Pakistan J. Zool., vol. 51(6), pp 2225-2231, 2019. DOI: http://dx.doi.org/10.17582/journal.pjz/2019.51.6.2225.2231

Nigella sativa Oil Improves Physiological Parameters, Oocyte Quality after Ovarian Transplantation, and Reproductive Performance of Female Mice

Abd El-Nasser Ahmed Mohammed[1,2,*]

[1]*Department of Animal and Fish Production, College of Agriculture and Food Sciences, King Faisal University, Kingdom of Saudi Arabia*

[2]*Department of Animal production, Faculty of Agriculture, Assiut University 71526, Egypt*

ABSTRACT

The present study aimed to explore firstly the changes in physiological parameters and oocyte quality after ovarian transplantation upon dietary supplementation of 1.0 and 2.0% of *Nigella sativa* (*N. sativa*) oil to female mice and secondly to explore if dietary *N. sativa* is effective in alleviation of hypothermia and hyperglycemia due to general anesthesia. Seventy-five albino female mice (21.74 ± 0.19) were distributed into three groups; control (G1; N=25; not receive *N. sativa* oil) and two *N. sativa* oil groups supplemented with either 1.0% (G2; N=25) or 2.0% (G3; N=25) for 30 days. Changes of body temperature, heart rate, blood parameters (RBCs, WBCs, hematocrit, hemoglobin, glucose and total protein), oocyte quality after ovarian transplantation (cumlus enclosed, brilliant cresyl blue stain and diameter) and reproductive performances (litter size and weight) were determined and recorded. In addition, values of body temperature and blood glucose were determined after general anesthesia. The results indicated that *N. sativa* oil supplementation resulted in significant ($P < 0.05$) increase of RBCs, hematocrit, WBCs and plasma total protein values in addition to significant hypoglycemia compared to control. The quality of oocytes and reproductive performances were improved due to *N. sativa* oil supplementation. *N. sativa* oil rescued the depression in oocytes number and quality after ovarian transplantation. *N. sativa* oil could not improve hypothermia and hyperglycemia due to general anesthesia. In conclusion, supplementation of *N. sativa* oil could benefit animals' health and reproduction through the improvement of physiological parameters and oocyte quality.

Article Information
Received 22 May 2018
Revised 14 July 2018
Accepted 27 January 2019
Available online 23 August 2019

Key words
***Nigella sativa*, Oocytes quality, Ovarian transplantation, Glucose.**

INTRODUCTION

Nigella sativa supplementation to mammals has received increased attention over the past decades due to its potential effects on growth and development and reproduction (Mohammed and Al-Suwaiegh, 2016). *N. sativa* is an annual flowering plant that cultivates in Asia and the Middle East (Khare, 2004). It has been reported that *N. sativa* seeds contains active materials known as nogelleone, thymoquinome, and thymohdroquinone that were shown to possess antitoxic, antimicrobial and pharmacological activities via increasing the defense mechanisms against infectious diseases (Forouzanfar *et al*., 2014; Mohammed and Al-Suwaiegh, 2016).

There are several studies have been carried out on different species of animals to investigate the effects of *N. sativa* on growth performance. Khattab *et al*. (2011) found that supplementation of calves with *N. sativa* oil resulted in higher nutrient digestibility compared to calves fed free *N. sativa* oil diet. In addition, Abd El-Rahman *et al*. (2011) indicated that Demeshgi goats fed 20% *N. sativa* meal had significantly higher average daily gain compared to the control diet. The levels of blood profiles and plasma metabolites were investigated because they are an indicative of body's condition. The research on rats indicated a significant increase in RBC, WBC, PCV and Hb of Trypanosoma brucei infected rats when treated with black seed oil as compared to control ones (Justine and Oluwatosin, 2008). Supplementation of *N. sativa* seed or oil increased serum total protein, albumin and globulin in different species (Khattab *et al*., 2011; Zeweil *et al*., 2008; Zanouny *et al*., 2013). In addition, several studies have shown the antioxidants activities of *N. sativa* when supplemented to animals' rations and human diets (Badary *et al*., 2003; El-Far *et al*., 2014).

