

An Ultrastructural Study on the Effect of *Nigella Sativa* and Hydrocortisone on Adult Albino Rat Testis.Ashraf H. Abd El-Hakem, Sobhy H .A. Ewis, Mohamed Atif A. Said Ahmed ¹and Refaat A.M. Eid²¹ Anatomy and ² Pathology Departments, College of Medicine, King Khalid University, Abha, Saudi Arabia
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Abstract: *Background:* *Nigella sativa* is consumed excessively in Saudi Arabia and Gulf Countries. Hydrocortisone is a synthetic glucocorticoid widely used in the medical fields. The present study aimed to investigate the histological and ultrastructural changes in rat testis under the effect of *Nigella sativa* and hydrocortisone. *Material and method:* Forty male Wister strain male albino rats used in this study. The rats divided randomly into four equal groups. Group I: served as control. Group II: treated by *Nigella sativa* oil orally (500 mg/kg body weight), daily for 15 days. Group III: treated by intramuscular injection with hydrocortisone sodium succinate (300mg/kg body weight), daily for 15 days. Group VI treated by both *Nigella sativa* oil orally and intramuscular with hydrocortisone sodium succinate (by the same rout, dose and duration of groups II and III). The testis subjected to light and transmission electron microscopy study. *Results:* *Nigella sativa* treated animals showed seminiferous tubules surrounded by healthy basal lamina with normal thickness. The most dominant finding is that the spermatids are numerous. The electron microscopic picture of the seminiferous epithelial cells showed picture of increased activity. Hydrocortisone sodium succinate treated animals revealed irregular and thicker basal lamina relative to the control. Partial collapse of some tubules was obvious causing widening intercellular spaces. Marked reduction in the germ cells. The electron microscopic picture of the seminiferous epithelial cells showed degenerative changes. Animals treated by both *Nigella sativa* and hydrocortisone sodium succinate showed mixed picture of both effects. *Nigella sativa* ameliorate of the degenerative changes of the hydrocortisone sodium succinate on the seminiferous epithelial cells. *Conclusion:* *Nigella sativa* oil has a beneficial effect on the seminiferous epithelium while hydrocortisone administration causes an obvious destructive effects on the seminiferous epithelium. Combined administration of *Nigella sativa* oil and hydrocortisone ameliorate the destructive effects of hydrocortisone on the seminiferous epithelium. So, it is better to use *Nigella sativa* oil in combination with hydrocortisone.

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Key Words: ultrastructure, histology, *Nigella sativa*, hydrocortisone, rat, testis

1. Introduction

Nigella sativa used in Islamic medicine for medical purposes for centuries both as herbs and oil. It is consumed excessively in Saudi Arabia and Gulf Countries. It has been used traditionally for centuries in the Middle East, Northern Africa, Far East and Asia for the treatment of many diseases. The seeds of *Nigella sativa* commonly known as black seed, are used in folk medicine all over the world for the treatment and prevention of a number of diseases and conditions (El Dakhakhny *et al.*, 2000). The seeds contain both fixed and essential oils, proteins, alkaloids and saponin. Much of the biological activity of the seeds has been shown to be due to thymoquinone, the major component of the essential oil. The seeds are characterized by a very low degree of toxicity (Ali and Blunden, 2003).

Several studies have been carried out in recent years on the pharmacological effects of these seeds. It has various therapeutic values such as anti-inflammatory (Goreja, 2003), antioxidant (Abdel-Wahhab and Aly, 2005), antifungal (Aljabre *et al.*,

2005), antiparasitic (Randhawa *et al.*, 2005), antibacterial (Salman *et al.*, 2008; Al-Zubaydi *et al.*, 2009; Ababutain, 2011), antiasthma (Al obaidy and Al samarai, 2009; Bedi *et al.*, 2010) and anticancer (Mohamed *et al.*, 2010).

Nigella sativa plays a role against hepatotoxicity (El Dakhakhny *et al.*, 2000), promotion of healing (Goreja, 2003), inhibit renal calculi (Hadjzadeh *et al.*, 2007). It decreases blood pressure and plasma concentrations of glucose, increase both the packed cell volume and haemoglobin (Al Jishi and Abou Hozaiifa, 2003). It also used in diabetic foot (Alzahrani and Bakhotmah, 2010), improves serum lipid profile (Islam *et al.*, 2011) and potential antiosteoporotic agent (Shuid *et al.*, 2012).

Hydrocortisone is a glucocorticoid hormone produced by the zona fasciculata of the of the adrenal cortex. An excessively high hydrocortisone level can cause catabolism or break down of tissue, premature aging and decreasing the body natural defensive response (Margioris and Tsatsanis, 2011).

Various synthetic forms of hydrocortisone are used to treat a variety of diseases (Li *et al.*, 2008). It is used in cases of stress (Nosenko and Mishunina, 2005), in replacement therapy for adrenal insufficiency (Nieman *et al.*, 2006; Jung and Inder, 2008), in congenital adrenal hyperplasia (Schimmer and Parker, 2006), as anti-inflammatory (Wang *et al.*, 2007), as adjuvant in malignancies (Da Silva and Schiff, 2007), for some psychological disorders (Romer *et al.*, 2010 and Wirth *et al.*, 2011) and for some blood diseases (Casella *et al.*, 2010).

Several studies had been carried out to investigate the effects of hydrocortisone as a synthetic glucocorticoid drug on the reproductive system such as non mammalian testis (Consten *et al.*, 2001 and 2002; Suchiang *et al.*, 2012) and female reproduction (Piffer and Pereira, 2004). Elshennawy and Abo Elwafa (2011) was the only study up to our knowledge on its effect on mammalian testis.

The testis is the primary male reproductive organ responsible for sperm production. The internal testicular structure is dominated by lobules. Each contains one to three or more minutes convoluted seminiferous tubules. The seminiferous epithelial cycle occupies 12-13 days in rats (Rosiepen *et al.*, 1994). Sertoli cells play a key role in spermatogenesis (Strandberg *et al.*, 2004).

Aim of the Study

As the testis is one of the most important reproductive organ. Improvement or suppression of its reproductive function is important for both human and animals. Consumption of *Nigella sativa* and treatment with hydrocortisone now widely increased. So, the present study aimed to throw the light on the impacts of *Nigella sativa* oil and hydrocortisone on adult albino rat testis.

