Role of Nigella Sativa Seeds on modulation testicular toxicity of colchicine repeated use in adult albino rats

Said S. Elshama[1,5], Gaber M. G. Shehab[2,6], Ayman E. El-Kenawy[3,7], Hosam-Eldin H. Osman[4,8], Mostafa M. Farag[2,6].

Department of Forensic Medicine and Clinical Toxicology[1], Department of Biochemistry[2], Department of Pathology[3], Department of Anatomy[4], College of Medicine, Taif University, kingdom Saudi Arabia. Department of Forensic Medicine and Clinical Toxicology, Suez Canal University, Egypt[5]. Department of Biochemistry, Faculty of Agriculture, Cairo University, Egypt[6]. Department of Molecular Biology. Genetic Engineering and Biotechnology Inst., Menoufiya University, Egypt[7]. Department of Anatomy, Al-Azhar University, Egypt[8].
Corresponding author: Said S. Elshama: saidelshama@yahoo.com.

Abstract: Introduction: Gout is one of the most common diseases among humans in the world. Colchicine is an important drug for the treatment of this disease. Colchicine has a narrow therapeutic index; its poisoning is serious and associated with high mortality rate. *Nigella sativa* seeds are used for treatment of many diseases in traditional medicine. **Aim of the study:** Investigation of the role of nigella sativa seeds on modulation toxic effect of colchicine in the testis of rats. **Materials and Methods**: 120 of male adult albino rats divided into three equal groups (each n = 40). First group (control) received distilled water daily; the second was given 2mg / kg/day of colchicine. Third group was given 2 mg/kg/day of cholchicine with 1000 mg/kg/day of aqueous suspension of powdered *nigella sativa* seeds. Administration of drug and aqueous suspension were achieved by gastric gavage for 12 weeks. Testicular toxicity was investigated by assessment of histopatholgical changes, plasma testosterone level and semen analysis. **Results:** Repeated use of colchicine induced testicular toxicity manifested by histopathological changes such as marked degeneration of seminiferous tubules germinal cells, interstitial cells of Leydig and spermatid in the form irregular acrosomal cap with degenerated sperms, swollen vacuolated mitochondria and periphery clumped chromatin particles in cytoplasm of spermatogonia with low positive reaction of PAS. It led to decrease of testosterone plasma level, normal sperm count, sperm motility and liveability with increase of abnormal sperm count. Administration of *Nigella sativa* seeds with colchicine induced significant improvement of testicular toxicity manifestations **Conclusions:** Repeated use of colchicine caused testicular toxicity of rats which was improved by administration of *nigella sativa* seeds.
[Said S. Elshama, Gaber M. G. Shehab, Ayman E. El-Kenawy, Hosam-Eldin H. Osman, Mostafa M. Farag. **Role of Nigella Sativa Seeds on modulation testicular toxicity of colchicine repeated use in adult albino rats.** *Life Sci J* 2013;10(4):1629-1639]. (ISSN:1097-8135). http://www.lifesciencesite.com. 216

KEYWORDS: *Nigella sativa*, colchicine, testis

1. Introduction

Colchicine is a natural alkaloid with weak anti-inflammatory activity. It has been used extensively for gout treatment from centuries and also been recommended in preventing attacks of familial Mediterranean fever, treatment of primary biliary cirrhosis; amyloidosis and condyloma acuminate **(Guven et al., 2002).** It is an anti-mitotic agent and can be either cytotoxic or protective against cytotoxicity, but it has a direct toxicity on cells of skeletal muscle. Colchicine has been reported to protect against a variety of hepatotoxic insults and improve survival in a clinical trial for alcoholic cirrhosis **(Maxwell et al., 2002)**.

Colchicine is rapidly absorbed from the gastrointestinal tract after ingestion. It undergoes significant first pass hepatic metabolism, which primarily involves deacetylation. Subsequent to this, the metabolites undergo widespread enterohepatic recirculation before being excreted in bile and faeces **(Borron et al., 1996).** The symptoms of gastrointestinal toxicity of colchicine such as nausea, vomiting, diarrhea and abdominal pain are seen in 80% of patients on full therapeutic doses and are used as the clinical endpoint in dose titration **(Milne and Meek, 1998).**

Nigella sativa is a plant of Ranunculaceae family that grows spontaneously and widely in several Southern Mediterranean and Middle Eastern countries. Its seed has over 100 different chemical constituents, including abundant sources of all the essential fatty acids. Although it is the oil that most often used medicinally, the seeds are a bit spicy and often used whole in cooking curries, pastries and Mediterranean cheeses **(Hajhashemi et al., 2004).** *Nigella sativa* seeds are used extensively in traditional medicine of many countries. It has been used for treatment of many diseases owing to the reported antiviral, anti-inflammatory, anti-schistosomiasis and immunomodulatory activities **(Uz et al., 2008).** Furthermore, it was found that *nigella sativa* extract has anti-tumor properties attenuating toxic side effects caused by several chemotherapeutic agents and protects against gentamicin-induced nephrotoxicity **(Yaman**

and Balikci, 2010). So, the present study aimed to investigate the effect of nigella seeds on modulation testicular toxicity of colchicine by assessment histopatholgical changes of testis, plasma testosterone level and semen analysis.

2. Material and methods

120 male adult albino healthy rats weighing (250–300 g) were used for the study. They were housed in air- conditioned, humidity-controlled cages. Rats had free access to water and food during the experimental period. The rats were caged in three equal groups (each n=40). The first was control group; each rat received equal volume of distilled water per day orally for 12 weeks. The second group was given 2 mg/kg/day of cholchicine orally for 12 weeks (**Terkeltaub et al., 2010)** and the third group was given 2 mg/kg/day of cholchicine with 1000 mg/kg/day of aqueous suspension of powdered *nigella sativa* seeds orally for 12 weeks also **(Gali et al., 2006)**. Oral administration was achieved by gastric gavage. Rats of each group were euthanized at the end of experimental period under anesthesia by administration 0.5 cm of ketamine intaperitoneal.

Cholchicine drug was in the tablet form and obtained from El-Nasr pharmaceutical Chemicals Company (ADWIC) Abu-Zaabal-Egypt. One tablet contains 500 microgram of active ingredient, was dispersed in 5 ml distilled water.

