



## **Comparative study between the flavonoids extracted from *Nigella sativa* seeds and vitamin E to ameliorating the effect of sodium nitrate on some testicular functions in adult male rats**

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### **Summery**

The present study was designed to investigate the protective effect of flavonoids extracted from *Nigella Sativa* seeds comparing to vitamin E in ameliorating the deleterious effect of sodium nitrate on some testicular function in adult male rats. Twenty adult male rats were randomly divided into four equal groups and treated for 120 days as follows: Group T1 (control) animals in this group were treated with normal saline; while animal in group T2 were daily treated with sodium nitrate 30mg/kg. B.W; animals in group T3 were daily treated with sodium nitrate 30 mg/kg B.W plus 50 mg of flavonoids extracted from *Nigella Sativa* and animals in group T4 were daily treated with sodium nitrate 30 mg/kg B.W plus 40 mg/kg B.W vitamin E. At the end of experiment animals were scarified and right testes were obtained to calculate testicular weight / body weight ratio and histological sections were prepared for measuring the thickness of cell lining the seminiferous tubules and the diameter of the tubules. Live and dead sperms were calculated by using left epididymis. The results pointed that a significant decrease in testes to body weight ratio and significant decrease in live sperms percentage, increased dead sperms percentage as well as decreased both seminiferous tubules diameter and cell lining seminiferous tubules in group T2. Administration of flavonoids extracted from *Nigella sativa* seeds (group T3) and vitamin E (group T4) ameliorated the deleterious effect of sodium nitrate on male reproductive system. Histological examination showed edema of basement membrane of seminiferous tubules and fibrous connective tissue proliferation and incomplete spermatogenesis in animals treated with sodium nitrate (group T2) comparing to control, while animals in group T3 showed moderate edema and thickness of basement membrane of seminiferous tubules and normal spermatogenesis, the results also showed moderate edematous interstitial tissue and congestion of blood vessels and moderate to normal spermatogenesis in group T4. In conclusion, flavonoids extracted

from *Nigella sativa* seeds and vitamin E play an important role as anti-oxidant against the oxidative effect induced by sodium nitrate on testicular function in adult male rats.

**Keywords: Sodium Nitrate, *Nigella sativa*, Vitamin E, Testicular Functions**

### Introduction

Nitrate salts are used widely as inorganic fertilizers, food preservatives, and are also used in explosives as oxidizing agents in the chemical industry (1 and 2). Dietary nitrate intake is considerable and many vegetables are particularly rich in this anion (3), for example, a plate of green leafy vegetables such as lettuce or spinach contains more nitrate (3 and 4). Nitrite can be found in some food stuffs, as a preservative in cured meat (2). Drinking water may contain variable amounts of nitrates which accounts for up to 21% of total nitrates intake in a typical human diet (4 and 5). Nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) are known predominantly as undesired residues in the food chain with potentially carcinogenic effects (6 and 7) or as inert oxidative end products of endogenous nitric oxide (NO) metabolism (8). It has been concern of possible health effects related to high nitrate consumption, including some forms of cancer (9 and 10), thyroid disorders (11) and reproductive effects (5,12 and 13).

Reactive oxygen species (ROS) can have beneficial effects on reproductive biology and sperm functions depending on the nature and the concentration of ROS as well as the location and length of exposure to ROS (14 and 15). Aitken *et. al.* (1989) explained that mammalian sperm cells have high content of spermatozoa's lipids which include polyunsaturated fatty acids, plasmalogenes and sphingomyelins which are responsible for the functional ability of sperm cells, are the main substrates for peroxidation (LPO) and sperm disorder (16).

The short term administration of ethylene glycol monoethyl ether (EGEE) have hemato-toxic and gonado-toxic effect on male reproduction could be due to the induction of oxidative stress in testes and spermatozoa (17). Besides, TawfeeK *et. al.* (2006) showed that *Nigella sativa* oil possess an anti-oxidative action to counteract the impairment in the epididymal sperm characters caused by H<sub>2</sub>O<sub>2</sub> (18), while Rahim *et. al.* (2013) demonstrate that aqueous extract of the *Cymbogon Citratus* reduced oxidative stress and protected male rats against H<sub>2</sub>O<sub>2</sub>-induced reproductive system injury (19).

