

Correlation between fennel- or anise-oil administration and damage to the testis of adult rats

Amal Attia El-Morsy Ibrahim

Department of Zoology, Women's Faculty of Arts, Education & Science, Ain Shams University, Cairo

Abstract

Tradition in Egypt indicates that anise oil damages testicular development in boys. This study compares the effect of fennel oil and anise oil on the histopathology and immunology of the testes of young adult rats. Sections from testicular tissue of rats treated with fennel oil showed no histopathological changes relative to control animals in all testicular cell types. On the other hand, anise-oil-treated rats showed several histopathological changes (inhibition in Sertoli cell numbers, necrotic spermatocyte cells, etc), Sertoli cells with marked changes in the boundaries of the nuclear membrane and cytoplasmic vacuoles of various sizes, spermatids with acrosomal vesicles and different pieces of spermatozoa with obvious lesions and distorted arrangements of microtubules, vacuoles around their heads and acrosomes, Leydig cells distorted nuclear membranes, distribution of heterochromatin in the inner surface and small lipid droplets in the cytoplasm. Anise oil administration also inhibited inhibin and GST expression, besides decreasing testosterone, T3 and T4 hormones and inhibiting sperm counts and sperm motility.

Keywords: Inhibin, GST

Introduction

Foeniculum vulgare (Fennel) and *Pimpinella anisum* (Anise) are traditional Egyptian herbs, and anise-seed beverages are very popular in Egypt. The plants are members of the Umbelliferae, and have a very sweet smell. They have been used extensively in folklore medicine to treat common ailments, and their oils contain trans-anethole, a flavouring substance.

Fennel oil helps clear up pulmonary congestion, bronchitis, and asthma (Haggag *et al.* 2003; Choi & Hwag 2004). Anethole and fenchone are the main components of fennel (Parejo *et al.* 2004; Tognolini *et al.* 2006). The oral LD50 of anethole in rats is 900 mg/kg body weight, and it does not induce any unscheduled DNA synthesis in rat hepatocytes (Howes *et al.* 1990). Very high doses of trans-anethole are hepatotoxic, inducing a small number of hepatocarcinomas in female rats, but not in male rats or mice of either gender (Newberne *et al.* 1989; Truhaut *et al.* 1989). The antioxidative properties of these components include radical scavenging effects, inhibition of H₂O₂ and Fe²⁺ chelating activity by more than 70% (El & Karakaya 2004), as well as anti-inflammatory and immunomodulatory activities by enhancing natural killer cell functions, the effectors of the innate immune response (Ng & Figg 2003; Choi & Hwag 2004; Ibrahim 2007a&b).

Anise has been used as a popular aromatic herb and spice since ancient times. Anise oil is used as an exportant, antispasmodic and bronchial aid. It has a mild hormonal action which is mostly estrogenic, so it has been used for female problems such as promoting milk, easing birthing and allaying menopausal symptoms. Its fruit has been used for medicine and in cooking, and is listed in European pharmacopoeia (Ishikawa *et al.* 2002). For medicinal purposes, it is used to treat dyspeptic complaints and catarrh of the respiratory tract. The taste and smell of the fruit are mainly due to the essential oil, which is 80-90% trans-anethole, with other components consisting of cis-anethole, safrole, estragole, p-anisaldehyde, anisketone, linalool and b-farnesene (Ishikawa *et al.* 2002). Safrole occurs naturally in a number of herbs and spices such as nutmeg, mace, cinnamon, anise, black pepper and sweet basil and their essential oils. Safrole is also present in Cola drinks, and associated with the presence of safrole-DNA adduct in human (Jeurissen 2007).

According to the traditional thinking, drinking anise by boys may be harmful to their reproductive system. Tyler & Foster (1999), found that safrole is toxic in a concentration of 1% of the diet, producing weight loss, testicular atrophy, bone marrow depletion and also produced tumors in two-thirds of the animals treated with it. On the other hand, Ibrahim (2007b) found that fennel oil administration could ameliorate the destructive effect of cigarette smoke in rat testis. Despite belonging to the same family (Umbelliferae), these two herbs apparently have opposite effects on the testis.

The aim of the present study is therefore to clarify the histopathological, immunological and hormone level changes in testicular tissue and blood under the influence of either fennel or anise oral intake.

Materials & Methods

Fennel and anise oil extracts were purchased from Cap Pharm for Extracting Natural Oils & Herbs, Cairo, Egypt. The extract of fennel contains 50% of trans-anethole and 25% of fenchone. The oil extract of anise containing 50% trans-anethole, linalool and about 5% safrole.

Young adult male albino rats of the *Rattus rattus* strain weighing 100 ± 10 g were obtained from the Serum & Antigen Laboratories at Helwan, Egypt. They were housed in a controlled environmental room, with a 12L:12D photoperiod. The animals were allocated to three groups of 7 rats each: *control* (administered saline), *fennel* (receiving 1 ml per kg body wt of fennel oil orally for 30 days, containing 500 mg kg^{-1} trans-anethole and 250 mg kg^{-1} fenchone) and *anise* (1 ml kg^{-1} of anise oil orally for 30 days, containing 500 mg kg^{-1} trans-anethole and 5 mg kg^{-1} safrole).

Small pieces of testis were collected from all dissected animals, immersed in saline and then put in 10% neutral-buffered formalin for histopathological studies, and stained with haematoxylin and eosin. For electron-microscope studies, fresh small pieces of testis were fixed in 3% glutaraldehyde-formaldehyde for 5 h, then in (0.2 M) Na cacodylate for 2 h at 4°C , then washed in phosphate buffer pH 7.2 for 30 min and post-fixed in 1% osmic acid (2% OsO_4 + 0.3 M Na cacodylate) for 2 h at 4°C . The tissue pieces were then washed in phosphate buffer (pH 7.2) for 30 min at 4°C . Samples were dehydrated through ascending grades of ethanol and embedded in epoxy resin in an oven at 60°C for 14 h to produce a firm block. Ultra-thin sections (about 80 nm) were cut with ultramicrotome, stained with uranyl acetate and lead citrate, and examined by Transmission EM JOEL 1200 EX II at the Central Laboratory, Faculty of Science, Ain Shams University.

For immunohistochemical studies, slides were deparaffinized, submerged in H_2O_2 to block endogenous peroxidase, and washed three times with PBS for 2 min. Sections were then incubated for 10 min with serum-blocking solution to block non-specific binding. This was followed by incubation with primary antibodies for 30-60 min at room temperature, rinsed three times with PBS for 2 min, incubated with biotinylated secondary antibody for 20 min, rinsed with PBS, incubated with enzyme conjugate for 10 min, and then washed. The color was developed using an AEC kit according to the manufacturer's directions, and sections counterstained with haematoxylin (Zymed).