2. Material and Methods

All experiments approved by the Ethics Committee of King Khalid University. Forty male Wister strain male albino rats housed at the animal house of King Khalid University, and gave pellet rodent diet, and water *ad-libitum*. They kept under controlled environmental conditions, including the room temperature and normal light/dark cycle.

The rats divided randomly into four equal groups. Group I: kept as control (injected intramuscular with 0.6ml of bacteriostatic water and toke water by gasteric tube). Group II: take *Nigella sativa* orally in a dose of 500 mg/kg body weight, daily for 15 days. Group III: injected intramuscular with hydrocortisone sodium succinate (300mg/kg body weight), daily for 15 days. Group VI treated by both *Nigella sativa* orally and intramuscular hydrocortisone sodium succinate (the same dose and

duration of groups II and III). *Nigella sativa* oil described dose according to Al-Ghamdi (2003) while hydrocortisone dose described according Elshennawy and Abo Elwafa (2011).

The testes removed from the animals and fixed in 10% formaldehyde, dehydrated in ascending grades of alcohol, and then after embedded in paraffin wax serial sections (10 µm) thickness were prepared and stained with Haematoxylin and Eosin stain and others by Van Gisson stain, then examined by light microscopy (Drury and Willington, 1980).

For evaluation by transmission electron microscopy as described by Dykstra *et al.* (2002), the testes excised and fixed directly in cold 1% glutaraldehyde (pH 2.2) for 24 hours, then post fixed in 1% osmium tetroxide in 0.1M phosphate buffer (pH 7.3), dehydrated in an ethanolic series culminating in 100% acetone, and will infiltrate with epoxide resin. After polymerization over night at 60°C, semithin sections (0.5 µm) stained with 1% toluidine blue in 1% sodium borate and examined with light microscope. Areas of seminiferous tubules selected and the blocks proceed to trimming accordingly. Ultrathin sections (80-90 nm) cut, mounted on 200 mesh copper grids, and stained with uranyl acetate and lead citrate. The stained grids examined and photographed at the Central Laboratory of College of Medicine, King Khalid University using JEOL 1200 EX TEM.

3. Reasults

Control Animals

Normal histological pictures of the testes of control animals noticed by Haematoxylin and Eosin stain (Fig. 1), by Van Gisson stain (Fig. 5) and by toluidine blue stain (Fig. 9). The seminiferous tubules surrounded by basal membrane formed of myoid cells (Fig.1). The basal lamina especially by Van Gisson stain appears regular and with normal thickness (Fig. 5). Each tubule composed of Sertoli cells and germ cells of various stages of development (Figs. 1and5).

Sertoli cells appear as tall cells extending from the basal lamina of the seminiferous tubules to their lumena. It is difficult to determine the outline of the cells as it is obscured by the surrounding germ cells. The nuclei appeared oval in shape, basal in location and pale stained (Fig. 9).

The spermatogonia are in contact to the basal lamina (Fig. 9). Dark and pale type-A spermatogonia is characterized by its large size and extensive contact with the basal lamina relative to type-B. The dark type-A is distinguished from the pale type-A by its dark nucleoplasm. Type B-spermatogonia, the cell size and nuclear size is relatively smaller than that of type-A. The contact with the tubular basal lamina is

also less than that of type-A. The nucleus is nearly spherical.

The spermatocytes are completely separated from the basal lamina. The primary spermatocytes are in different stages of maturation (Figs. 1 and 9). The preleptotene spermatocyte is characterized by its spherical nucleus which contains well-stained chromatin granulation. Leptotene primary spermatocyte, the chromatin granulations assume a filamentous texture and become deeply stained. The zygotene primary spermatocytes are characterized by the presence of coarser filaments which, progressively shorten and thicken to give the typical pachytene configuration.

The spermatids (Figs. 1 and 9) begins as small rounded cells with spherical nuclei. Their nuclei are eccentrically located and pass into serial different stages and shapes during the process of spermiogenesis. The nucleus is surrounded by irregular spherical zone in Golgi phase. The cap phase, head cap of the spermatid surrounds nearly one third of the nucleus. The acrosomal granule expands over the nucleus giving the characteristic picture of the acrosomal phase. At the end of the maturation phase the nucleus and the overlying acrosomic structure of the spermatid elongate, flatten and become condensed.

The interstitium between seminiferous tubules contain distinct Leydig cells and blood vessels (Figs. 1 and 9).

Electron microscopic picture:

The Sertoli cell (Fig. 13) rests on the basal lamina. The indented nucleus located in the basal region. The mitochondria in the basal region are small, spherical and reveal a dense matrix. No mitochondria in the apical region. The endoplasmic reticulum is well developed in the basal region. The rough type is infrequent and free ribosomes are scattered.

Spermatogonia (Fig. 17) are in contact with the basal lamina. Dark type-A spermatogonia is characterized by its large size and extensive contact with the basal lamina. The nucleus contains dispersed chromatin material. Many polymorphous mitochondria with lamellar cisternae are randomly distributed in the cytoplasm, but sometimes gathered in-groups. Free ribosomes are abundant.

Primary spermatocyte nucleus (Fig. 21) is rounded with heterochromatin and euchromatin and surrounded by intact nuclear membrane. The cytoplasm appear granular, characterized by dispersed oval and rounded mitochondria and cisternae of smooth endoplasmic reticulum.

Cap phase spermatid (Fig.25), the acrosome spread over part of nucleus. The nuclear membrane is highly electron dense under the acrosomal cap. The

chromatin material is evenly distributed. The mitochondria and smooth endoplasmic reticulum are numerous.

Nigella Sativa Treated Animals

The seminiferous tubules surrounded by basal lamina formed of myoid cells (Fig.2). The basal lamina by Van Gisson stain appears with normal thickness (Fig. 6). The light microscopic picture is more or less similar to that of the control animals. The most dominant finding is that the spermatids are numerous with only two or three rows of the spermatocytes obvious (Figs. 2and 10).

Electron microscopic picture:

The Sertoli cell (Fig.14) nucleus is indented, and has two prominent nucleoli. The cytoplasm is darker and contains numerous, more dilated endoplasmic reticulum relative to the control.

Type-B spermatogonia (Fig. 18) are in contact with the basal lamina, but the contact with the basal lamina is less than that of type-A. The nucleus is nearly spherical. The cytoplasmic organelles are similar to that of type-A in which abundant free ribosomes, polymorphic mitochondria, endoplasmic reticulum and lamellated cisternae are present.