Antioxidants are found in varying amounts in foods such as vegetables, fruits, grain cereals, eggs, meat, legumes, and nuts (20). Many medicinal plants contain substantial amounts of antioxidants such as vitamin C, vitamin E, flavonoids, and carotenoids (21). Antioxidants superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX) have been reported to prevent oxidative damage caused by free radicals and may prevent the occurrence of disease, cancer and aging. It can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals and also by acting as oxygen scavengers (22). Thus, they can be utilized to scavenge the excessive free radicals generated from human body (23) with no side effects and economic viability (22 and 24).

The present study focused on investigation the possible preventive role of flavonoid extracted from *Nigella sativa* seeds comparing to vitamin E

### Materials and Methods

*Nigella sativa* seeds were obtained from commercial sources (from Baghdad) and the vouchers specimen of the plant were deposited to be identified and authenticated at the National Herbarium of Iraq Botany Directorate in Abu-Ghraib, under scientific name *Nigella sativa* belongs to the family *Ranunculaceae*. The method of Harborne (1973) modified by Al-Kawary (2000) was employed for the extraction of flavonoid (25).

Forty male Albino Wister rats (200-250gm) were used in this study, their ages ranged between (2.5-3) months. Animals housed in plastic cages in conditioned room (22-25°C) in the animal house of department of physiology and pharmacology at the College of Veterinary Medicine - University of Baghdad. Rats were randomly divided into four groups (5 rat/group) and treated as follows for 120 days: Group T1: animals in this group were administered normal saline and served as control. While animals in group T2 were administered sodium nitrate 30 mg/kg B.W by gavage needle (26). Animals in group T3 were administered sodium nitrate 30 mg/kg B.W plus 50 mg of flavonoids extracted from *Nigella Sativa* by gavage needle and animals in group T4: were administered sodium nitrate 30 mg/kg B.W plus 40 mg/kg B.W Vitamin E by gavage needle.

At the end of experiment, animals were weighed by sensitive balance and were anesthetized by intramuscular injection of (Ketamine 90mg /Kg B.W and Xylazine 40mg/kg B.W). Right testes were obtained and weighed by sensitive balance after being cleaned from the accessory

connective and adipose tissues. Testicular weight to body weight ratio was calculated as in the following equation: Testicular weight-to-body weight ratio = wt. of testes (gm) /wt. of animal (gm) × 100. Histological sections of testes were prepared according to Luna (27) to measuring the thickness of cell lining seminiferous tubules and diameter of seminiferous tubules by calculated horizontal and vertical views of the 5 tubules which were selected randomly for each sample by using Ocular micrometer (28). The tail of the left epididymis was taken and embedded in one ml of normal saline at 37 0C in a watch glass ,and then the tail was cut into at least 200 sections by microsurgical scissor ,to perform the following microscopical examination on sperm parameter.

Assessment of alive and dead sperms was carried out by method as described by Chemineau et.al. (29). By adding one drop of sperms suspension on a slide, a drop of eosin nigrosine stain was added and mixed, two smears of each sample were made and 200 sperms were examined in each smear. (30).

All data were expressed as mean ± SE. Analysis of data was statistically performed by using (ANOVA) one – way analysis of variance and LSD to determine the difference between mean values P.value (0.05) was considered significantly different (31).

### Results and Discussion

The results of testes to body weight ratio in the control and three treated groups were described in table (1). It was showed significant differences (p<0.05) in the mean value testes to body weight ratio in experimental groups when compared between each other.

**Table (1): The preventive role of vitamin E and flavonoid extracted from *Nigella sativa* seeds against the effects of sodium nitrate on testes – body weight percentage in rats.**

Groups Parameters	Control (T1)	Sodium Nitrate (T2)	Sod. Nit + Flavonoids (T3)	Sod. Nit + Vit E (T4)
Testes / body %	0.927 ± 0.01 A	0.314 ± 0.011 D	0.675 ± 0.009 B	0.632 ± 0.016 C

Mean values are expressed as mean ±SE. N=5 rats/group.