The left epididymis was placed in a pre-warmed Petri-dish containing 5 ml of sodium citrate solution (2.9%) at 37°C . The cauda was then nicked with a scalpel blade to allow sperm to emerge from the engorged cauda epididymis, and placed in an incubator at 37°C for 15 min. The suspension was stirred and one drop placed on a warmed slide under a cover slip. At least five fields were observed at 400x magnification, using a standard light microscope. Motility was reported as an average percent motile. One drop of the previous suspension of live sperm was placed on a slide, and a smear made using the edge of another slide (as in the procedure

for preparing blood smears). The slides were air-dried and stained with 1% eosin at room temperature. Approximately 200 sperm from each rat were examined by light microscopy and classified as normal and abnormal.

The right epididymis and specimens of the right testis were homogenized manually in 0.5 ml of 0.9% NaCl solution. The homogenates were diluted with 1.5 ml saline, and spermatozoa counted using Neubauer haemocytometer at 400x magnification in five squares. Five counts per sample were averaged.

The serum level of thyroid hormones, total triiodothyronine (T3) and total thyroxine (T4) and testosterone were measured using Adaltis ElAgen kits (Italy).

Statistical analysis used one-way ANOVA with the conventional significance level of $P < 0.05$, implemented by SPSS for Windows, release 10. The least significant difference (LSD) test was carried out for multiple *post-hoc* comparisons, to compare the treated groups with control group.

Results

Testis sections from control rats showed normal microscopical features of testicular tissue (Fig.1A). Sections from rats treated with fennel oil also revealed normal appearance of the testicular tissue architecture (Fig.1B). Sections of testicular tissues from rats administered anise oil revealed several histopathological changes such as inhibition in Sertoli cell numbers, necrotic cells of the spermatocytes, spermatogonia leaving the basement membrane, and marked reduction of the germ cells (Fig.2A). Some seminiferous tubules had irregular boundaries and reduction in germ cells as shown in Figure 2B.

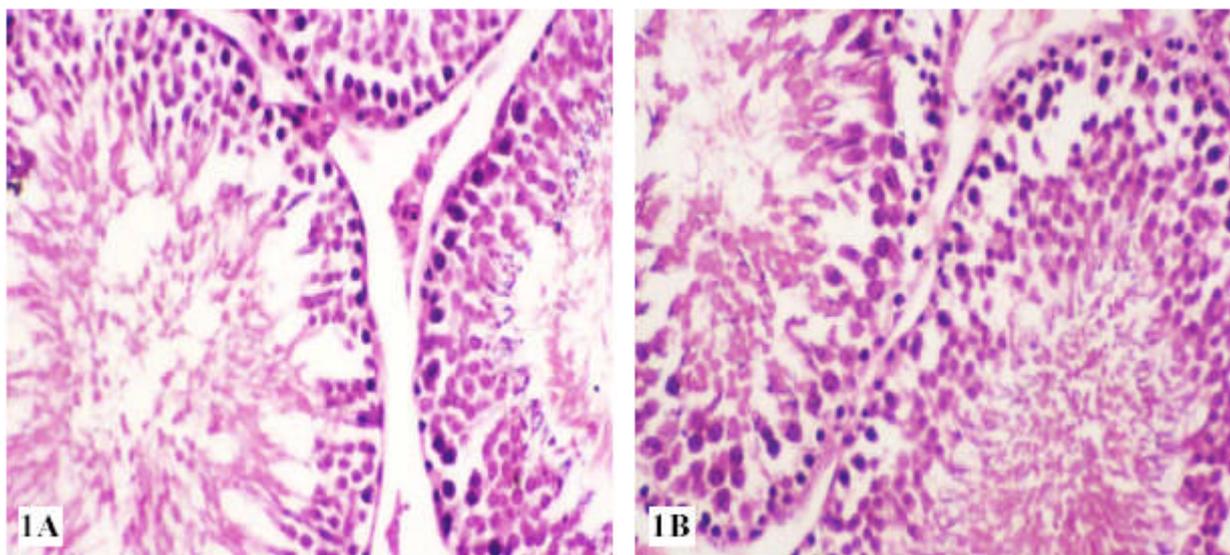


Fig. 1: Photomicrographs of testis sections from (A) a control rat, showing normal testicular tissue architecture; and (B) from a rat treated with fennel-oil, showing normal features of testicular tissue; (H-E, x200).

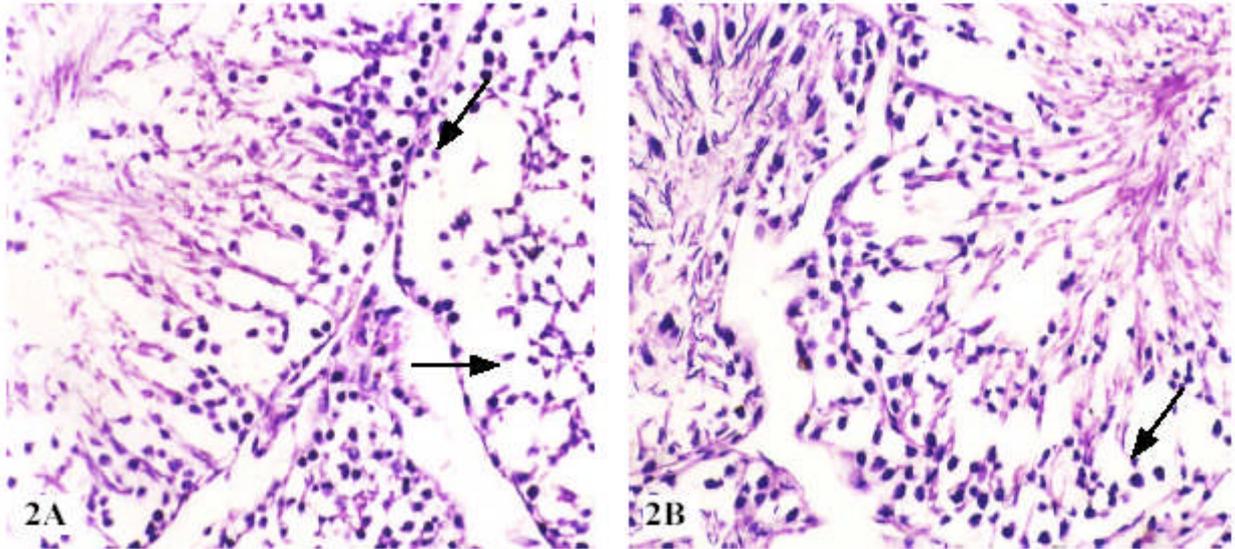


Fig. 2: Photomicrographs of testis sections from rats administered anise oil orally for 30 days, showing (A) inhibition in Sertoli cell numbers, appearance of spermatocytes as necrotic cells (arrows), spermatozoa leaving the basement membrane, marked reduction of germ cells and degeneration of the sperms; and (B) depletion of basal germ cells, decrease in the number of Sertoli cells and irregular boundaries of some seminiferous tubules; (H-E, x200).