Primary spermatocyte (Fig. 22) nucleus is rounded with heterochromatin and euchromatin and surrounded by intact nuclear membrane. The electron density of the nucleus increased. The cytoplasm appear more granular, characterized by more dispersed oval and rounded mitochondria and more cisternae of smooth endoplasmic reticulum.

The spermatid (Fig.26) reveals that the cytoplasmic organelles are more numerous. There is increase in the smooth endoplasmic reticulum, secretory vesicles, mitochondria and the free ribosomes. The acrosome spread over part of nucleus. The nuclear membrane is highly electron dense under the acrosomal cap. The chromatin material is evenly distributed.

Hydrocortisone Sodium Succinate Treated Animals

The seminiferous tubules surrounded by myoid cells (Fig. 3). By Van Gisson stain the basal lamina appears irregular and thicker than normal (Fig. 7). Partial collapse of some tubules are obvious causing widening intercellular spaces (Figs. 3, 7 and 11). The interstitium between seminiferous tubules appear edematous in some areas (Fig. 11). Marked reduction in the germ cells, only two or three rows if present (Figs. 3, 7 and 11). Spermatocyte nuclei of variable size (Fig. 11). The elongated spermatid nearly absent from many tubules and accordingly the spermatozoa.

Electron microscopic picture:

Sertoli cells (Fig. 15) showed no nuclear changes. The nucleus indented and has a well prominent nucleolus. The cytoplasm contains electron dense mitochondria, fragmented smooth endoplasmic reticulum, lysosomes and numerous vacuoles.

Type-B spermatogonia (Fig. 19) are in contact with the basal lamina, but The contact with the basal lamina is less than that of type-A. The nucleus is nearly rounded. The cytoplasm contains fragmented mitochondria, smooth endoplasmic reticulum, lysosomes and numerous vacuoles.

The primary spermatocyte (Fig. 23) nucleus is smaller in size, elongated and revealing condensed chromatin materials. The cytoplasm appears less granular with less cytoplasmic organelles. The mitochondria are rounded and oval.

The spermatid (Fig. 27) reveals less developed head cap, less numerous cytoplasmic organelles with cytoplasmic vacuoles.

Nigella Sativa And Hydrocortisone Sodium Succinate Treated Animals

The seminiferous tubules surrounded by myoid cells (Fig.4). By Van Gisson stain the basal lamina appeared irregular and thicker than normal (Fig. 8). Collapse of some tubules were obvious causing widening intercellular spaces (Figs. 8 and 12). The irregularity, the increased thickness and the collapse was less than that of hydrocortisone treated alone. The number of seminiferous epithelial rows were

more or less similar to control. Spermatocyte nuclei of variable size. The spermatids are more or less similar to the control (Figs. 4, 12).

Electron microscopic picture:

Sertoli cell (Fig. 16) nucleus deeply indented with well prominent nucleolus. The cytoplasm contains electron dense mitochondria, fragmented smooth endoplasmic reticulum and vacuoles. The cytoplasmic findings were less than that of the hydrocortisone treated animals alone.

Pale type-A spermatogonia (Fig. 20) are in contact with the basal lamina. The nucleus contains dispersed chromatin material. Many polymorphous mitochondria with lamellar cisternae are randomly distributed in the cytoplasm. Free ribosomes are abundant.

Primary spermatocyte (Fig.24) nucleus is rounded with heterochromatin and euchromatin and surrounded by intact nuclear membrane. The cytoplasm appear granular, characterized by dispersed oval and rounded mitochondria, cisternae of smooth endoplasmic reticulum and cytoplasmic vacuoles.

The spermatid (Fig. 28) reveals that the cytoplasmic organelles are numerous. There is increase in the smooth endoplasmic reticulum, secretory vesicles, mitochondria and the free ribosomes. The acrosome spread over part of nucleus deep to it an electron dense nuclear membrane. The chromatin material is evenly distributed.

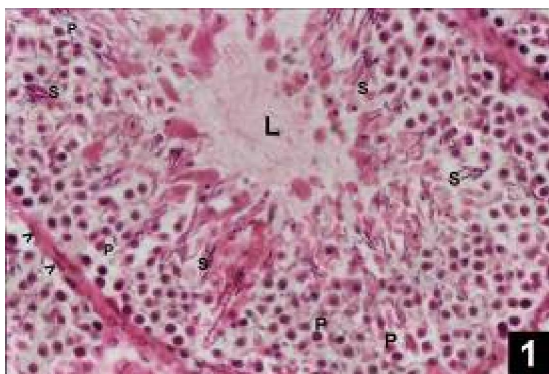


Fig. 1:- A photograph of part of seminiferous tubule of control rat showing the basal lamina (head of arrow) containing myoid cells, primary spermatocytes (P) and elongated spermatids (S) towards the lumen (L). (Hx&E X 400)

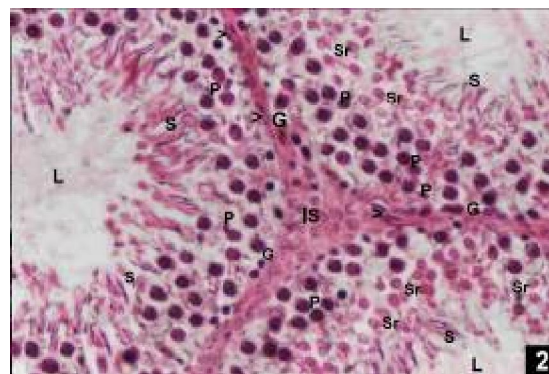


Fig. 2:- A photograph of part of seminiferous tubules of *Nigella sativa* treated rat showing the basal lamina (head of arrow) containing myoid cells, spermatogonia (G), primary spermatocytes (P), elongated spermatids (S) and rounded spermatids (Sr) towards the lumen (L). Normal interstitial tissues (IS) between the tubules. (Hx&E X 400)

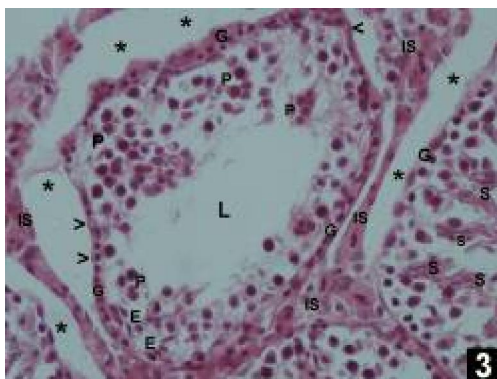


Fig. 3:- A photograph of part of seminiferous tubule of hydrocortisone treated rat showing irregular collapsed basal lamina (head of arrow) containing myoid cells leaving empty spaces (*), spermatogonia (G), primary spermatocytes (P) of variable size, abnormal elongated cells (E), no spermatids towards the lumen (L). The other tubule shows few elongated spermatids (S). Less cellular interstitial tissues (IS). (Hx&E X 400)