Deferent letters denoted significant differences between groups (p<0.05).

T1 Control group.

T2 animals treated with Sodium Nitrate 30 mg/kg .

T3 animals treated with Sodium Nitrate 30 mg/kg plus 50mg of flavonoids extracted from *Nigella Sativa*.

T4 animals treated with Sodium Nitrate 30 mg/kg plus 40mg/kg. B.W Vit E

**Table (2): The preventive role of vitamin E and flavonoid extracted from *Nigella sativa* seeds against the effects of sodium nitrate on live and dead sperms percentage in rats.**

Groups Parameters	Control (T1)	Sodium Nitrate (T2)	Sod. Nit + Flavinoids (T3)	Sod. Nit + Vit E (T4)
Live sperms %	68 ± 0.64 B	26.66 ± 1.043 C	82.64 ± 2.45 A	79.96 ± 1.042 A
Dead sperms %	31.86 ± 0.64 B	73.34 ± 1.043 A	16.7 ± 2.47 D	20.04 ± 0.053 C

Mean values are expressed as mean ±SE. N=5 rats/group.

Deferent letters denoted significant differences between groups (p<0.05).

T1 Control group.

T2 animals treated with Sodium Nitrate 30 mg/kg.

T3 animals treated with Sodium Nitrate 30 mg/kg plus 50mg of flavonoids extracted from *Nigella Sativa*.

T4 animals treated with Sodium Nitrate 30 mg/kg plus 40mg/kg. B.W Vit E

**Table (3): The preventive role of vitamin E and flavonoid extracted from *Nigella sativa* seeds against the effects of sodium nitrate on Thickness of cells lining and diameter of seminiferous tubules in rats.**

Groups Parameters	Control (T1)	Sodium Nitrate (T2)	Sod. Nit + Flavonoids (T3)	Sod. Nit + Vit E (T4)
Thickness of cells lining S. T. (mm)	0.108 ± 0.005 A	0.067 ± 0.002 C	0.092 ± 0.004 A	0.085 ± 0.021 B
Diameter of S. T. (mm)	0.321 ± 0.009 A	0.241 ± 0.009 C	0.287 ± 0.003 B	0.280 ± 0.010 B

Mean values are expressed as mean ±SE. N=5 rats/group.

Deferent letters denoted significant differences between groups (p<0.05).

T1 Control group.

T2 animals treated with Sodium Nitrate 30 mg/kg.

T3 animals treated with Sodium Nitrate 30 mg/kg plus 50mg of flavonoids extracted from *Nigella Sativa*.

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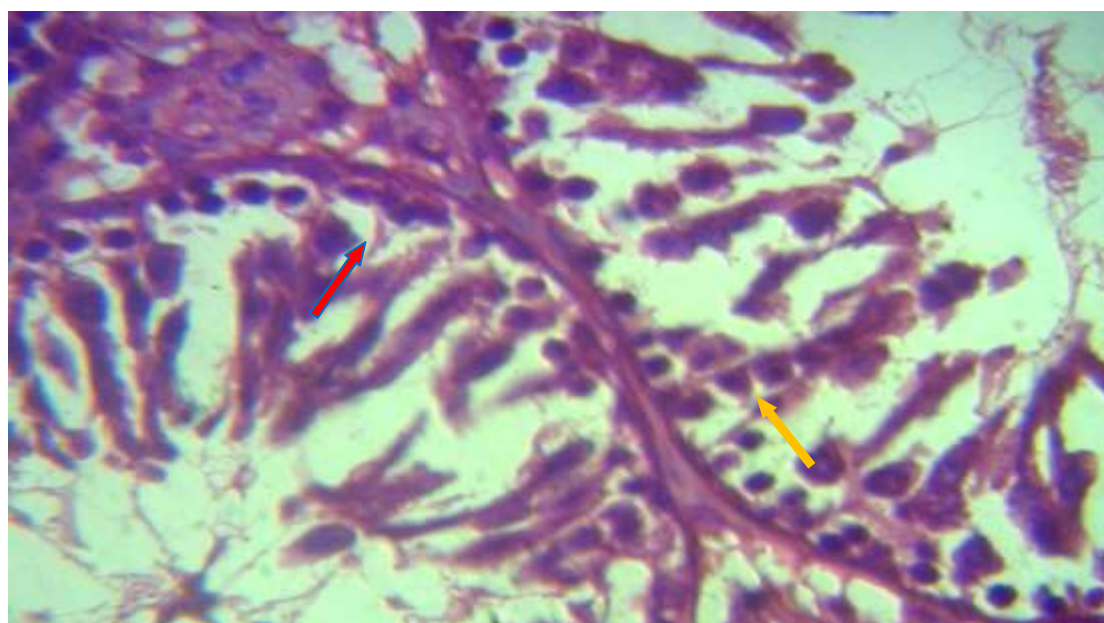