The ultrastructure examination of sections from rat testes of the control group showed Sertoli cells of normal appearance, with oval nuclei with a prominent nucleolus; the cytoplasm was filled with ribosomes, lysosomes, mitochondria and filamentous proteins (Fig.3A). In the fennel-oil-treated rats, there were no apparent ultrastructure changes in Sertoli cells, which had dumbbell-shaped mitochondria (Fig.3B). However, Sertoli cells from anise-oil-treated rats showed marked changes in the boundaries of the nuclear membrane and cytoplasmic vacuoles of various sizes appeared (Fig. 3C). The ultrastructure of the spermatids from control rat testis manifested spermatids in different phases of spermatogenesis; these cells have developing acrosomal vesicle elaborated by Golgi apparatus (Fig. 4A). Normal spermatids with mitochondria massed beside the cell membranes appeared in testis sections from fennel-oil-treated rats (Fig. 4B), but testis sections from anise-oil-treated rats revealed spermatids with swollen acrosomal vesicles, and different pieces of spermatozoa that appeared with distorted arrangement of microtubules (Fig. 4C).

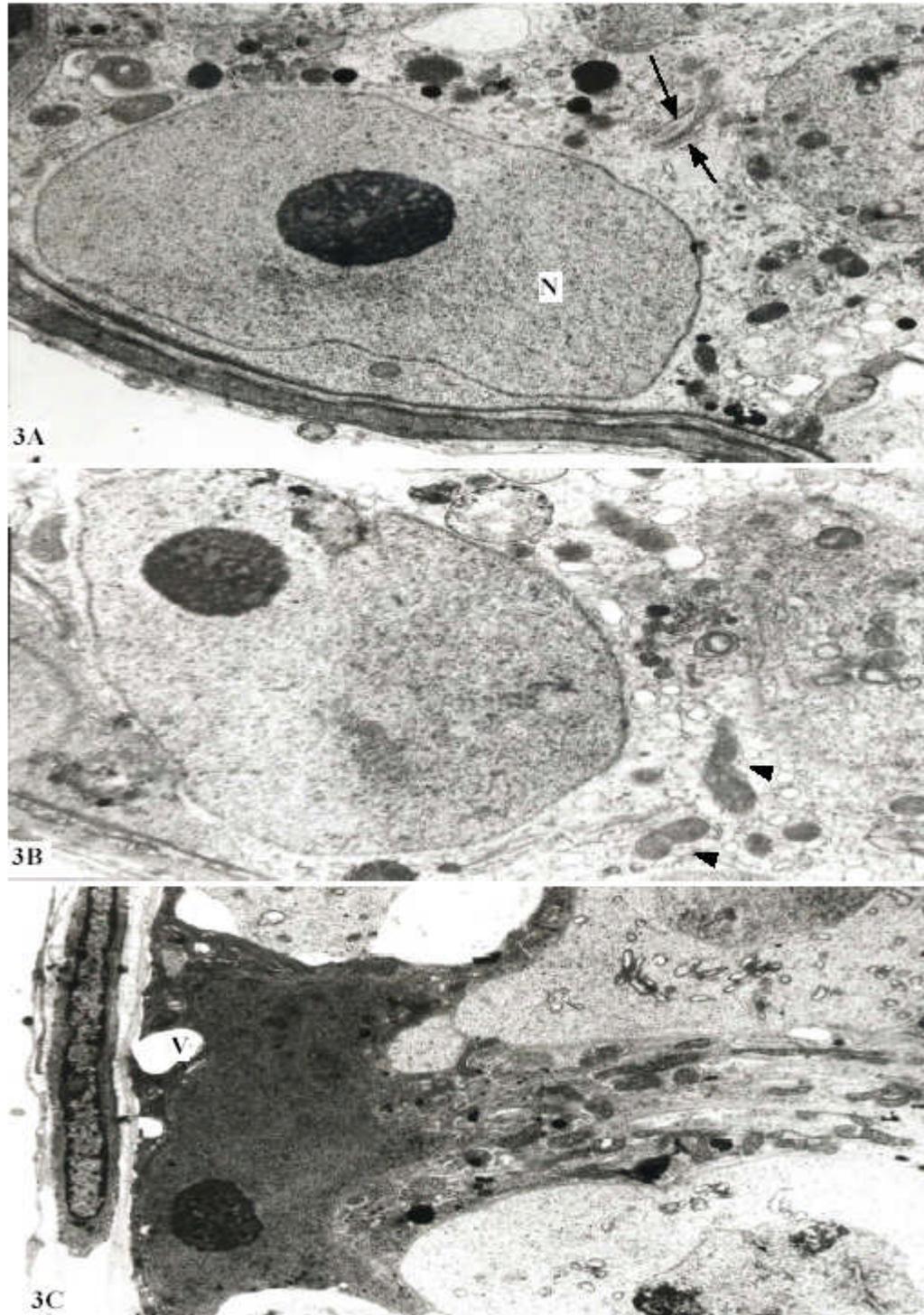


Fig. 3: Electron micrographs of testis sections showing Sertoli cells in the different experimental groups. (A) Sertoli cell from a control rat, showing the oval nucleus (N) with a prominent nucleolus, the cytoplasm full of ribosomes, lysosomes, mitochondria and filamentous proteins such as intermediate filaments (arrows), (EM, x5000); (B) A Sertoli cell from a fennel-treated rat, showing the typical ultrastructure with dumbbell-shaped mitochondria (head arrows) (EM, x6000); (C) The nucleus of a Sertoli cell from rat treated with anise oil, showing marked changes in its boundaries and cytoplasmic vacuoles (V) of varying sizes (EM, x4000).