One gram of *nigella sativa* seeds were added to 100 ml of distilled water, mixed and grinding by a blender, this was made for 5-6 intervals, 60 seconds each time, at room temperature until complete grinding and mixing to obtain a stock solution of crude suspension (**Tekeoglu et al., 2007).**

Estimation of plasma testosterone level:

The thoracic cavity was opened and blood samples were collected directly after puncture of rat's hearts in the morning in order to minimize the diurnal variation of hormone levels. Collection of plasma was done by using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay of testosterone quantitative measurement was performed immediately by Testosterone Rat/Mouse ELISA method using Kits obtained from Roche Diagnostics GmbH. D-68298 Mannheim, USA, according to the method of **Sakuma, 2009.**

Semen analysis:

Seminal content of epididymis was obtained by cutting of cuda epididymis using surgical blades and squeezed in a sterile clean watch glass. This content was diluted with normal saline on warm slide and thoroughly mixed to estimate the progressive motility. One drop of the suspension was smeared on a glass slide and stained by an equal drop of Eosin-nigrosin stain (1% eosin + nitrogen dissolved in 20.9% sodium citrate dehydrate buffer). The film is spread on clean slide and dried quickly on a hot plate to determine the percentage of sperm cell viability; morphological abnormalities such as abnormal head and tail **(Mori et al., 1991; Okomura et al., 2005).** The haemocytometer pipette of erythrocyte was used for counting sperms. The diluted epididymal content (diluted with 100 ul eosin) was withdrawn up to mark 0.5 and the pipette was filled up to the mark by 2% aqueous solution of eosin. The eosin killed sperms so that counting could be accomplished as well as staining the sperm heads so that they were easier to be counted. The contents of the pipette were mixed and the sperms in five large squares (80 small squares) were counted using the high power of the light microscope. The sperm concentration per epididymis was calculated by the following equation:

Sperm concentration / epididymis = N/80 X 400 X 200 X 10 X 100

Where:

N = Count sperm number.

80 = Number of small square in five large squares.

400 = Total number of small squares in chamber.

10 = Depth of haemocytometer chamber.

200 = Dilution factor on pipette.

100 = Dilution factor of epididymal content. **(Bearden et al., 2003).**

Histopathological Examination:

Following the semen collection, the testes of rats were dissected out; its tissues were cut and immediately fixed in Bouins' fluid. It processed for preparation of paraffin blocks (paraffin method). Sections were cut by rotatory microtome and mounted on glass slides. The sections were stained by Hematoxylin & Eosin, Mallory and Periodic acid Schiff (PAS). The sections were examined by light microscope (Leica DM 1000) **(Drury and Wallington, 1980)**.

Ultrastructural studies were done by Transmission Electron Microscope (TEM). Testicular tissue was cut into small pieces 1 mm thick and fixed in 4% formaldehyde in phosphate buffer solution (pH 7.2) for 3 hr at 4 °C, after which the tissues were removed and post fixed in buffered 2% osmium tetroxyde for one hour at 4°C. Post fixed tissues were rinsed in the buffer and dehydrated at 4°C through a graded series of ethanol. Then they were embedded in Epon Araldite mixture in labeled beam capsules. Ultrathin sections (50 nm thick) were cut, collected on naked copper-mesh grids and stained with uranyl acetate for 10-15 min and lead citrate for 5-10 min **(Bancroft and Gamble, 2002).**

Statistical analysis

Statistical analysis was performed using SPSS version 16. Variability of results was expressed as

mean ± SD. The significance of differences between mean values was determined using one way analysis of variance (ANOVA) test and followed by Tukey (H.S.D) test. P <0.05 represents level of significance.

Ethical considerations

The most appropriate animal species was chosen for this research. Promotion of a high standard of care and animal well-being at all times was done. Appropriate sample size was calculated by using the fewest number of animals to obtain statistically valid results. Painful procedures were performed under anesthesia to avoid distress and pain. Our standards of animal care and administration met those required by applicable international laws and regulations.

3. Results

1- Testicular histopathological findings by light microscope:-

Examination of testicular tissue rats of first group (control), showed normal structure of seminiferous tubules, basement membrane, germ cells, interstitial tissue, interstitial cells of Leydig and tunica albuginea **(Fig.1&2)** with normal sperms **(Fig.3)** and positive reaction of PAS **(Fig.4).** But testicular section rats of second group which received colchicine alone, showed marked disorganization and necrotic germ cells lining of the seminiferous tubules, degenerated sperms and interstitial cells of Leydig, irregular thin basement membrane and tunica albuginea **(Fig.5&6)** with degenerated sperms **(Fig.7)** and low positive reaction of PAS **(Fig.8)**. Testicular section rats of third group which received colchicine and aqueous suspension of powdered *nigella sativa* seeds showed marked recovery of germ cells lining of seminiferous tubules with normal sperms, normal interstitial cells of Leydig and basement membrane **(Fig.9&10)** with normal sperms **(Fig.11)** and positive reaction of PAS **(Fig.12).**

2- Testicular histopathological findings by electron microscope:-

Examination of testicular tissue ultrastructures in rats of first group (control) showed normal histological structure of seminiferous tubule with various stages of spermatogenesis and interstitial of Lidge cells. The spermatogonia have two types, (A and B type). It lies on the basement membrane of seminiferous tubules. There are two types of spermatocytes (primary and secondary). The spermatids appear as rounded cells with large spherical nuclei and lightly stained cytoplasm. The Sertoli Cells are a large cell that extends from the basement membrane to the lumen of the seminiferous tubule **(Fig.13)**. And testis in rats of second group showed thin irregular basal lamina of the seminiferous tubules with fibrous connective tissue and degenerative changes in the germinal cells. The cytoplasm of spermatogonia (type A and B) showed swollen vacuolated mitochondria, vascular endoplasmic reticulum and periphery clumped chromatin particles. There are degenerative changes of spermatid which appeared in the form irregular acrosomal cap **(Fig. 14).** In regards ultrastructures of testis in rats of third group showed an improvement of histopathological changes of testis which manifested by normal basal lamina of seminiferous tubules, normal interstitial cells of Leydig and lack of degenerative changes in seminiferous tubules. There are marked improvement of germinal cells lining of seminiferous tubules particularly in primary spermatocytes and spermatids **(Fig.15).**

3- Effect of colchicine repeated use alone or with *nigella sativa* seeds on testicular weight and body weight of rats:-

Table (1) represents mean ± SD values of testicular weight and body weight of rats.