Fig. 5:- A photograph of part of seminiferous tubule of control albino rat showing basal lamina (head of arrow), interstitial tissues (IS) between them, seminiferous epithelium with elongated spermatids (S) towards the lumen (L). (Van Gieson X 400)

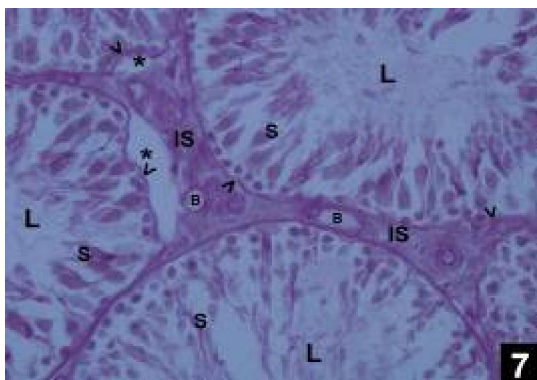


Fig. 7:- A photograph of part of seminiferous tubules of hydrocortisone treated rat showing irregular thick and shrunken basal lamina (head of arrow) leaving empty spaces (*). Scanty seminiferous epithelium with little elongated spermatids (S) towards the lumen (L). The interstitial tissue (IS) has thick walled blood vessels (B). (Van Gieson X 400)

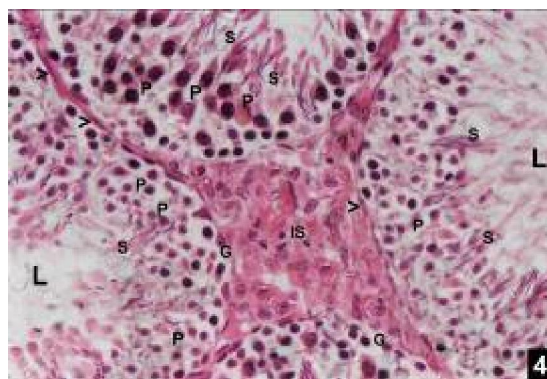


Fig. 4:- A photograph of part of seminiferous tubules of *Nigella sativa* and hydrocortisone treated showing the basal lamina (head of arrow) containing myoid cells, spermatogonia (G), primary spermatocytes (P) of variable sized and elongated spermatids (S) towards the lumen (L). Normal interstitial tissues (IS) between the tubules. (Hx&E X 400)

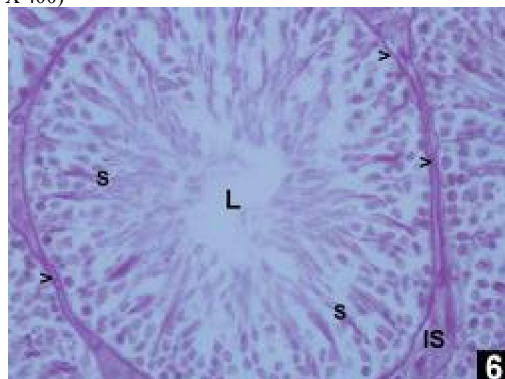


Fig. 6:- A photograph of part of seminiferous tubule of *Nigella sativa* treated rat showing basal lamina (head of arrow), interstitial tissues (IS) between them, seminiferous epithelium with elongated spermatids (S) towards the lumen (L). (Van Gieson X 400)

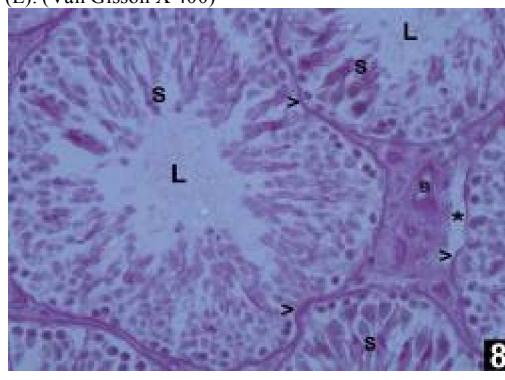


Fig. 8:- A photograph of part of seminiferous tubules of *Nigella sativa* and hydrocortisone treated rat showing irregular thick and shrunken basal lamina (head of arrow) leaving empty spaces (*). The seminiferous epithelium with numerous elongated spermatids (S) towards the lumen (L). The interstitial tissue (IS) has thick walled blood vessels (B). (Van Gieson X 400)

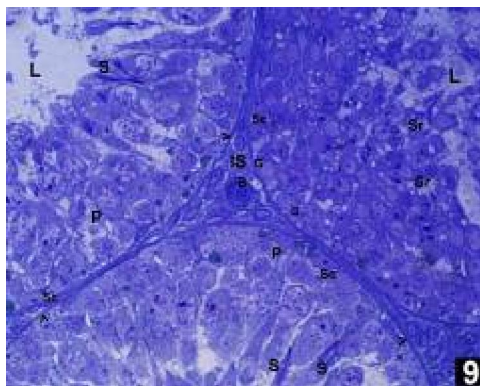


Fig. 9:- A photograph of part of seminiferous tubules of control rat showing the basal lamina (head of arrow) containing myoid cells, Sertoli cell (Sc), spermatogonia (G), primary spermatocytes (P), rounded spermatid (Sr) and elongated spermatids (S) towards the lumen (L). The interstitial (IS) tissues contains normal blood vessel (B). (toluidine blue X 400)

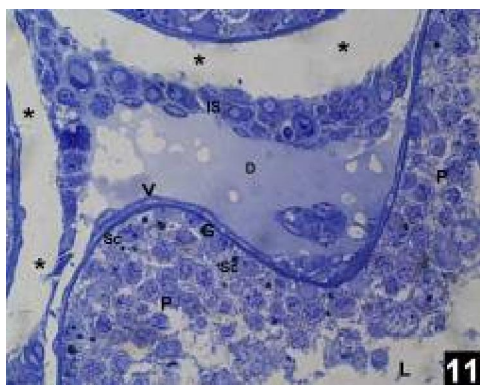


Fig. 11:- A photograph of part of seminiferous tubules of hydrocortisone treated rat showing the irregular collapsed basal lamina (head of arrow) containing myoid cells, Sertoli cell (Sc), spermatogonia (G), primary spermatocytes (P) towards the lumen (L). The interstitial (IS) tissues contains area of exudates (D) and empty spaces (*). (toluidine blue X 400)



Fig. 13: An electron micrograph of Sertoli cell of control rat rests on basal lamina (head of arrow) containing myoid cell (MY) nucleus. The nucleus (N) is indented (arrow) with prominent nucleolus. The cytoplasm contains mitochondria (M), lysosomes (Ly), smooth endoplasmic reticulum (R) and free ribosomes (r). (X 4800).