Figure 1: Testes of animals treated with sodium nitrate alone (T2) showed edema and fibrous connective tissue proliferation (→) and incomplete spermatogenesis (→) H & E stain 40X.

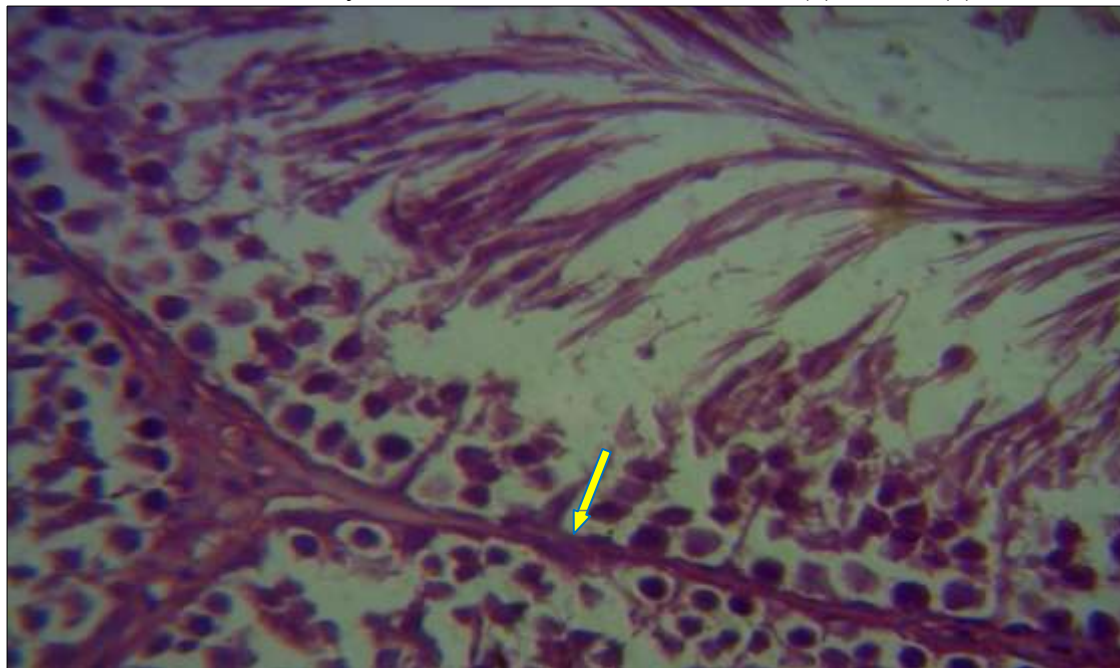



Figure 2: Testes of animals treated with sodium nitrate and flavonoids extracted from *Nigella sativa* seeds (T3) showed moderate edema and thickness of basement membrane of seminiferous tubules (  ) with normal spermatogenesis H & E stain 40X