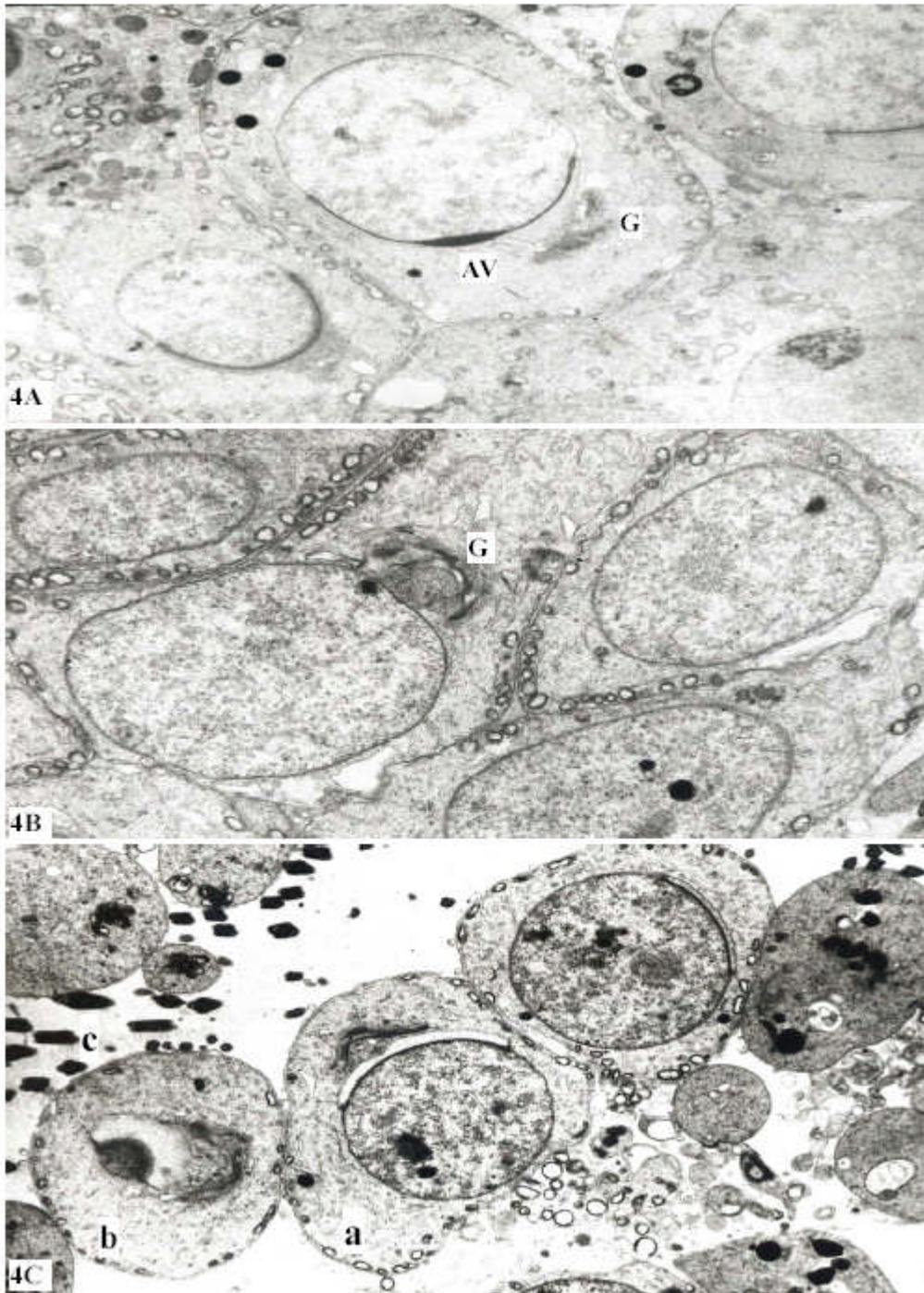


Fig. 4: Transmission electron micrographs of rat testes, showing spermatids in different phases of spermatogenesis. (A) Normal (control) rats, whose cells have developing acrosomal vesicles (AV) elaborated by a large Golgi apparatus (G) and the presence of lysosomes (EM, x3000). (B) Normal spermatids from fennel-treated rats with dense mitochondria along the cell membranes (EM, x4000). (C) Photo showing various parts of spermatids with (a) irregular boundaries of some spermatids; (b) some with abnormal nuclei; (c) swollen acrosomal vesicles and distorted arrangement of microtubules and various amorphous changes (EM, x3000).

Rats from the control group showed typical spermatozoa with the tail divided into three regions (middle, principal and end pieces). The elongation process is typically associated with the appearance of a ring of microtubules, the manchette, that extends posteriorly from the base of acrosome parallel to the axis of elongation (Fig. 5A). The different parts of the spermatozoa

tail with mitochondria wrapping the nine distinct densities around the axoneme appeared in testis section from fennel-oil-treated rats (Fig.5B). Anise-oil-treated rat showed sperm with vacuoles around their heads and acrosomes (Fig.5C). Normal Leydig cells appeared in electron micrographs of control and fennel-oil-treated rats with the nucleus, lipid droplets, mitochondria, and rough and smooth endoplasmic reticulum (Fig. 6A). Leydig cells from anise-oil-treated rats appeared with distortion on the nuclear membrane, distribution of heterochromatin in the inner surface, numerous lysosomes and small lipid droplets in the cytoplasm (Fig. 6B).