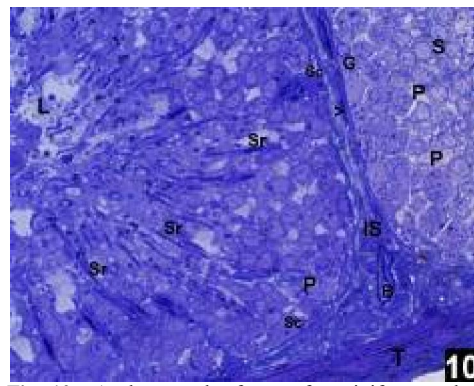


Fig. 10:- A photograph of part of seminiferous tubules of *Nigella sativa* treated rat showing tunica albuginea (T), basal lamina (head of arrow) containing myoid cells, Sertoli cell (Sc), spermatogonia (G), primary spermatocytes (P), rounded spermatid (Sr) and elongated spermatids (S) towards the lumen (L). Normal interstitial (IS) tissues contains blood vessel (B). (toluidine blue X 400)

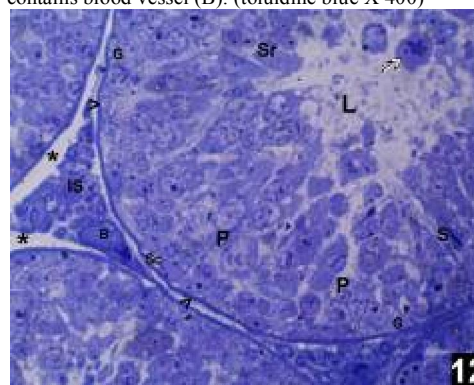


Fig. 12:- A photograph of part of seminiferous tubules of *Nigella sativa* and hydrocortisone treated rat showing the irregular collapsed basal lamina (head of arrow) containing myoid cells, Sertoli cell (Sc), spermatogonia (G), primary spermatocytes (P), rounded spermatid (Sr), elongated spermatids (S) towards the lumen (L) and spermatocyte in a stage of cell division (white arrow). The interstitial (IS) tissues contains empty spaces (*) and blood vessel (B). (toluidine blue X 400)

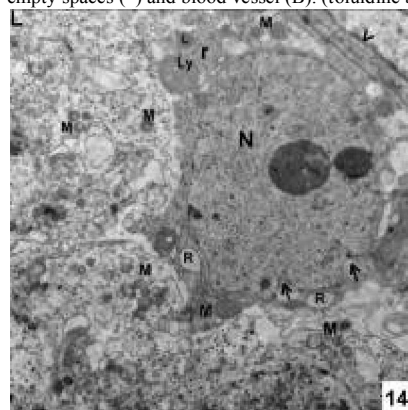


Fig. 14: An electron micrograph of Sertoli cell of *Nigella sativa* treated rat rests on basal lamina (head of arrow). The nucleus (N) is indented (arrow) with two prominent nucleoli. The cytoplasm is darker with numerous mitochondria (M), lysosomes (Ly), lipid droplet (L), smooth endoplasmic reticulum (R) and free ribosomes (r). (X 4800).

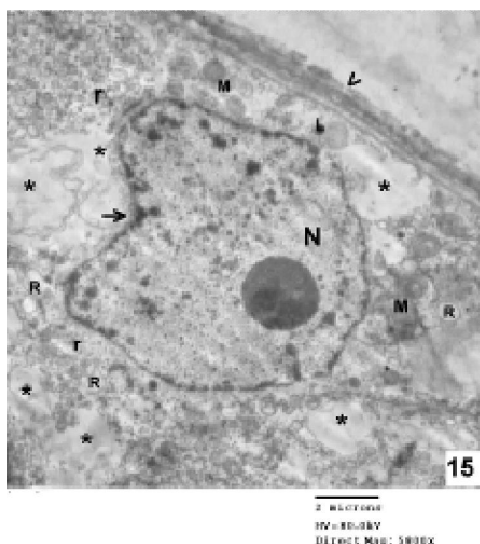


Fig. 15: An electron micrograph of Sertoli cell of hydrocortisone treated rat rests on basal lamina (head of arrow).The nucleus (N) is indented (arrow) with prominent nucleolus. The cytoplasm has little mitochondria (M), lipid droplet (L), smooth endoplasmic reticulum (R), free ribosomes (r) and empty spaces (*). (X 5800).

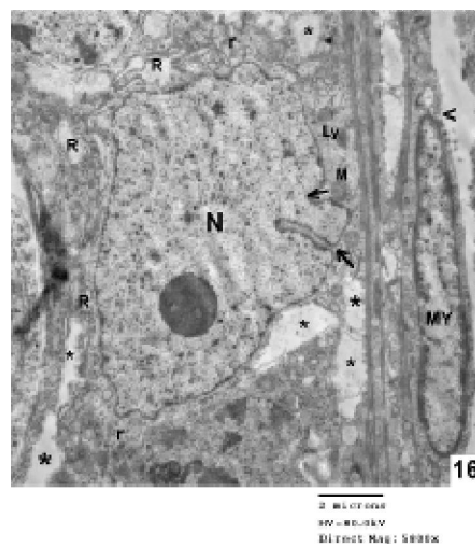


Fig. 16: An electron micrograph of Sertoli cell of *Nigella sativa* and hydrocortisone treated rat rests on basal lamina (head of arrow) containing myoid cell (MY) nucleus. The nucleus (N) is indented (arrow) with prominent nucleolus. The cytoplasm has little mitochondria (M), lysosomes (Ly), smooth endoplasmic reticulum (R), free ribosomes (r) and empty spaces (*). (X 5800).