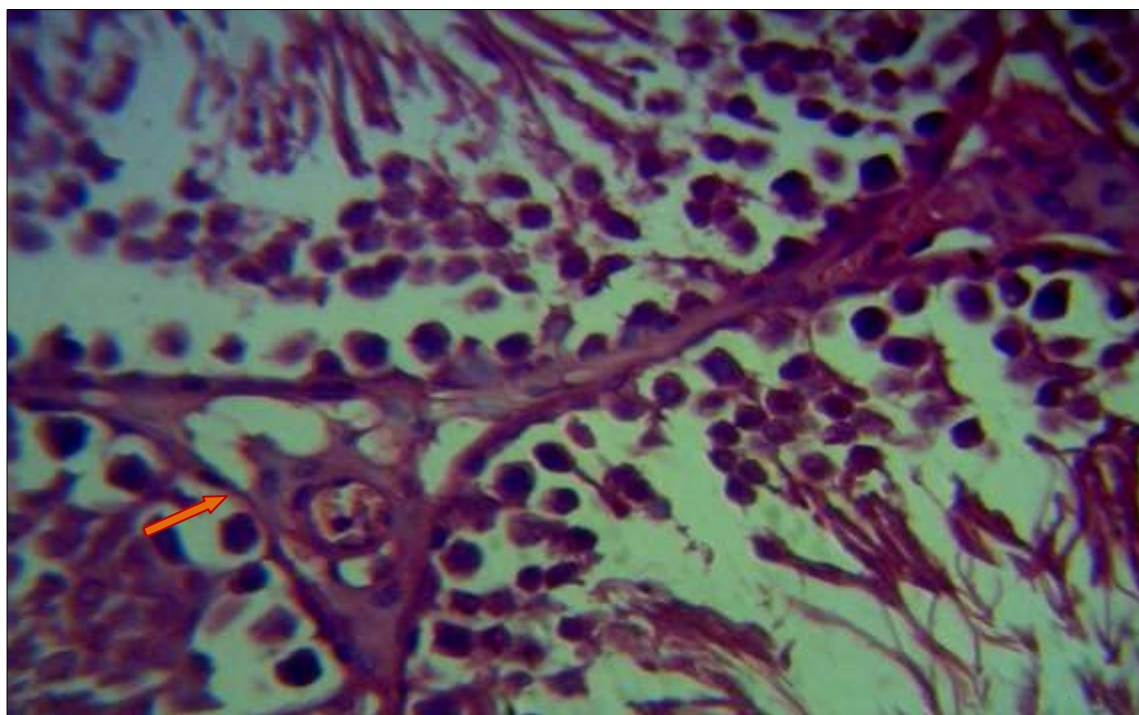
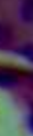


Figure 3: Testes of animals treated with sodium nitrate and vit E (T4) showed edematous interstitial tissue and congested blood vessels (  ) with moderate to normal spermatogenesis H & E stain 40X