Mean ±SD values of rats testicular weight in control group which received distilled water, second group which received cholchicine dissolved in distilled water and third group which received cholchicine dissolved in distilled water and *nigella sativa* seeds were 1.55±.15; 74±.17; and 1.04±.065 respectively. The Mean ±SD values of rat's body weight of control group, second group, and third group were 2.73±13.9; 2.29±13.5; and 2.42±11.4, respectively.

Table (1): Effect of colchicine repeated use alone or with *nigella sativa* seeds on Mean ± SD of testicular weight and body weight of rats.

Group / Parameter	First	Second	Third
Testicular weight (gm)	1.55±.15	.74±.17*	1.04±.065**
Body weight (gm)	2.73±13.9	2.29±13.5*	2.42±11.4**

Number per group = 40 SD = standard deviation

First group (control) received equal volume of distilled water/day.

Second group received 2 mg/kg/day of cholchicine.

Third group received 2 mg/kg/day of cholchicine with 1000 mg/kg/day of aqueous suspension of powdered *nigella sativa* seeds.

* = $p < 0.05$ (significant difference in comparison with control group)

** = $p < 0.05$ (significant difference in comparison with second group)

4- Effect of colchicine repeated use alone or with *nigella sativa* seeds on plasma testosterone level and semen analysis of rats:

Table (2) represents mean ±SD values of plasma testosterone level and semen analysis of rats. Mean ±SD values of rat's testosterone level in control group, second group, and third group were 3.06±.82; 93±.17; and 2.30±.6 respectively. Mean ±SD values of normal sperm count of rats in control group, second group, and third group were 4.34±1.05, 2.02±1.69, and 3.80±1.19, respectively. Mean ±SD values of abnormal sperm count of rats in control group, second group, and third group were 22.33±1.39, 46.90±2.36, and 30.90±6.54, respectively. Mean ±SD values of sperm motility of rats in control group, second group, and third group were 83.71±1.84, 28.67±4.37, and 74.56±.17, respectively. Mean ±SD values of sperm liveability of rats in control group, second group, and third group were 88.68±.18, 77.72±4.07, and 84.56±.17, respectively.

Table (2): Effect of colchicine repeated use alone or with *nigella sativa* seeds on Mean ± SD of plasma testosterone level and semen analysis of rats.

Group / Parameter	First	Second	Third
Testosterone (ng/ml)	3.06±.82	.93±.17*	2.30±.66**
Normal Sperm count (million/ml)	4.34±1.05	2.02±1.69*	3.80±1.19**
Abnormal sperm count (%)	22.33±1.39	46.90±2.36*	30.90±6.54**
Sperm motility (%)	83.71±1.84	28.67±4.37*	74.56±.17**
Sperm liveability (%)	88.68±.18	77.72±4.07*	84.56±.17**

Number per group = 40; SD = standard deviation

First group (control) received equal volume of distilled water/day.

Second group received 2 mg/kg/day of cholchicine.

Third group received 2 mg/kg/day of cholchicine with 1000 mg/kg/day of aqueous suspension of powdered *nigella sativa* seeds.

* = $p < 0.05$ (significant difference in comparison with control group)

** = $p < 0.05$ (significant difference in comparison with second group)

Semen analysis of rats in the first group (control), showed normal shaped sperms **(Fig.16. A)**. in contrast, in second group rats showed abnormal shaped sperms such as, thin elongated head, club-shaped head, incorrect head-neck connection, tailless head, headless tail, bent mid-piece and curved mid piece **(Fig.16.B)**. And in case of the semen of third group rats showed improvement in reduction of abnormal shaped sperms **(Fig.16.C)**.

4. Discussion

Colchicine has a narrow therapeutic index; its poisoning is a serious and associated with high mortality rate. Gout is a chronic disease and needs repeated doses of colchicine over a long period inducing multi-organ dysfunction if it is given in high doses exceeding the recommended dose because there does not seem to be any clear cut separation between non toxic and toxic doses of colchicine **Wagenaar (2004).** Thus, the present study investigates testicular toxicity induced by repeated use of colchicine and how we can modulate this toxicity by studying plasma testosterone level, semen analysis and testicular histopathological changes using *nigella sativa* seeds.

The present study showed that one of major manifestations of testicular toxicity induced by colchicine was histopathological changes which represented as marked degeneration of germinal cells of the seminiferous tubules, sperms, spermatid and interstitial cells of Leydig, irregular thin basement membrane and tunica albuginea with low positive reaction of PAS. This is consistent with the result obtained by **Nabila (2006).**

Finkelstein et al., (2010), explained that histopathological changes of testis depending on colchicine toxicity due to arrest of cell division in metaphase by intervention with spindle fibers formation because of mitotic inhibition property of colchicine. It is taken up equally by all cells except highest cell turnover such as testicular cells which have the greatest mitotic activity and then it is more affected. **Brncic et al., (2001),** indicated that mechanism of colchicine toxicity due to its binding selectively and reversibly to 65 subunits of microtubules, altering cellular processes such as cell shaping, division, mobility and ability to exhibit phagocytosis. This is in contrast with **Klintschar et al., (1999)** who refereed that mechanism of toxicity is unrelated to binding microtubules.

Our results revealed that colchicine toxicity led to damage of seminiferous tubules with germ cell loss and then defect of spermatogenesis causing low normal sperm count, increase of abnormal sperm number, decrease of sperm motility and liveability, increase of abnormal shaped sperms and acrosome of testicular spermatids. This is consistent with **Harris and Gillet (1998).**

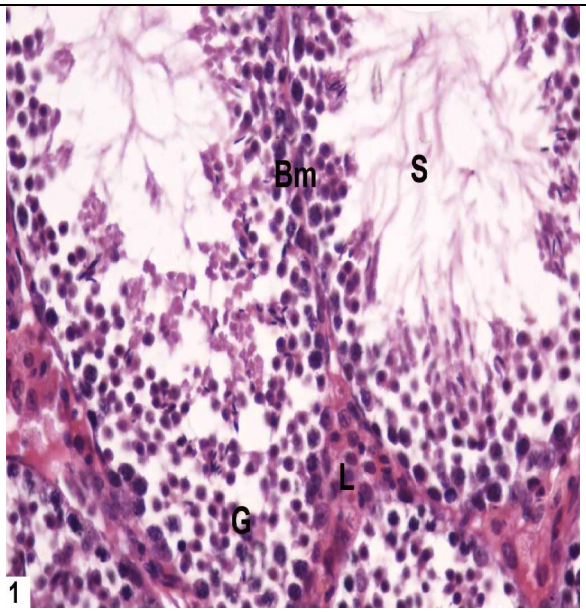

Fig (1) a photomicrograph of a transverse section of a control rat testis showing a group of seminiferous tubules which are surrounded by the basement membrane (bm). The germ cells (G) are arranged in layers. The sperms are seen in the lumen (s) & the interstitial tissue is present in between the tubules containing Leydig cells (L). (H&E X400)