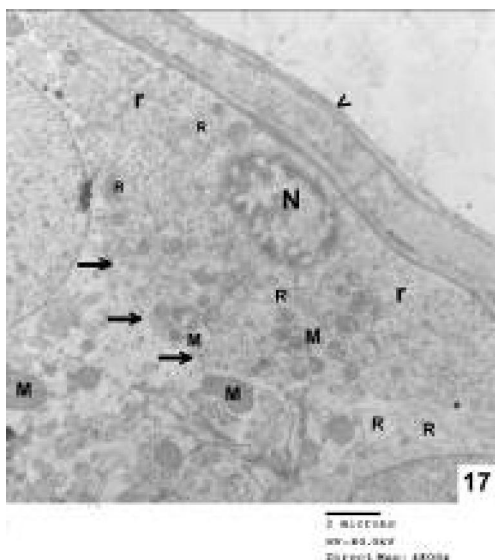


Fig. 17: An electron micrograph of dark type-A spermatogonia of control rat rests on basal lamina (head of arrow).The nucleus (N) is oval with heterochromatin along the nuclear membrane. The cytoplasm contains mitochondria (M), smooth endoplasmic reticulum (R) and free ribosomes (r). Junction with the neighboring Sertoli cell represented by (arrow). (X 4800)

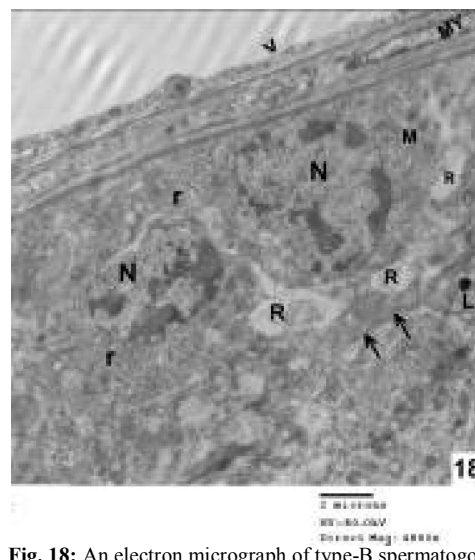


Fig. 18: An electron micrograph of type-B spermatogonia of *Nigella sativa* treated rat rests on basal lamina (head of arrow) containing myoid cell (MY) nucleus. The nucleus (N) is nearly rounded. The cytoplasm contains mitochondria (M), dilated smooth endoplasmic reticulum (R) and free ribosomes (r). Junction (arrow) with the neighboring Sertoli cell. That rich in organelles and contains Lipid droplets (L). (X 4800)

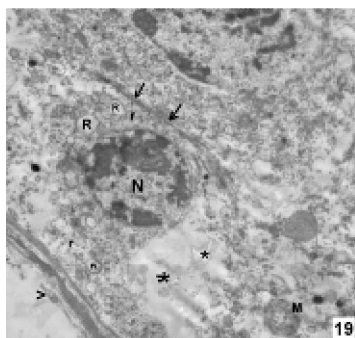


Fig. 19: An electron micrograph of type-B spermatogonia of hydrocortisone treated rat rests on basal lamina (head of arrow). The nucleus (N) is rounded. The cytoplasm contains fragmented mitochondria (M), little smooth endoplasmic reticulum (R), free ribosomes (r) and empty spaces (*). Junction with the neighboring Sertoli cell represented by (arrow). (X 5800).

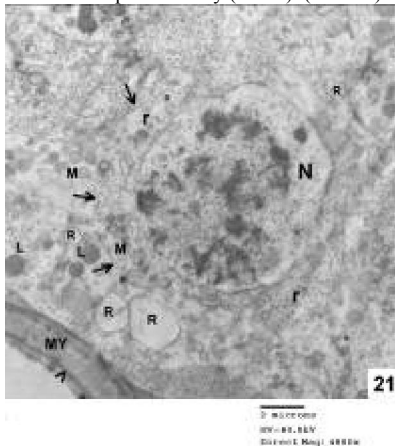


Fig.21: An electron micrograph of primary spermatocyte of control rat. The nucleus (N) is rounded with condensed chromatin. The cytoplasm contains mitochondria (M), smooth endoplasmic reticulum (R) and free ribosomes (r). Junction with the neighboring Sertoli cell (arrow) that rich in organelles and lipid droplet (L). The basal lamina (head of arrow) contains myoid cell nucleus (MY). (X 4800)

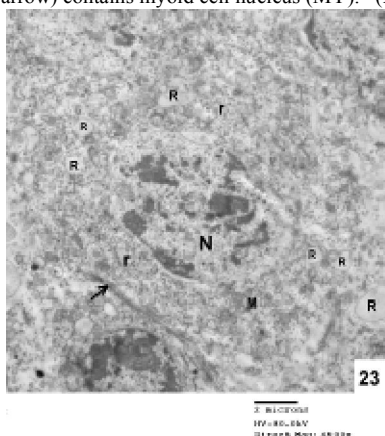


Fig. 23: An electron micrograph of primary spermatocyte of hydrocortisone treated rat. The nucleus (N) is elongate with condensed chromatin and irregular outline. The cytoplasm contains mitochondria (M), smooth endoplasmic reticulum (R) and free ribosomes (r). Junction with the spermatogonia represented by (arrow). (X 4800).

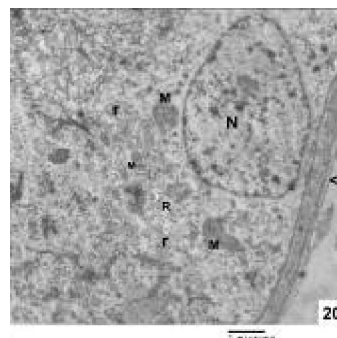


Fig. 20: An electron micrograph of pale type-A spermatogonia of *Nigella sativa* and hydrocortisone treated rat rests on basal lamina (head of arrow). The nucleus (N) is oval with more euchromatin. The cytoplasm contains mitochondria (M) of variable size, smooth endoplasmic reticulum (R) and free ribosomes (r). (X 4800).