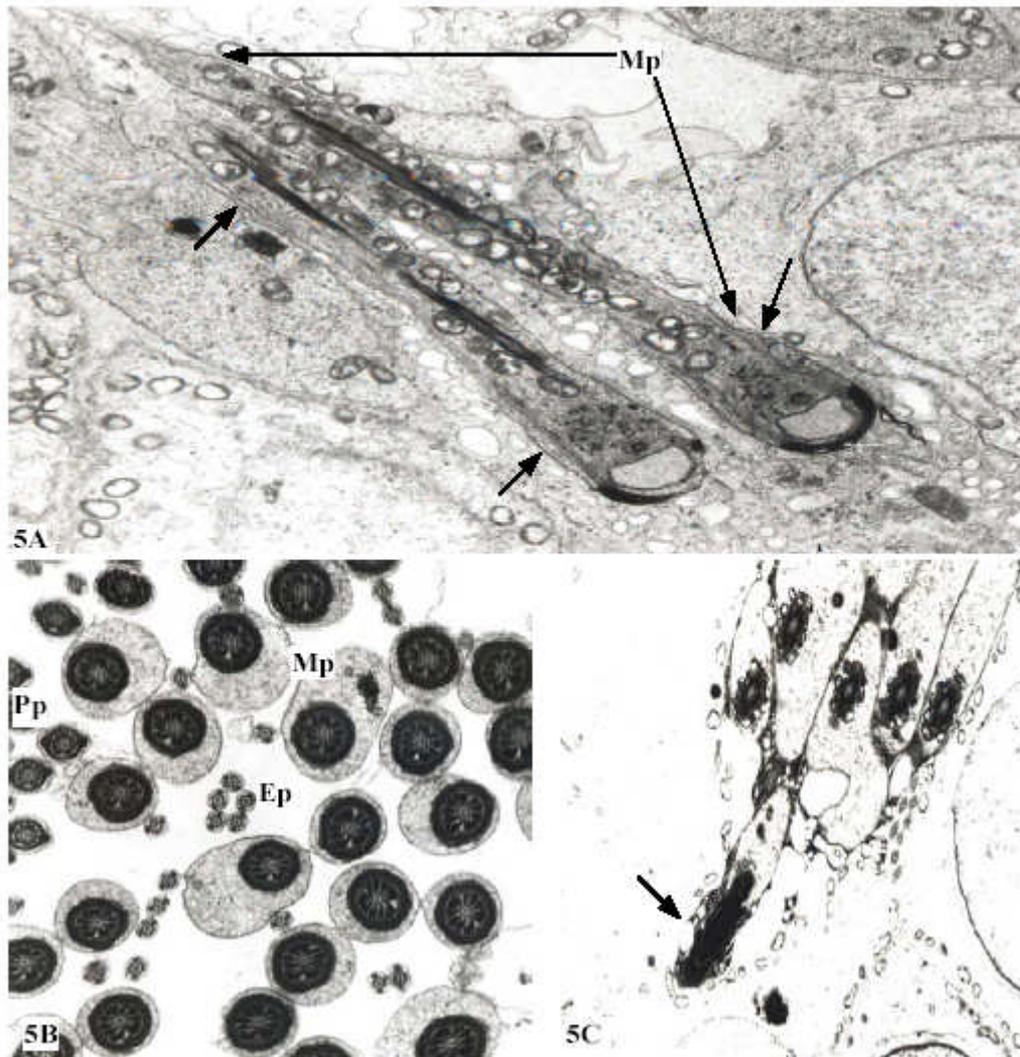


Fig. 5: Electron micrographs of testis sections from: (A) control rats, showing part of a spermatid and typical spermatozoa with a middle piece (Mp) and a ring of microtubules, the manchette (arrows), that extends posteriorly from the base of acrosome parallel to the axis of elongation (EM, x5000); (B) fennel-oil-treated rats, showing transverse sections of the different parts of spermatozoa tails, end piece (Ep), principle piece (Pp) and middle piece (Mp), with the mitochondria wrapping the nine distinct densities around the axoneme (EM, x7500); and (C) anise-oil-treated rats, showing amorphous changes in sperm, with vacuoles (arrow) around their heads and acrosomes (EM, x5000).

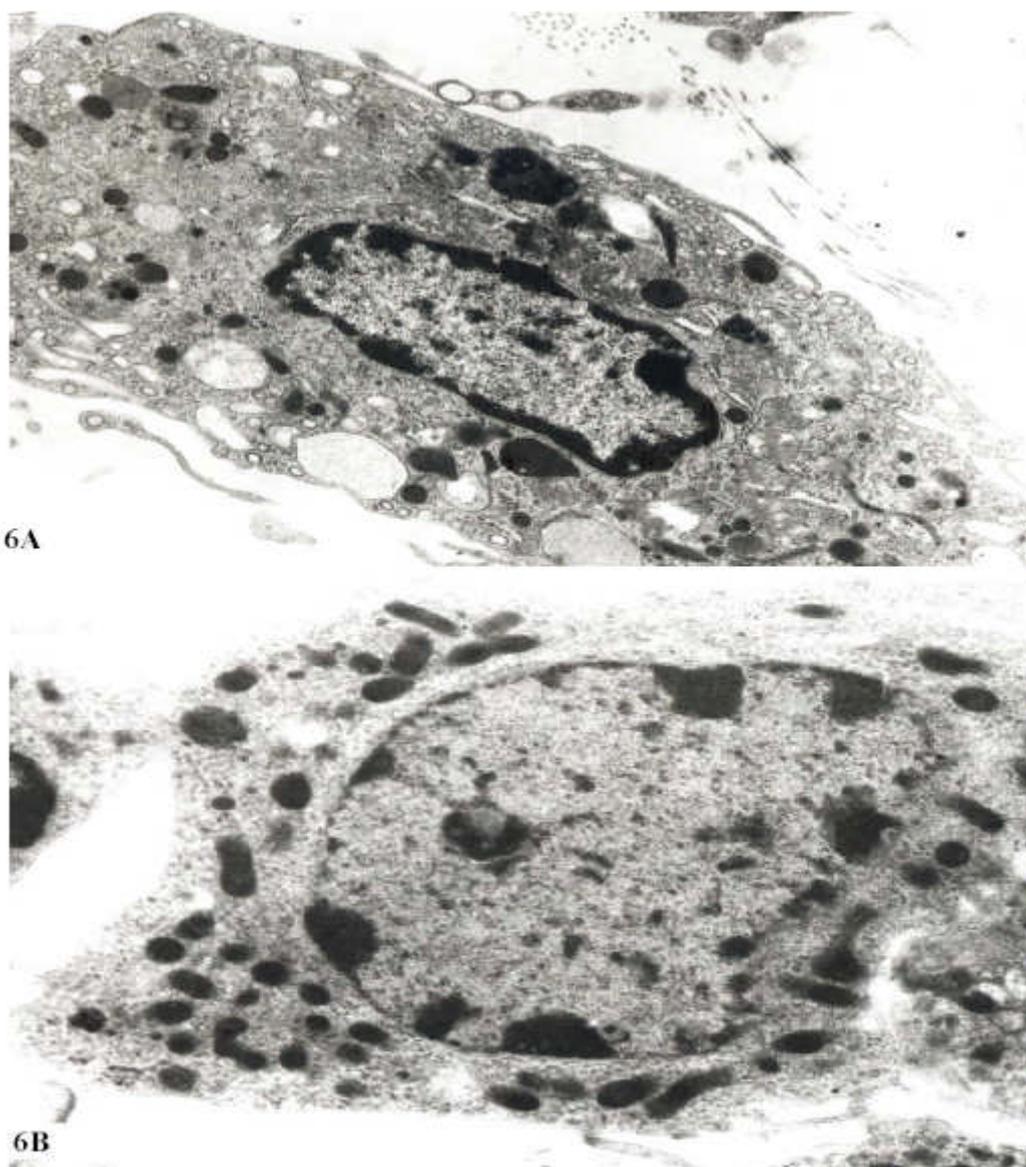


Fig. 6: Transmission electron micrographs of rat testis showing, (A) in control rats, a typical Leydig cell with its nucleus, lipid droplets, mitochondria, rough and smooth endoplasmic reticulum (EM, x7500); and (B) in anise-oil-treated rats, a Leydig cell with distortion in the nucleus membrane, distribution of heterochromatin on the inner surface, numerous lysosomes and small lipid droplets in the cytoplasm (EM, x6000).

Sections of testis from anise oil treated rats showed that inhibin expression detected faint positive reaction in Leydig cell (Fig.7C) as compared with control and fennel oil treated groups as shown in figures (7A&B) where the positive immunoreactivity against anti-inhibin intensely appeared in Leydig cells. Also, sections of testis from anise oil treated rats showed decrease in GST expression in the nuclei and the cytoplasm of Sertoli and spermatogenic cells (Fig.8C) as compared with control and fennel oil treated groups as shown in figures (8A&B).