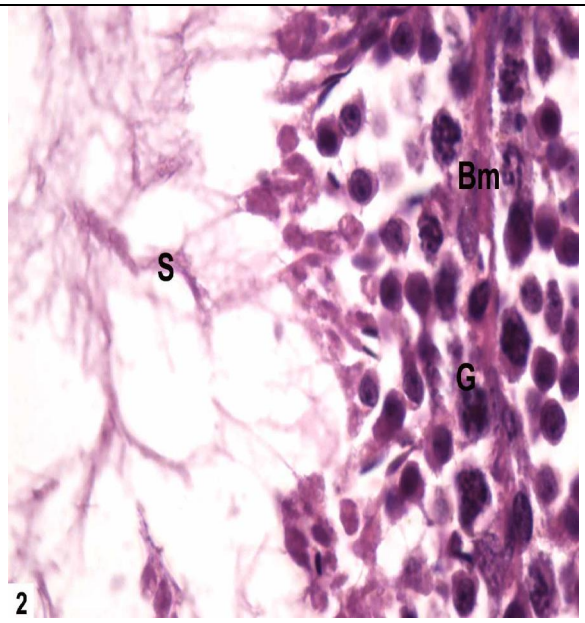

Fig (2) a photomicrograph of a transverse section of a control rat testis showing a group of seminiferous tubules which are surrounded by the basement membrane (bm). The germ cells (G) are arranged in layers. The sperms are seen in the lumen (s). (H&E X1000)

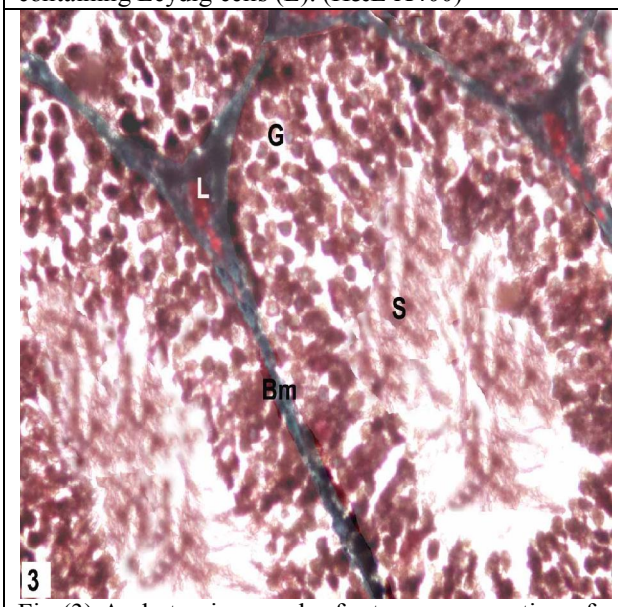

Fig (3) A photomicrograph of a transverse section of a control rat testis showing normal thickness of basement membrane (Bm), normal arrangement of germ cells (G)and normal sperms (S) (MalloryX400)

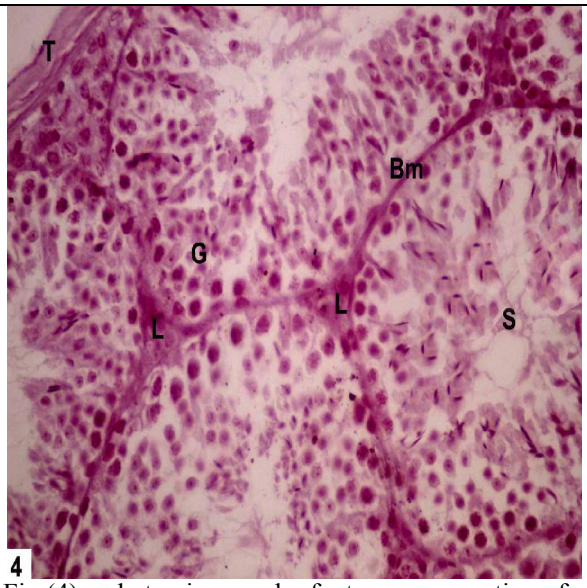

Fig (4) a photomicrograph of a transverse section of a control rat testis showing a positive reaction of PAS in all its components with normal thickening of tunica albuginea (T), normal thickness of the basement membrane (bm), normal histological structure of seminiferous tubules with normal arrangement of germinal cells (G) and normal sperm (S). (Periodic acid-Schiff's X400)

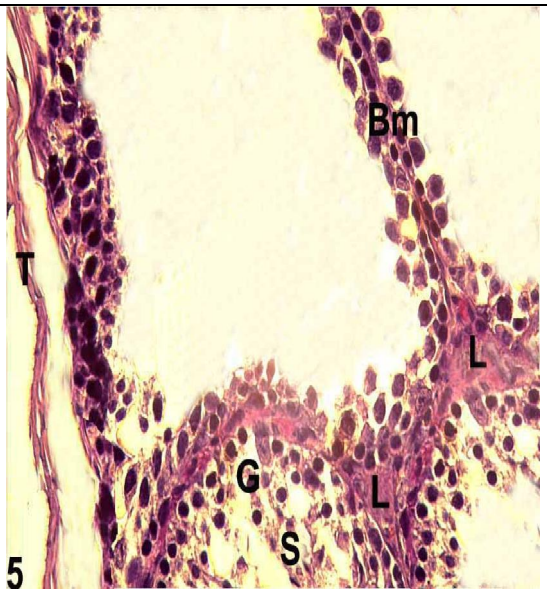

Fig (5) a photomicrograph of a transverse section of a second group rat testis showing marked disorganized and necrotic germ cells (G) lining of the seminiferous tubules degenerated sperms (S), with degenerated interstitial cells of Leydig (L) and irregular thin basement membrane (Bm) and tunica albuginea (Ta). (H&E X400)