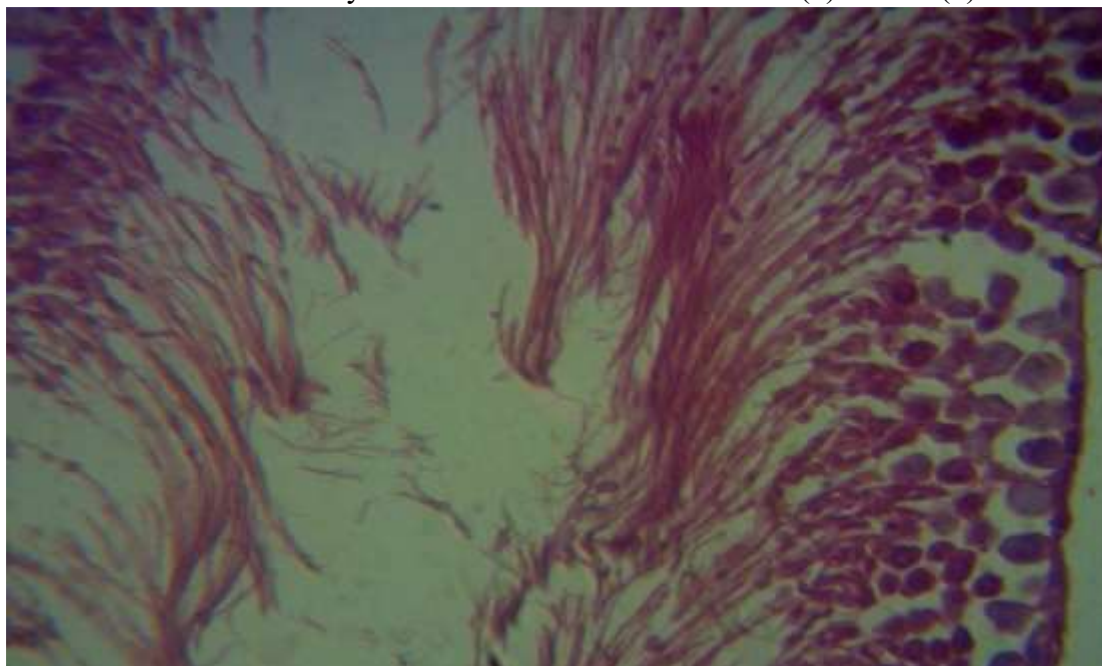


Figure 4: Testes of control animals (T1) showed normal structure of seminiferous tubules with normal spermatogenesis H & E stain 40X.

Treatment of adult male rats with sodium nitrate (group T2) orally for 120 days caused a significant decrease in mean values of testes to body weight ratio, alive sperms and thickness and diameter of seminiferous tubules indicating a case of testicular toxicity which may be attributed to testicular oxidative stress (32). Decrease in testes: body weight ratio may be due to the inhibiting effect of  $\text{NaNO}_2$  on biosynthesis of protein. Effect of  $\text{NaNO}_2$  on thyroid and adrenal gland lead to block in protein biosynthesis and decrease protein turnover (33) Besides, nitric oxide release from nitrite interaction, may cause inhibition of total protein synthesis (34).

Administration of sodium nitrate significantly enhanced  $\text{H}_2\text{O}_2$  production (35), and induce oxidative stress leading to generation of high amount of reactive oxygen species (ROS) (32), associated with lipid peroxidation which may be due to high level of polyunsaturated fatty acids in

rat testes that makes plasma membranes of testicular cells highly susceptible to oxygen radical attack (35). Oxidative stress may create a ground for sperm deterioration or persistent infertility (36). Peroxidation of polyunsaturated fatty acids are accompanied with a wide variety of pathological condition and infertility, so peroxidation of sperm lipids may disturb sperm function with complete inhibition of spermatogenesis (37). Furthermore, the expected lowering thyroid hormone concentration accompanied sodium nitrate treatment have also been reported to affect semen quality (38).

Our results demonstrate the beneficial effect of treatment with flavonoids extracted from *Nigella sativa* as manifested by increase in values of testes: body weight ratio, percentage of alive sperm and decrease in percentage of dead sperm, reflecting the antioxidant properties that counteract the oxidative stress induced by sodium nitrate (39).

This activity is attributed to their hydrogen-donating ability (40). Flavonoids are capable of modulating the activity of enzymes and affect the behaviour of many cell systems and exerting beneficial effects on the body (41). They terminate chain radical reaction by donating hydrogen atom to a peroxy radical, forming flavonoids radical, which, further reacts with free radicals thus terminating propagating chain leading to suppression of oxidative stress (42). On the other hand treatment of animals with sodium nitrate plus vitamin E orally for 120 days, caused improvement in testicular function.