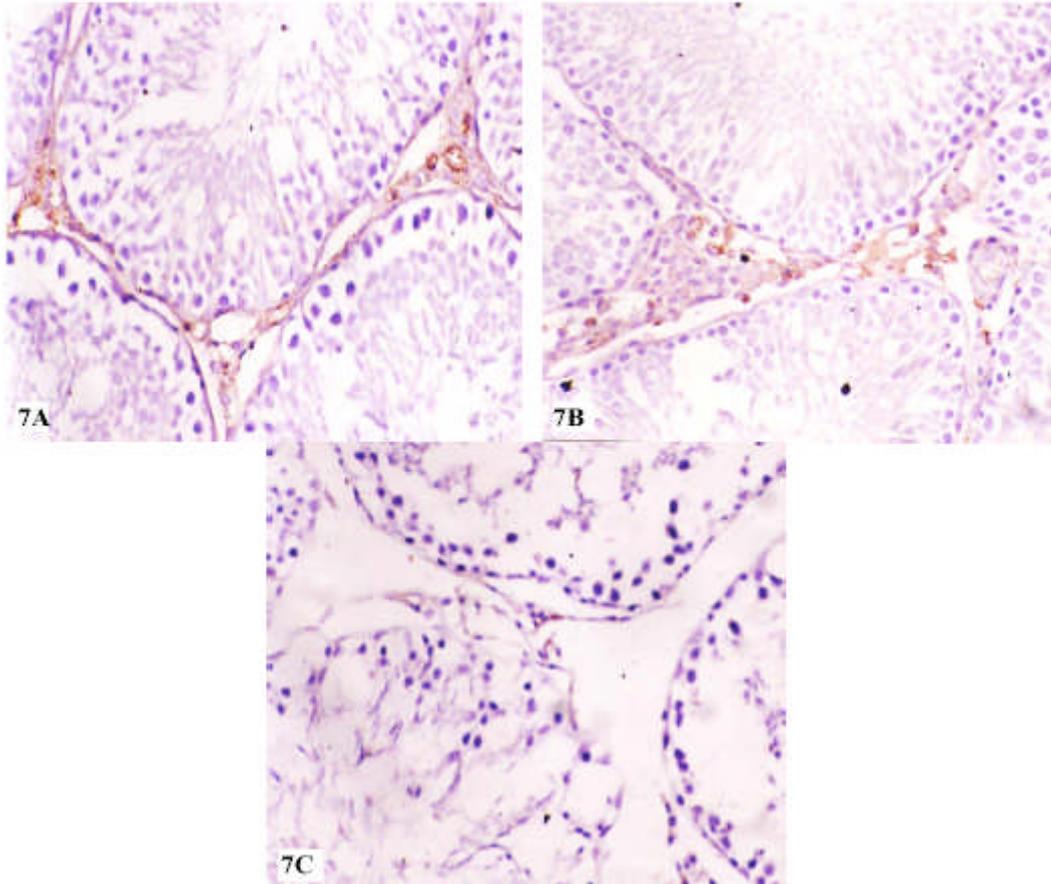


Fig. 7: Photomicrographs of testis sections from, (A) control rats, showing normal immunohistochemical positive reactivity against anti-inhibin in the Leydig cells; (B) fennel-oil-treated rats, showing the normal expression of inhibin in the interstitial tissue; and (C) anise-oil-treated rats, showing decrease in inhibin expression in the damaged Leydig cells (immunohistochemical stain, x200).

The results of semen analysis (Table 1) show no significant change (from the control) in sperm abnormalities caused by fennel-oil administration, but sperm motility increased. Anise oil administration caused a significant decrease in sperm motility and sperm count, and increase in levels of sperm abnormalities, such as sperm with double heads or amorphous heads (Fig. 9).

Table 1: Indicators of sperm quality and quantity in control and treated groups of rats.

	Control	Fennel oil	Anise oil
Sperm motility (%)	57.7± 0.7	61.4 ± 1.5 ^a	48.7 ± 1.0 ^a
Sperm abnormalities (%)	5.9± 0.3	6.3 ± 0.3	9.0 ± 0.3 ^a
Sperm count (x 10 ⁶ / ml)	147.6± 1.8	151.9 ± 2.3	128.7 ± 1.0 ^a

^a = significantly different from control rats (p<0.05, LSD post-hoc comparisons)

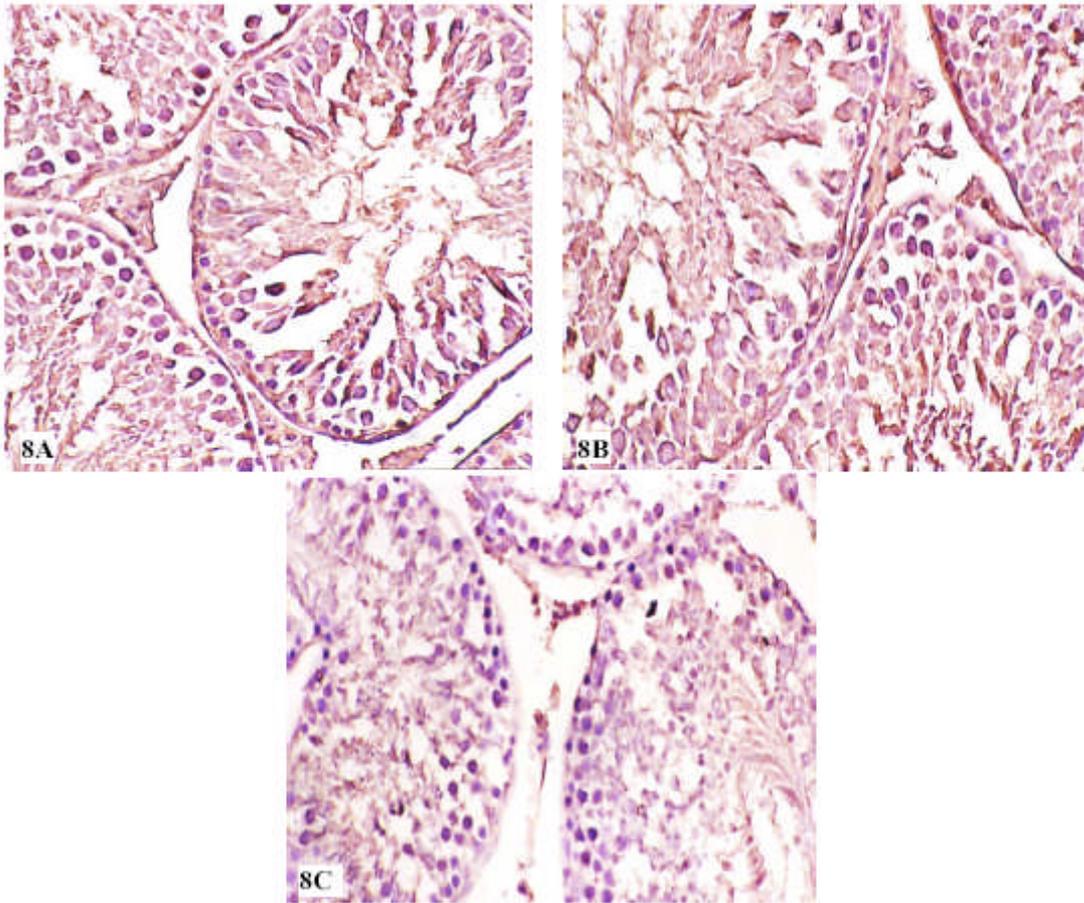


Fig. 8: Photomicrographs of testes sections from, (A) control and (B) fennel-oil-treated rats, showing the normal expression of GST in the Sertoli and spermatogenic cells; and (C) anise-oil-treated rats, showing a decrease in GST expression in the Sertoli and spermatogenic cells (immuno-histochemical stain, x200).