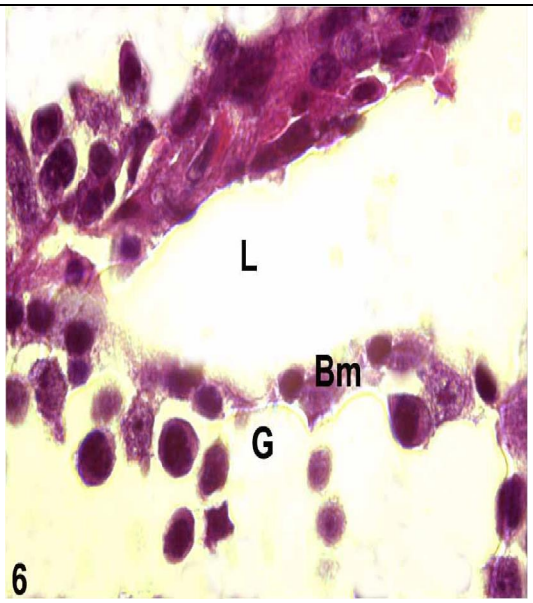

Fig (6) a photomicrograph of a transverse section of a second group rat testis showing marked disorganized and necrotic germ cells (G) lining of the seminiferous tubules with degenerated interstitial cells of Leydig (L) and irregular thin basement membrane (Bm). (H&E X1000)

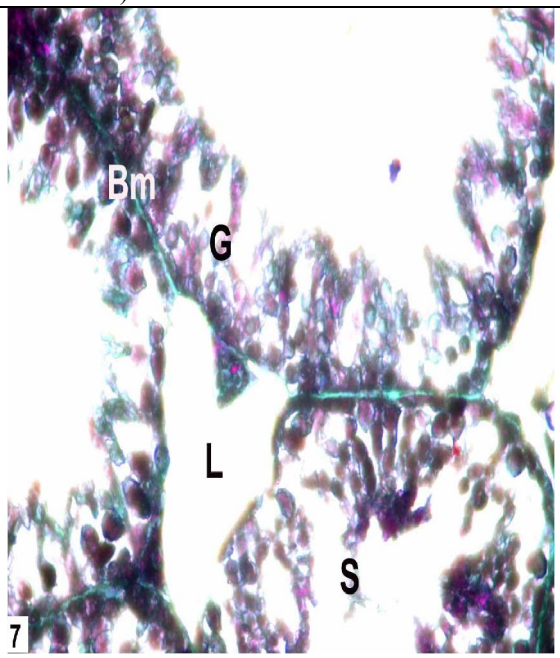

Fig (7) a photomicrograph of a transverse section of a second group rat testis showing marked disorganized and necrotic germ cells (G) lining of the seminiferous tubules with degenerated interstitial cells of Leydig (L) and damaged irregular thin basement membrane (Bm). (Mallory X400).

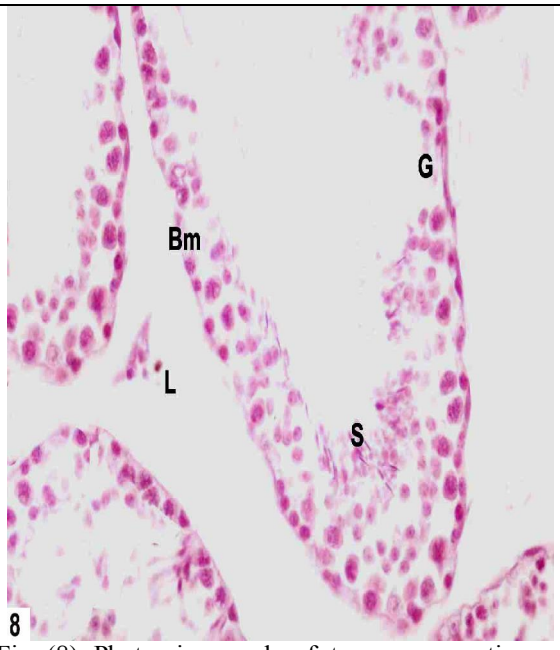

Fig (8) Photomicrograph of transverse section of testis of colchicines treated group showing low PAS positive reaction in the disorganized germ cells (G) lining of the seminiferous tubules, irregular thin basement membrane (Bm) low density of sperm(S) and degeneration of lydeing cells (L). (Periodic acid-Schiff's X400)

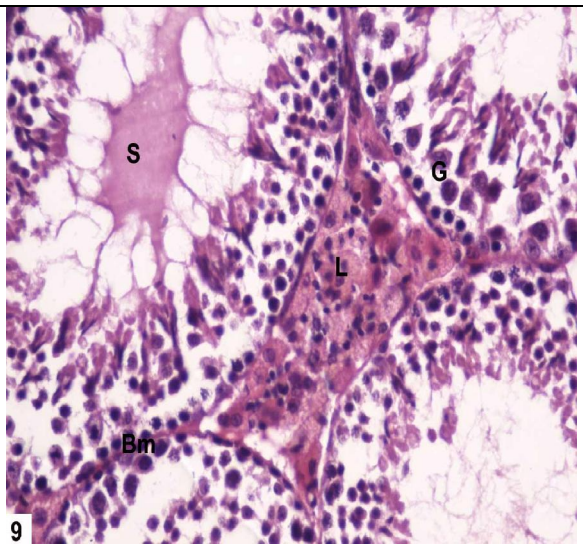

Fig (9) Photomicrograph of transverse section of testis of third group showing recovery of histological features of germ cells lining of the seminiferous tubules (G), basement membrane (Bm) normal sperms in the lumen (S) as well as the interstitial cells of leydig (L). (H&E X400)

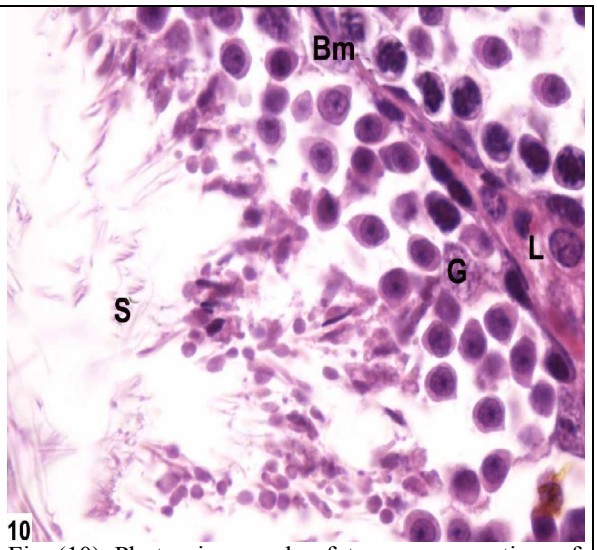

Fig (10) Photomicrograph of transverse section of testis of third group showing recovery of histological features of germ cells lining of the seminiferous tubules (G), basement membrane (Bm), normal sperms in the lumen (S) well as the interstitial cells of leydig (L). (H&E X1000)