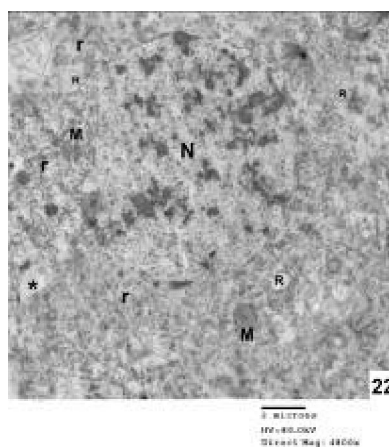


Fig.22: An electron micrograph of primary spermatocyte of *Nigella sativa* treated rat. The nucleus (N) is rounded. The cytoplasm contains rounded mitochondria (M), smooth endoplasmic reticulum (R), free ribosomes (r) and empty spaces (*). (X 4800)

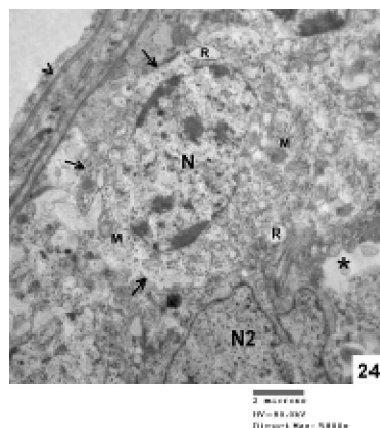


Fig. 24: An electron micrograph of primary spermatocyte of *Nigella sativa* and hydrocortisone treated rat separated from the basal lamina (head of arrow) by part of Sertoli cell. Junction between it and the Sertoli cell represented by (arrow). The nucleus (N) is elongated. The cytoplasm contains mitochondria (M), smooth endoplasmic reticulum (R) and empty spaces (*). The Sertoli cell nucleus represented by (N2). (X 5800).

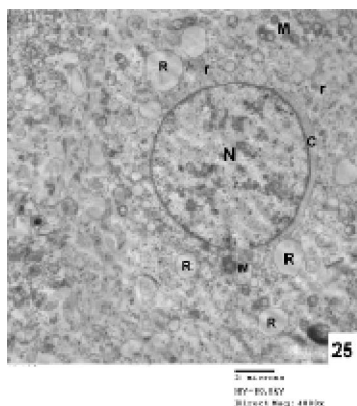


Fig.25: An electron micrograph of cap phase spermatid of control rat. The head cap (C) covers nearly 1/3 of the nucleus (N) which is rounded. The cytoplasm contains mitochondria (M), cisternae of the smooth endoplasmic reticulum (R) and free ribosomes (r). (X 4800)

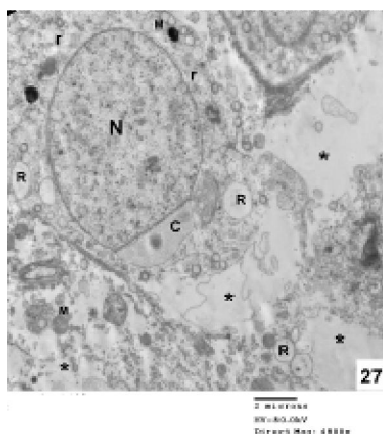


Fig. 27: An electron micrograph of cap phase spermatid of hydrocortisone treated rat. The head cap (C) covers nearly 1/5 of the nucleus (N) which is nearly rounded. The cytoplasm contains fragmented mitochondria (M), cisternae of the smooth endoplasmic reticulum (R), free ribosomes (r) and empty spaces (*). (X 4800)

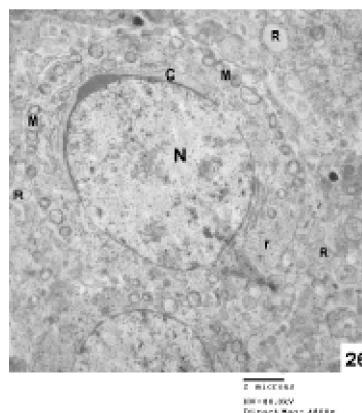


Fig.26: An electron micrograph of cap phase spermatid of *Nigella sativa* treated rat. The head cap (C) covers nearly 1/3 of the nucleus (N) which is rounded. The cytoplasm contains mitochondria (M), cisternae of the smooth endoplasmic reticulum (R) and free ribosomes (r). (X 4800)

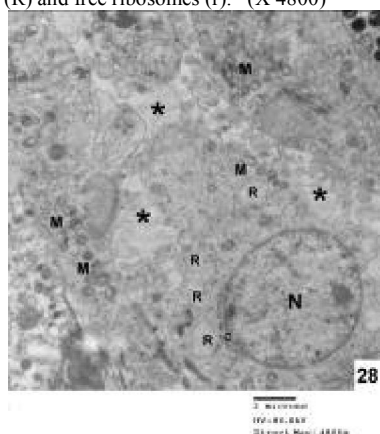


Fig. 28: An electron micrograph of cap phase spermatid of *Nigella sativa* and hydrocortisone treated rat. The head cap (C) covers nearly 1/5 of the nucleus (N) which is nearly rounded. The cytoplasm contains fragmented mitochondria (M), cisternae of the smooth endoplasmic reticulum (R), free ribosomes (r) and empty spaces (*). (X 4800).

4. Discussion

Nigella Sativa is of great uses in Islamic countries (Bakathir and Abbas, 2011; Shuid *et al.*, 2012), and hydrocortisone now widely used in medical field. So, the present study try to clarify the effects of *Nigella Sativa* oil and hydrocortisone on mammalian testes both the separate and the combined effect of both. Up to our knowledge Elshennawy and Abo Elwafa, 2011 was the only study on the effect hydrocortisone on mammalian testes and nothing on effect of *Nigella Sativa* on mammalian testes.

We chose the testis as it is one of the most important organs in reproduction (Johnson and Everett, 2000). The reproductive function depend on partially integrity of seminiferous tubule epithelium which show a cyclic pattern of renewal and development (Standring *et al.*, 2004).

The present study is performed on rat. The choice of that animal is based on several factors including easy handling and manipulation, economically good, they do well at any reasonable temperature and lastly they are sexually active all over the year. The seminiferous epithelial cycle lasts for 12-13 days in rats, they clarify good and rapid model for both types of cell division (Melby and Altman, 1974). From these considerations the seminiferous epithelial cells in our model are exposed to the experimental drugs nearly for one complete cycle which make them more vulnerable to the effect of our experimental drugs.

The present results on light microscopic level revealed histological pictures of the seminiferous tubules of control animals agree with that described by Fawcett, 1993. The basal lamina is regular and of normal thickness. Normal basal lamina plays an

important role in maintaining the structural and functional integrity spermatogenic epithelium (Richardson *et al.*, 1998). The electron microscopic picture of Sertoli cells and primary spermatocytes of control animals are in agreement with that reported by Kuehnel (2003).

