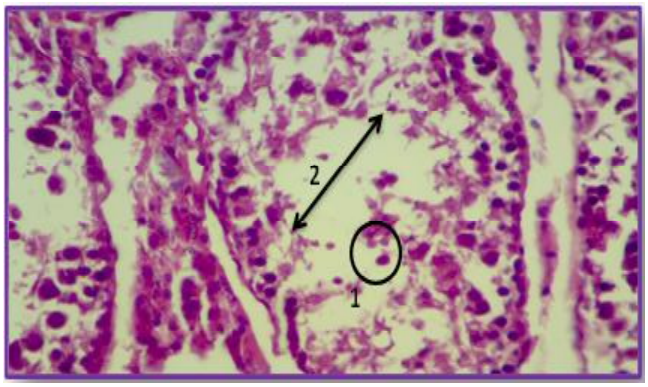

Fig. 21 : Section of testis from rat groups treated with 200g/kg of bw fennel for 30 days. The seminiferous tubules showing more development of necrosis to spermatogonia cells, 1. Necrosis debris inside the lumen, 2. No sperm inside the lumen. (400x) H & E.

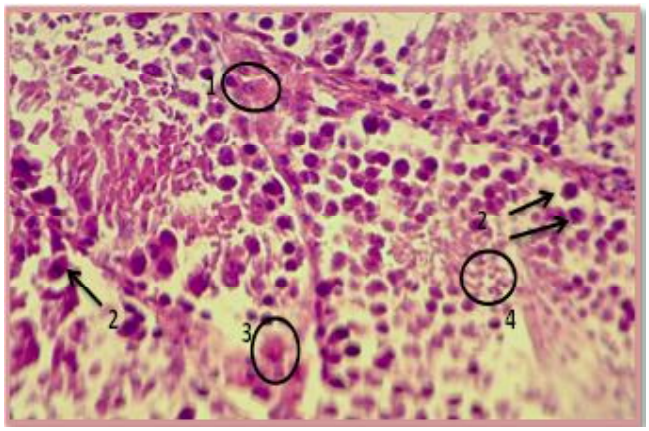

Fig. 18 : Section of testis from rat groups treated with 100g/kg of bw fennel for 10 days, showing : 1. Leydig cells, 2, Seminiferous tubules with certain degenerative of sp;ermatogonia cells, 3. Necrosis 4. Less sperms inside the lumen. (400x) H & E.

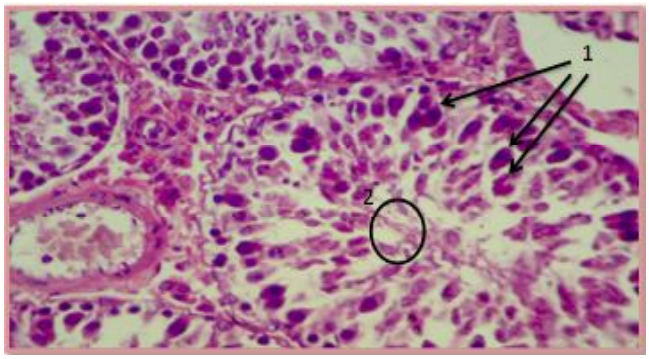

Fig. 20 : Section of testis from rat groups treated with 200g/kg of bw fennel for 10 days. The seminiferous tubules showing : 1. necrosis of spermatogonia cells, 2. Rare sperm development with necrotic debris inside the lumen. (400x) H & E.

weight, weight of testes and other accessory organs also exhibited atrophic testis, but subchronic treatment showed reduced testosterone levels (Zanoli *et al*., 2004). In agreement with previous studies, exposure of rats to leave extract of *Centella asiatica* was followed by weight decrease of reproductive organs and a decrease in testosterone level with an increment in body weight which suggests reproductive toxicity that includes degenerative changes in the seminiferous tubules, absence of spermatozoa in the testes, and germ cell apoptosis (Heidari *et al*., 2012), 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 in leptin receptors and leptin expression in germ cells inside the testis (Lampiao *et al*., 2009). 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 (Parejo *et al*., 2004). On the other hand, destructive effects of anise oil extract are probably related to its safrole component (Ibrahim, 2008).

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 Zhou *et al.* (2007) considered that safrole biotransformation is related with the outcome of safrole-DNA adduct. Spermatogenesis is stimulated by androgen but inhibited by estrogen and progesterone (Junqueira, 2003). 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 inhibit 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 (Crissman *et al*., 2000). Thyroid gland tissue changes reflect its function by the hormonal production on the maturation of spermatozoa and the function of sex hormones (Al-Sudany, 2004), so deficiency of thyroid hormones retards maturation of Sertoli cells, thyroid hormones delay will prolong the period of proliferation. (Waner *et al*., 2008) reported that thyroid hormones have significant effects on testis function in general, and in 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 (Mendis-Handagama *et al*., 1998). 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 missed sperms in the lumen of seminiferous tubules. The hormonal inhibition and the decrement in sperm count and sperm motility may be perhaps of low androgen levels (Ibrahim, 2008). 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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