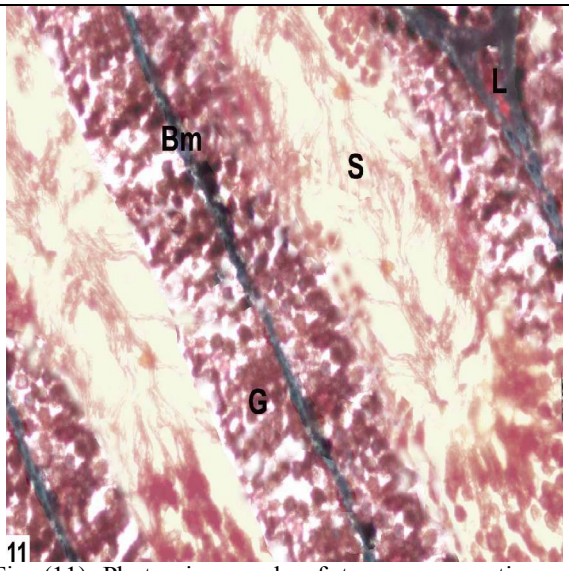

Fig (11) Photomicrograph of transverse section of testis of third group showing recovery of histological features of germ cells lining of the seminiferous tubules (G), basement membrane (Bm), normal sperms in the lumen (S) well as the interstitial cells of leydig (L). (Mallory X400)

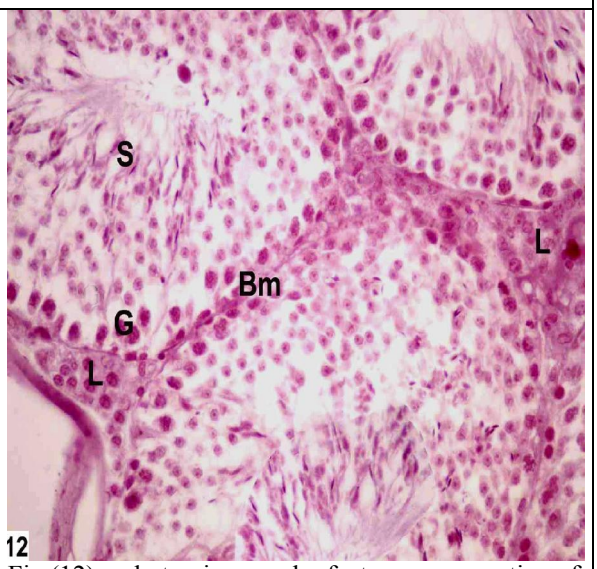

Fig (12) a photomicrograph of a transverse section of third group showing a positive reaction of PAS with recovery of histological features in all its components with normal thickness of the basement membrane (bm), normal histological structure of seminiferous tubules with normal arrangement of germinal cells (G) and normal sperm in the lumen(S). (Periodic acid-Schiff's X400

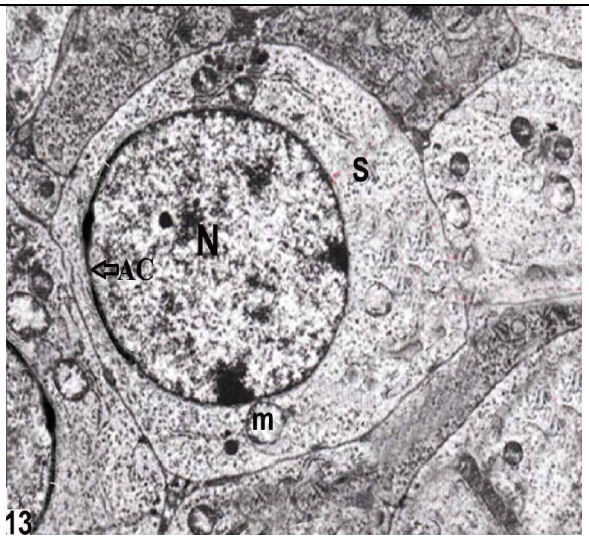

Fig (13): Electron micrograph of the testis of a control rat, showing normal sperm (S) and acrosomal cap of spermatid (AC), normal nucleus (N) and mitochondria(m) X15000

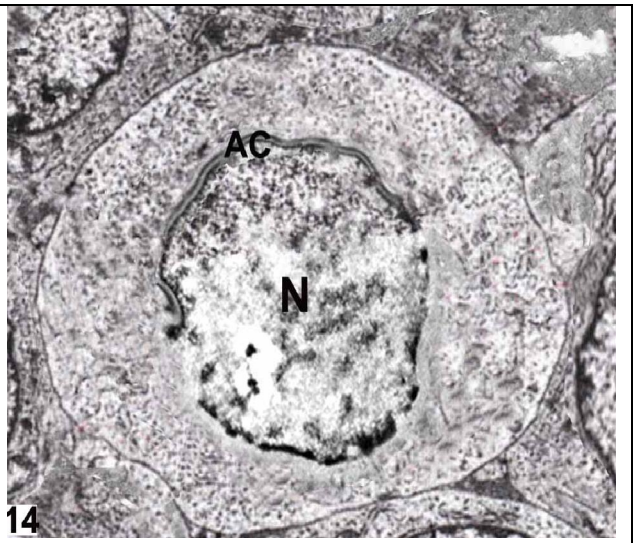

Fig (14): Electron micrograph of the second group rat testis showing irregular acrosomal cap of spermatid (AC) and irregular nuclear membrane (N). X17000

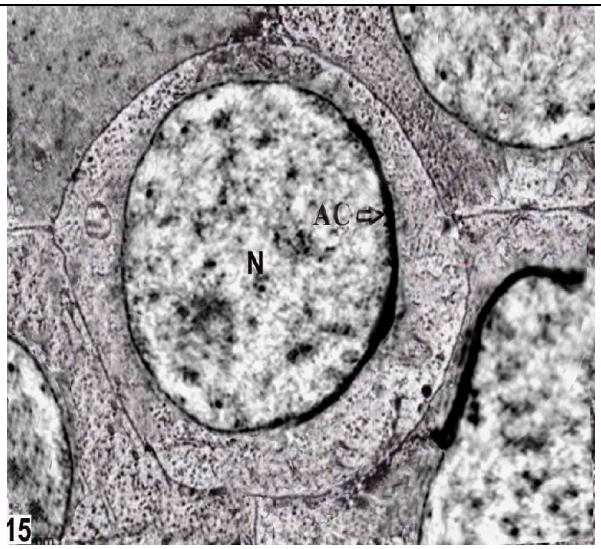

Fig (15): Electron micrograph of the third group rat testis showing recovery of sperm (S), acrosomal cap of spermatid (AC) and recovery of nuclear membrane (N). X14000