The results of the present study on light microscopic level revealed that *Nigella Sativa* oil causes an obvious increase in the spermatids relative to the control. The spermatocytes are two or three rows. This rapid turnover of the spermatocytes to spermatids may be due to stimulation of the cell division between these types of cells.

In the present study most of the Sertoli cells of *Nigella Sativa* oil treated animals have two nucleoli and the chromatin material appears in reticular pattern. Numerous mitochondria in the basal and middle regions and numerous smooth endoplasmic reticulum observed relative to the control group. Sinowatz and Ameslgruber (1988) correlate these findings to increased metabolic activity of the cells. The smooth endoplasmic reticulum is associated with synthesis of lipids, cholesterol and other steroids in addition to other metabolic processes (Dorrington and Khan, 1993). According to Sawada and Esaki (2003) Sertoli cells foster the development and maintain the viability of germ cells by secreting hormonal and nutritive factors. Manivannan *et al.* (2009) reported that proteins necessary for the differentiation of germ cells are secreted at their highest rates in the testis during spermatid elongation and the process of spermiation.

Results of the present study revealed nuclear and cytoplasmic changes of the seminiferous epithelial cells indicating increased activity among *Nigella Sativa* oil treated animals. The nucleus showed increased electron density, indicating stimulation of cell division. Standring *et al.*, 2004 reported that during normal cell division, chromosomes undergo a gradual process of condensation. The cytoplasm in the present study appeared more granular, characterized by more dispersed oval and rounded mitochondria and more cisternae of smooth endoplasmic reticulum. All these elements associated with increased cellular activity (Bermudez *et al.*, 1993).

The results of the present study under the effect of hydrocortisone revealed thick, collapsed, irregular basal lamina of the tubules. This is in agreement with the results reported by Elshennawy and Abo Elwafa (2011).

On light microscopic level we also found dilated intercellular spaces between the seminiferous epithelium, marked reduction in the germ cells, only two or three rows, some of the spermatocytes revealed mega nuclei others reveal small sized nuclei

and lastly the elongated spermatid nearly completely absent from many tubules and accordingly the spermatozoa. These findings are in accordance with the results reported by Elshennawy and Abo Elwafa (2011). The first suggestive explanation for these findings is the direct effect of hydrocortisone on the Sertoli cell. Sertoli cells plays major role in the integrity and viability of the germ cells (Monsees *et al.*, 2002; Krishnamoorthy *et al.*, 2005). Although our results show minimal structural changes on the Sertoli cells, still functional changes may attribute to these changes. The second explanation for the marked reduction in the germ cells was reported by Goos and Consten (2002). They noticed retarded testicular development on non mammalian testes under the effect of hydrocortisone. They concluded that hydrocortisone has a direct inhibitory effect on the testicular androgen secretion.

In the present study the Sertoli cells of hydrocortisone treated animals showed no nuclear changes. This may be explained by the resistance of Sertoli cells to different physical agent. Sertoli cells considered as resistant and stable cells against irradiation, hypophysectomy, estrogen and F.S.H. treatment (Sinha-Hikim, 1986), constant darkness (Selim *et al.*, 1986) and cisplatinum (Galal *et al.*, 1994). As it is consider as a resistant cells, they taken as a reference cells for the germinal epithelium under different physiological and pathological conditions (Sinha-Hikim *et al.*, 1986). But still this explanation is not accepted completely as the Sertoli cells show in our study a group of structural changes on the cytoplasmic level as well as absolute resistance is not reported (Hassanein and Mohammed, 1991). The cytoplasm of Sertoli cells of hydrocortisone treated animals contains fragmented smooth endoplasmic reticulum, lysosomes and empty vacuoles. The cytoplasmic changes are in agreement with that reported by Elshennawy and Abo Elwafa (2011).

Under the effect of hydrocortisone alone the nuclei of the germinal epithelium cells in our results is smaller in size with condensed chromatin materials, sometimes irregular outline. The cytoplasm appears less granular with less cytoplasmic organelles and dilated vacuoles. These nuclear and cytoplasmic changes considered as degenerative (Izunya1 *et al.*, 2010) and are in agreement with that reported by Elshennawy and Abo Elwafa (2011). These findings may explained either by direct inhibitory effect on the testicular androgen secretion by hydrocortisone and hens retarded spermatogenesis (Goos and Consten, 2002) or its effect on Sertoli cells that retard spermatogenesis (Elshennawy and Abo Elwafa, 2011).

Our results as regard the effect of combined administration of *Nigella Sativa* oil and

hydrocortisone showed mixed picture of both effects. The hydrocortisone effect on the basal lamina was obvious but on the cells was less marked. The basal lamina appeared irregular and thicker than normal. Collapse of some tubules were obvious causing widening of the intercellular spaces. The irregularity, the increased thickness and the collapse was less than that of hydrocortisone alone. The number of seminiferous epithelial rows were more or less similar to control. Some of the spermatocytes revealed mega nuclei others reveal small sized nuclei. Empty spaces and necrotic cells were less marked relative to hydrocortisone administration alone. This picture may be due to protective effect of *Nigella sativa* oil (Abdel-Wahhab and Aly, 2005) on the seminiferous epithelial cells.

The electron microscopic picture of the seminiferous epithelial cells of combined administration of *Nigella Sativa* oil and hydrocortisone in the present study showed picture more or less similar to control on the nuclear and cytoplasmic level. The cytoplasm appeared granular but less than that of the control, characterized by dispersed oval and rounded mitochondria and cisternae of smooth endoplasmic reticulum. Still it contains dilated vacuoles. The destructive cytoplasmic changes of the hydrocortisone was not marked. This may explained by the protective effect of *Nigella Sativa* oil on the seminiferous epithelial cells. Ali and Blunden 2003 reported that much of the biological activity of *Nigella Sativa* has been shown to be due to thymoquinone, the major component of the oil. But still the actual mechanism of protection needs further study.

5. Conclusion:

Nigella sativa oil has a beneficial effect on the seminiferous epithelium while hydrocortisone administration causes an obvious destructive effects on the seminiferous epithelium. Combined administration of *Nigella sativa* oil and hydrocortisone ameliorate the destructive effects of hydrocortisone on the seminiferous epithelium. So, it is better to use *Nigella sativa* oil in combination with hydrocortisone.

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