There were significant increases over the controls in T3 and testosterone levels (Table 2) in the serum of rats treated with fennel oil, but not in T4 levels; significant inhibition of all three measures occurred in anise-oil-treated rats.

Table 2: Serum levels of T3, T4 and testosterone in control and treated groups of rats.

	Control	Fennel oil	Anise oil
T3 (ng/ml)	2.51± 0.004	2.39 ± 0.006 ^a	0.95 ± 0.005 ^a
T4 (g/dl)	2.11± 0.008	2.40 ± 0.131	1.43 ± 0.005 ^a
Testosterone (nmol/l)	1.78± 0.006	1.98 ± 0.006 ^a	0.71 ± 0.006 ^a

Values are means ± SE (n=7); a = significantly different from control at P<0.05.

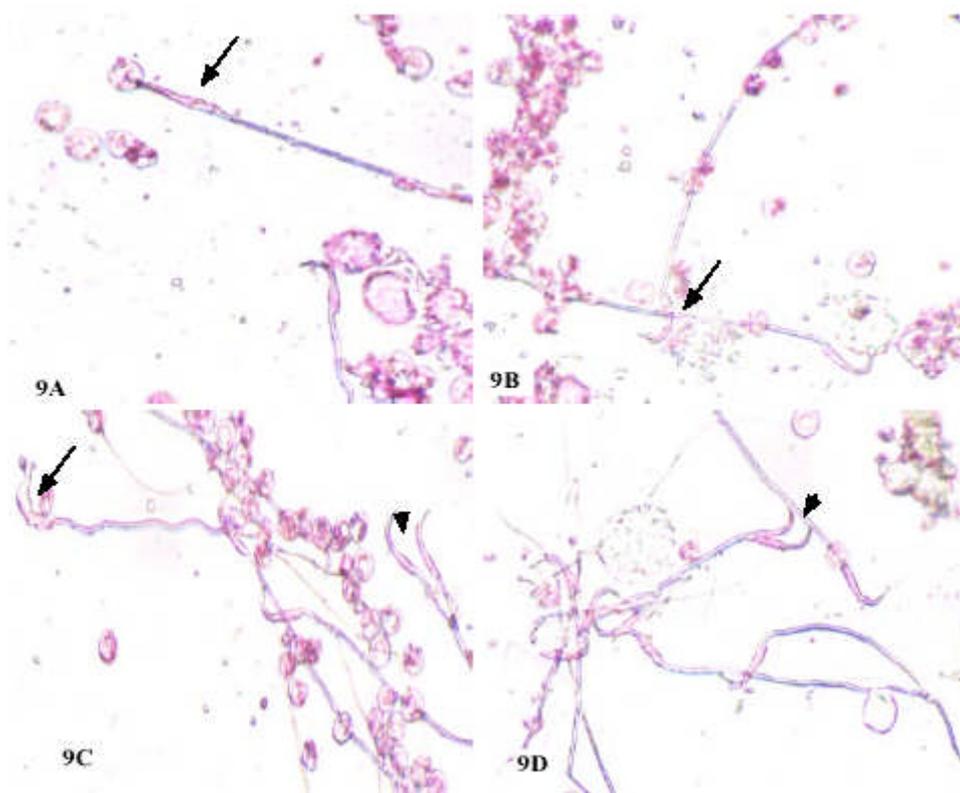


Fig. 9: Photomicrographs of sperm smears showing sperm with amorphous heads (arrow) and double heads (head arrow) (Eosin, x400).

Discussion

Sertoli cells are somatic cells of the testis essential for testis formation and spermatogenesis. They facilitate the progression of germ cells to spermatozoa via direct contact and by controlling the environment milieu within the seminiferous tubules. The regulation of spermatogenesis by FSH and testosterone occurs by the action of these hormones on the Sertoli cells, and the action of testosterone is necessary for spermatogenesis (Griswold 1998). Fennel-oil-treated rats had normal testicular tissue, and fennel does not affect lung tissue either (Ibrahim 2007a,b). Shah *et al.* (1991) also reported that fennel extract failed to show spermatotoxic effects. This benign effect may be related to the antioxidant components of fennel (Parejo *et al.* 2004; Tognolini *et al.* 2006): it contains many powerful active components that might be effective in increasing human immunity and preventing cancer (Esiyok *et al.* 2004).

Rats treated with anise oil had several histopathological changes, including in the Sertoli and Leydig cells. The destructive effect of anise oil may be related to its safrole content. Safrole has been associated with the presence of safrole-DNA adduct in human (Jeurissen 2007), and Zhou *et al.* (2007) reported that some safrole (and other naturally occurring alkenylbenzenes) can undergo metabolic activation by sequential 1-hydroxylation and sulfation, resulting in reactive intermediates capable of forming DNA adducts and finally genotoxicity.

Inhibin is a peptide hormone produced in the gonads which may act as an autocrine and/or paracrine regulator of testicular function. Inhibin consists of two distinct subunits α and β , linked by disulfide bonds. Fennel-oil-treated rats had a normal distribution of inhibin in the Leydig cells. In adults, binding of [125 I] inhibin localizes primarily to the interstitial

compartment of the testis (Krummen *et al.* 1994), Leydig cells show strong positive staining for the inhibin β A subunit (Jin *et al.* 2001), and *in situ* ligand-binding studies show that [125 I] inhibin β A binds specifically to Leydig cells throughout rat testis development: thus inhibin is probably a regulator of Leydig cell differentiated function (Lejeune *et al.* 1997; Matzuk 2000). Recently, additional inhibin-specific binding proteins were identified in inhibin target tissues, including pituitary and Leydig cells (Chong *et al.* 2000; Bernard *et al.* 2002). From these receptors, betaglycan (the TGF β type III receptor) and InhBP/p120 (a membrane-tethered proteoglycan) were identified as putative inhibin receptors, all present in Leydig cells. A faint positive reaction was detected in Leydig cell cytoplasm from rats treated with anise oil, perhaps related to damage to the Leydig cells, as a result of the decreasing of inhibin expression. This may be related to safrole.