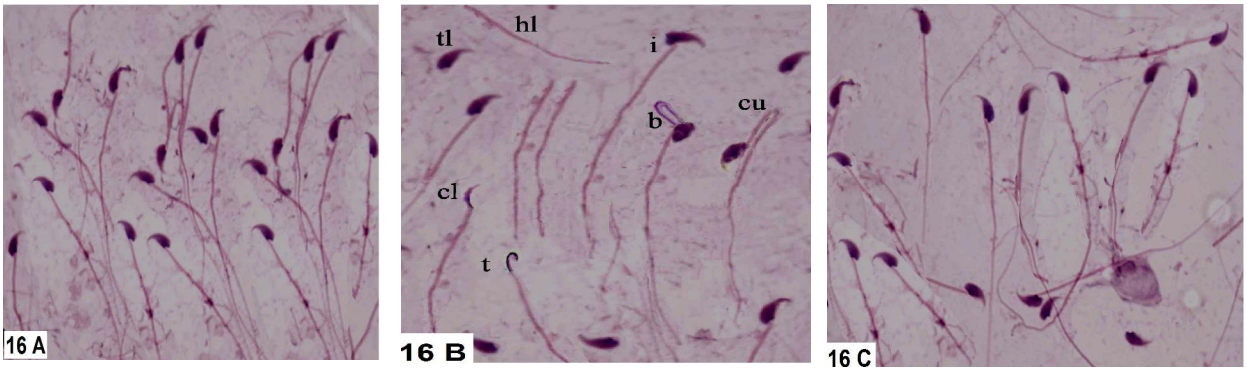

Fig (16.A): A photomicrograph of normal shaped sperms of control rats. HE X 1000
Fig (16.B): A photomicrograph of abnormal shaped sperms of second group rats.
t: Thin elongated head.
cl: Club-shaped head.
i: Incorrect head-neck connection.
tl: Tailless head.
hl: Headless tail.
b: Bent mid-piece.
cu: curved mid piece.
HE X 1000
Fig (16.C): A photomicrograph of improvement of abnormal shaped sperms in third group rats. HE X 1000.

According to **Hess and Nakai (2000),** sperm morphogenesis is a complex process. Germ and Sertoli cells contain numerous microtubules which are essential for normal morphogenetic processes of spermatogenesis. Colchicine interacts with microtubules inducing sperm structural abnormalities. **Allard et al., (1993)** indicated that testicular reduction weight is due to decrease of germ cells number and volume of other testicular compartments such as interstitial and seminiferous epithelium. According to **Katsumi et al., (2004),** the weight of testes is the most sensitive parameter indicating the sperm toxicity and this is consistent with the current study.

The present study demonstrated reduction in plasma testosterone level as a result of colchicine treatment. Our result was in agreement with **Dianne (2001),** who showed that male reproductive system toxicity may result in reduced sperm output or reduced testosterone secretion from the testis and quality of the sperm available for fertilization may also be impaired. Testicular spermatogenesis is dependent on a mixture of hemodynamic and androgenic factors as well as central and autonomic nervous input. Interference with any of these processes has the potential to reduce fertility of the male. He indicated also to a relationship between body weight reduction of rat due to colchicine intoxication, fat deposition and decrease of testosterone level.

El Shenawy et al., (2008), confirmed that colchicine intoxication induced testicular toxicity due to oxidative damage because the high lipid content of the reproductive organs such as testis makes it particularly susceptible to free radicals mediated insult.

Results of the current study showed an improvement in third group rats after adding aqueous suspension of powdered *nigella sativa* seeds with colchicine administration. This improvement represented in histopathological changes of testis such as marked recovery of germ cells lining of seminiferous tubules, normal interstitial cells of Leydig and basement membrane with normal positive reaction of PAS. Testosterone level returns approximately to normal level with reduction of abnormal shaped sperms and improvement sperm motility and liveability.

Chandra et al., (2009), referred that main constituent of *nigella sativa* seeds is thymoquinone (TQ) which possesses an important analgesic, anti-inflammatory properties and protects organs against oxidative damage induced by a variety of free radical generating agents because it is antioxidant agent involved in defense system against free radical mediated tissue or cellular damage.

The present data indicated the protection effect of *nigella sativa* seeds against testicular toxicity of colchicine and this may be due to its antioxidant action and free radical scavenger. **Burits and Bucar (2012)** showed that nigella sativa seeds have preventive effect against ischemia reperfusion injury to various organs. Furthermore, **Parveen and Shadabig, (2011),** indicated that generation of reactive oxygen species (ROS) as result of oxidative stress of any toxic agent were depressed by antioxidant effect of nigella sativa seeds and this consistent with (**Ragheb et al., 2009)**

and (**Uzma et al., 2012)** who showed importance of thymoquinone which is a pharmacologically active quinone and its antioxidant effect.

Conclusion

Repeated use of colchicine leads to testicular toxicity which represents as histopatholgical changes, reduction of testosterone level and normal sperm count associated with decrease of sperm motility and liveability in adult albino rats. Administration of *nigella sativa* seeds as antioxidant agent modulates testicular toxicity induced by colchicine.

Recommendations

Results of this study may be significant to animal but we need further researches in human to investigate our results. We suggest further studies to determine oxidative stress parameters such as lipid peroxidation level, reduced glutathione tissue level "GSH", superoxide dismutase "SOD" and catalase activity to complete this work. According to this study, repeated use of colchicine leads to toxic effect in testis and administration of *nigella sativa* seeds has a protective action against colchicine testicular toxicity.