Vitamin E is one of the natural antioxidants with low toxicity (43). In animals supplemental vitamin E affords protection against various drugs, metals and chemicals that can initiate free radical formation (44 and 45). Also it is a chain breaking anti-oxidant and singlet oxygen quencher and it is thought to be an immune modulator enhancing cell mediated as well as, humeral immunity (46). Vitamin E was observed to repair the genotoxicity and improved the hematological and biochemical changes (47). Its protective role against the effects of nitrate/nitrite exposure has been attributed to its ability to decrease the formation of peroxynitrite and ROS production (48 and 49) and increased GSH levels in RBCs, indicating a decrease in their oxidative status (50). Accordingly, its anti-oxidant effect prevent lipid peroxidation and oxidative stress induced by sodium nitrate, thus maintaining the integrity of testicular membrane and spermatozoa with maintaining testicular function and sperm characteristic.

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دراسة مقارنة بين دور الفلافونيدات المستخلصة من بذور الحبة السوداء وفيتامين هـ في التقليل من التأثير  
المستحدث بنترات الصوديوم على بعض وظائف الخصى في ذكور الجرذان البالغة  
عمار احمد عبد الواحد الحداد

فرع الفلسفة والكيمياء الحياتية والادوية كلية الطب البيطري- جامعة بغداد- العراق

### الخلاصة

صممت هذه الدراسة لمعرفة دور الفلافونيدات المستخلصة من بذور الحبة السوداء وفيتامين هـ في التقليل من التأثير المستحدث بنترات الصوديوم على بعض وظائف الخصى في ذكور الجرذان البالغة. تم استخدام عشرون جرذاً بالغاً قسمت عشوائياً إلى أربعة مجاميع متساوية وعولمت كالتالي: مجموعة السيطرة (T1) اعطيت المحلول الملحي، مجموعة المعاملة الثانية (T2) عولمت بنترات الصوديوم بتركيز 30 ملغم / كغم من وزن الجسم يومياً عن طريق الأنبوب المعدي، مجموعة المعاملة الثالثة (T3) اعطيت نترات الصوديوم بتركيز 30 ملغم / كغم من وزن الجسم يومياً بالإضافة إلى مستخلص الحبة السوداء بتركيز 50 ملغم يومياً عن طريق الأنبوب المعدي في حين مجموعة (T4) عولمت بنترات الصوديوم بتركيز 30 ملغم / كغم من وزن الجسم يومياً بالإضافة إلى فيتامين هـ بتركيز 40 ملغم / كغم من وزن الجسم. عند نهاية التجربة تم التضحية بالحيوانات واخذت الخصية اليمينية ووزنت وتم حساب نسبة وزن الخصية إلى وزن الجسم وتم تحضير الشرائح النسجية لغرض قياس سمك طبقة الخلايا المبطنة للنبيبات المنوية كذلك قطر النبيب المنوي أما نسبة الحيامن الميتة والحية فقد حسبت من اليربخ الايسر لحيوانات التجربة. اظهرت النتائج حصول انخفاض معنوي في الوزن النسبي لخصى حيوانات التجربة بالمقارنة مع السيطرة وكان هذا الانخفاض واضحاً جداً في المجموعة المعاملة بنترات الصوديوم (T2) فضلاً عن الانخفاض المعنوي في نسبة الحيامن الحية والذي يقابله الارتفاع المعنوي في نسبة الحيامن الميتة إضافة إلى الانخفاض المعنوي في أقطار النبيبات المنوية وسمك طبقة الخلايا المبطنة لها ولنفس مجموعة المعاملة مقارنة مع المجاميع الثلاث T1, T3, T4. اظهر الفحص النسجي وجود خبز وتشنج في الغشاء القاعدي فضلاً عن تجمع النسيج الرابط في المجموعة المعاملة بنترات الصوديوم (T2) مقارنة مع المجاميع الأخرى وكانت التغيرات النسجية متوسطة في كل من (T3) و (T4). يستنتج من ذلك أن للفلافونيدات المستخلصة من بذور الحبة السوداء وفيتامين هـ تأثير واقياً ومضاداً للاكسدة الحاصلة بسبب نترات الصوديوم في فعالية الخصى لذكور الجرذان.

الكلمات المفتاحية: نترات الصوديوم، بذور الحبة السوداء، فيتامين هـ ، فعالية الخصى