GST enzymes play a major role in the detoxification pathway and help in the conversion of reactive chemicals to non-reactive polar compounds which can be excreted from the body (Gandy *et al.* 1996). Here there was normal expression of GST in testis sections from fennel-oil-treated rats, but a decrease in rats administered anise oil. Normal expression of GST in rats treated with fennel oil for 60 days was previously reported (Ibrahim 2007b), and fennel oil can stimulate GST expression again after exposure to tobacco smoke, indicating that it can confer protection against the toxic effect of xenobiotics (Ibrahim 2007b). The importance of GSTs in the protection against oxidative stress in testis is underscored by recent studies showing that when GST activity is inhibited, the products of lipid peroxidation accumulate, resulting in germ-cell apoptosis (Rao & Shaha 2000; Yang *et al.* 2001). Otieno *et al.* (1997) and Benbrahim-Tallaa *et al.* (2002) reported that GST α is permanently expressed in Leydig cells from the neonatal period to adulthood, whereas in Sertoli cells, GST α is expressed only in the adult testis and all stages of sperm-cell maturation were unreactive to antibodies against classes of GST (Papp *et al.* 1995). Thus the decrease in GST expression in testis tissue as a result of anise-oil administration may be explained by the reduction in the numbers of Sertoli cells and the destruction of Leydig cells.

Sperm counts of rats treated with fennel oil revealed levels near to normal, as found by Shah *et al.* (1991). Anise-treated rats had decreased sperm counts, perhaps related to the damaged Sertoli cells. Because each Sertoli cell is thought to support a finite number of germ cells, fewer or damaged Sertoli cell may predispose a lower sperm count (Winters *et al.* 2006). Safrole probably caused inhibition of sperm motility and the high proportion of abnormal sperm in anise-oil treated rats, since it is known to be a genotoxic and carcinogenic agent (Jeurissen 2007).

Decreases in Sertoli cells and damage to Leydig cells probably caused the decline in T3, T4 and testosterone hormone levels in the anise-oil administered rats. In the rat, Sertoli cells proliferate only during the fetal and early neonatal periods before assuming a terminally differentiated state. The ultimate number of Sertoli cells in the adult testis is determined by both the rate and the duration of the proliferative phase (Buzzard *et al.* 2000). It has been demonstrated that each Sertoli cell is capable of supporting a limited number of germ cells through to maturity; hence Sertoli cell number determines the maximum spermatogenic potential of the testis. The hormonal factors controlling the rate and duration of Sertoli cell proliferation are therefore critical determinants of fertility. Previous studies have demonstrated that FSH is a mitogenic factor during the Sertoli cell proliferative phase, while thyroid hormones (T3 and T4) influence the duration of proliferation (Buzzard *et al.* 2000).

Sustaining the normal levels of circulating testosterone is clearly important for the well-being of male reproductive function. Testosterone is primarily produced by the Leydig cells of the testis. Ariyarante *et al.* (2000) & Kim *et al.* (2002), reported that due to thyroid hormone deficiency, Leydig cells failed to differentiate and levels of T3 and testosterone hormones decreased. The deficiency of thyroid hormones could have originated from decreased iodide pump activity resulting in the inhibition of tyrosine iodination (Virion *et al.* 1980) or perhaps a

decrease in the synthesis of thyroxine-binding globulin (TBG), the major serum thyroid hormone-binding protein (Concannon *et al.* 1999).

In neonatal testes, Sertoli cells are rich in thyroid hormone receptors, and thus there are important effects of hypothyroidism in this tissue. The deficiency of thyroid hormone retards Sertoli cell maturation. This is the key to understanding the ultimate effect on the testes: the delay prolongs the period of proliferation, and thus ultimately increases the number of Sertoli cells (Crissman *et al.* 2000). Many studies point out that hypothyroidism may arrest differentiation of Leydig cell in neonatal and adult testis, and more importantly show that Leydig cells undergo atrophic changes in size and organelle content (Mendis-Handagama *et al.* 1998), and hence into a malfunctioning state.

Hormonal inhibition and the decrease in sperm motility and sperm count caused by anise oil is perhaps due to a low level of androgens. The androgen-receptor function is essential in males for proper sexual differentiation and for the maintenance of normal spermatogenesis. Androgen-receptor activity is regulated by the steroid ligand testosterone, and the receptor protein is expressed in the somatic Leydig and Sertoli cells. Loss of receptor activity from Sertoli cells is thus responsible for a whole range of testicular abnormalities (Holdcraft & Braun 2004).

This study is the first to have examined thoroughly the relation between anise and testicular damage and hormonal disturbance. Its results support the claim that moderate to high levels of anise intake cause testicular damage and hormonal disturbance. Due to the wide use and easy availability of herbal medicines, further research should be conducted to ensure the safety and quality of herbal medicine.

Acknowledgements

The author thanks Professor Şanaa M.R. Wahba (Professor of Histology & Histochemistry, Zoology Department, Faculty of Women, Ain Shams University) for critical reading of the manuscript.

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الملخص العربي

العلاقة بين تعاطي زيت الشمر والينسون و الضرر الناتج في خصية الجرذان البالغة

أمل عطية المرسي ابراهيم

قسم علم الحيوان، كلية النبات للأدب والتربية والعلوم، جامعة عين شمس، مصر.

تمت هذه الدراسة للمقارنة بين تأثير تعاطي زيت الشمر أو الينسون على التغيرات الهيستوباثولوجية والمناعية في خصي الجرذان البالغة. وقد قسمت الجرذان الى ثلاث مجموعات، حقنت المجموعة الأولى بمحلول ملحي متعادل كمجموعة ضابطة، و عوملت جرذان المجموعة الثانية بزيت الشمر (1مل/كجم) عن طريق الفم، أما جرذان المجموعة الثالثة فعوملت بزيت الينسون (1مل/كجم) عن طريق الفم، واستمرت التجربة مدة ثلاثون يوماً. وقد أوضحت قطاعات خصي الجرذان التي حقنت بزيت الشمر عدم حدوث تغيرات نسيجية سواء في قطاعات الميكروسكوب الضوئي أو الاليكتروني. بينما بينت قطاعات الخصية لجرذان المجموعة التي حقنت بزيت الينسون انخفاض في عدد خلايا سيرتولي، تنخر في الخلايا المكونة للحيوانات المنوية. كذلك أوضحت قطاعات الميكروسكوب الاليكتروني حدود غير منتظمة للغشاء النووي لخلايا سيرتولي ووجود العديد من الفجوات في سيتوبلازم هذه الخلايا. كما ظهرت قطاعات الحيوانات المنوية غير محددة الشكل، أما بالنسبة لخلايا ليدج فقد ظهرت وبها اختلال في تنظيم المادة الكروماتينية على السطح الداخلي للغشاء النووي مع نقص في محتوى الحبيبات الدهنية مقارنة بالمجموعة الضابطة والمجموعة المعاملة بزيت الشمر.

كما أوضحت النتائج علاقة وثيقة بين المعاملة بزيت الينسون والانخفاض الواضح في مستوى تعبير الأنهيبيين و الجلوتاثيون في قطاعات الخصية. كذلك انخفاض معدلات هرمون التستوستيرون وهرمونات الترياي أيودوثيروكسين و الثيروكسين، بالإضافة الى تأثيره المثبط على حركة وعدد الحيوانات المنوية.