Corresponding Author:
Said S. Elshama
Forensic Medicine & Clinical Toxicology Department, College of Medicine, Taif University, KSA; Suez Canal University, Egypt.
saidelshama@yahoo.com

References

1. Allard EK, Johnson KJ and Boekelheide K. Colchicine disrupt the cytoskeleton of rat testis seminiferous epithelium in a stage-dependent manner. Biol., Reprod., (1993) 48, 143-153.
2. Bancroft JD and Gamble M Theory and practice of histological techniques. 5th., Ed., Edinburgh and London, Churchill Livingstone Pub., (2002). 172-5, pp 593-620.
3. Bearden HJ, Fuquay JW and Willard ST Applied Animal Reproduction. 6th., Ed., Reston Publishing Co., Inc., Reston, Virginia, (2003) p: 448.
4. Borron SW, Scherrmann JM and Baud FJ. Markedly altered colchicine kinetics in a fatal intoxication: Examination of contributing factors. Hum., Exp., Toxicol., (1996) 15:885–890.
5. Brncic N, Ivica V, Relja P, Anoelko D, Dinko V and Draen C. Accidental Plant Poisoning with Colchicum autumnale: Report of Two Cases. Croat., Med., J., (2001) 42(6):673-675.
6. Chandra S, Murthy SN, Mondal D and Agrawal KC. Therapeutic effects of *Nigella sativa* on chronic HAART-induced hyperinsulinemia in rats. Can., J., Physiol., Pharm., (2009) 87: 300-309.
7. Dianne MC. Pathogenesis of Male Reproductive Toxicity. Toxicologic pathology, (2001), 29, 1, 64 – 76.
8. Drury RA and Wallington EA. Carlton Histological Technique (1980) 4th Ed.Oxford Press, 65-75.
9. El Shenawy NS, Soliman MF and Reyad SI. The effect of antioxidant properties of aqueous garlic extract and *Nigella sativa* as anti-schistosomiasis agents in mice. Rev., Inst., Med., Trop., São Paulo. (2008) 50: 29-36.
10. Finkelstein Y, Aks SE, Hutson JR, Juurlink DN, Nguyen P, et al. Colchicine poisoning: the dark side of an ancient drug. Clin., Toxicol., (Phila) (2010) 48: 407-414.
11. Gali MH, Roessner A and Schneider SR Thymoquinone: a promising anti-cancer drug from natural sources. Int., J., Biochem., Cell., (2006), 38: 1249-1253.
12. Guven AG, Bahat E, Akman S and Atran, EM Late diagnosis of severe colchicine intoxication. Pediatrics, (2002), 109(5):971-3.
13. Hajhashemi V, Ghannadi A and Jafarabadi H. Black cumin seed essential oil, as a potent analgesic and anti-inflammatory drug. Phytother. Res., (2004),18: 195-199.
14. Harris RD and Gillet MJ. Colchicine poisoning - overview and new directions. Emergency Medicine; (1998) 10: 161-8.
15. Hess RA and Nakai M. Histopathology of the male reproductive system induced by the fungicide benomyl. Histol., Histopathol., (2000) 15: 207-224.
16. Katsumi O, Shigeru Y, Hisayo K, Muneyuki M and Junzo S. Comparative Investigation of Several Sperm Analysis Methods for Evaluation of Spermatotoxicity of Industrial Chemical: 2-Bromopropane as an Example. Industrial Health, (2004), 42, 219–225
17. Klintschar M, Beham-Schmidt C, Radner H, Henning G and Roll P. Colchicine poisoning by accidental ingestion of meadow saffron (Colchicum autumnale): pathological and medicolegal aspects. Forensic Sci., Int., (1999),106: 191-200.
18. Maxwell MJ, Muth P and Priiy PE. Accidental colchicine overdose. A case report and literature review. Emerg., Med., J. (2002) 19(3):265-7
19. Milne ST and Meek PD. Fatal colchicine overdose: report of a case and review of the literature. Am., J., Emerg., Med., (1998) 16:603–608.
20. Mori K, Kaido M, Fujishiro K, Inoue N, Koide O, Hori H and Tanaka I. Dose dependent effects of inhaled ethylene oxide on spermatogenesis in rats. Brit., J., Ind., Med., (1991), 48, 270 - 274.
21. Nabila AR. Effect of colchicine on the histology of spleen and testis of albimo rats. The Egyptian Journal of Hospital Medicine, (2006), 23:268-276.
22. Okamura A, Kamijima M, Shibata E, Ohtani K, Takagi K, Ueyama J, Watanabe Y, Omura M, Wang H, Ichihara G, Kondo T and Nakajima T. A comprehensive evaluation of testicular toxicity of

dichlorvos in wistar rats. Toxicology (2005), 213, 129 - 137.
23. Parveen N and Shadabig GG Ameliorative action of *nigella sativa* against iron induced chromosomal aberrations in rat bone marrow cells in vivo. International Journal of Pharma and Bio Sciences, (2011), 2: 470-477.
24. Ragheb A, Attia A, Eldin WS, Elbarbary F and Shoker A. The protective effect of thymoquinone, an anti-oxidant and anti-inflammatory agent, against renal injury: a review.
25. Saudi J., Kidney, Dis.,Transpl., (2009), 20(5):741-52.
26. Sakuma Y. Gonadal steroid action and brain sex differentiation in the rat., J., Neuroendocrinol., (2009), 21 (4): 410-4
27. Tekeoglu I, Dogan A, Ediz L, Budancamanak M and Demirel A. Effects of thymoquinone (volatile oil of black cumin) on rheumatoid arthritis in rat models. Phytother., Res., (2007), 21: 895-897.
28. Terkeltaub RA, Furst DE, Bennett K. High versus low dosing of oral colchicine for early acute gout flare: Twenty-four-hour outcome of the first multicenter, randomized, double-blind, placebo-controlled, parallel-group, dose-comparison colchicine study. Arthritis Rheum., (2010) 62(4):1060-1068.
29. Uz E, Bayrak O, Uz E, Kaya A, Bayrak R, Uz B and Turgut FH. *Nigella sativa* oil for prevention of chronic cyclosporine nephrotoxicity: an experimental model. Am., J., Nephrol., (2008) 28: 517-522.
30. Uzma S, Bashir A and Kanwal R, Saeed M, Maqsood A and Alia E. Nephro-protective effect of vitamin C and *Nigella sativa* oil on gentamicin associated nephrotoxicity in rabbits. J., Pharm., Sci., (2012), 25, 4: 727-730.
31. Wagenaar Z. Accidental colchicines poisoning in a dog. Can., Vet., J., (2004), 45(1):55-57.
32. Yaman I and Balikci E. Protective effects of *Nigella sativa* against gentamicin induced nephrotoxicity in rats. Exp., Toxicol., Pathol., (2010), 62: 183-190.

9/21/2013