N. sativa effects on reproductive performances of females have been reported concerning ovarian follicle

* Corresponding author: aamohammed@kfu.edu.sa; elnasser@aun.edu.eg
0030-9923/2019/0006-2225 $ 9.00/0

development and pregnancy. The beneficial effects of *N. sativa* in gynecologic disorders have been reported in several studies. Parhizkar *et al*. (2016) investigated the effect of *N. sativa* on menopausal parameters of ovariectomized rats. The results indicated the probable beneficial role of *N. sativa* for the treatment of postmenopausal symptoms and its possibility to use as an alternative hormone replacement therapy in human for post menopause. El-Harairy *et al*. (2006) studied the effect of replacing 50% of concentrate feed mixture protein by *N. sativa* meal protein on reproductive performance of Rahmani ewe lambs. They concluded that feeding Rahmani ewe lambs with *N. sativa* meal protein had beneficial effect on oestrous activity in terms of length of estrous cycle and estrous duration as well as on conception rate.

In this study, the changes in blood parameters and oocyte quality after ovarian transplantation and litter size and weight upon dietary supplementation of 1.0 and 2.0% of *N. sativa* oil to female mice were investigated. In addition, because general anesthesia in several studies is imperative for experimental animals and resulted in hyperglycemia, hypothermia and bradycardia, therefore, the effects of the two levels of dietary *N. sativa* oil on values of body temperature and glucose after general anesthesia were investigated.

MATERIALS AND METHODS

Pure *N. sativa* (100% pure cold-pressed *N. sativa* oil) was used in this study. The remaining organic and inorganic compounds used in this study were purchased from Caisson Lab (USA), unless stated otherwise. All media were prepared fresh and sterilized through a 0.22-µm filter (Acrodisc; Pall Gelman Lab, Ann Arbor, MI).

Animal feeding and management

The experiment was carried out following the procedures approved by the Ethics Committee on Animal Experimentation of College of Agriculture and Food Sciences, King Faisal University.

The study was conducted from April to May. Sixty virgin female albino mice of 6-8 week old (21.74±0.19 g body weight) were kept in an environmentally controlled room set to maintain a temperature of 25.0±3°C and a relative humidity of 50.0±10.0% on a 12 h light/dark cycle (lights on at 7:00 AM). Mice were fed commercial pellets of basal control diet (Arasco, KSA) composed of 21.0% protein, 3.3% fiber, 2.9% fat, 1% mixture of vitamins and minerals, and 2800 kcal/kg energy. Animals had free access to water and food. The animals were randomly assigned to three groups. The control group (n=25; 21.78±0.35 g) was given basal control diet. The second (n=25; 21.46±0.28) and third groups (n=25; 21.98±0.34) were given basal control diet supplemented with 1.0 and 2.0% per kg feed.

Effect of N. sativa *oil body temperature, partial pressure of oxygen and heart rate*

Body temperatures were recorded using Digital LCD IR Infrared Thermometer Body Surface Temperature (Cofoe Portable Digital Termomete Infrared Thermometer Gun Non-contact IR LCD). The pulse oximeter and heart rate monitor was used (CMS60D-VET Handheld Veterinary Pulse Oximeter) to measure partial pressure of oxygen (PO2) and heart rate (Mohammed *et al*., 2018).

Blood sample collections and analysis

Blood samples were obtained in heparinized tubes from orbital sinus of animals in each group according to Hoff (2000). Hemocytometer was used to determine red (million/mm^3) and white (thousand/mm^3) blood cells in addition to micro-hematocrit centrifuge and micro-capillary reader were used to determine hematocrit (%). Blood glucose recorded using blood glucose meter (iCare advanced Medical). Thereafter, the remaining blood sample was centrifuged at 5000 rpm for 15 min to obtain blood plasma for determination of total protein (g/dl) using clinical refractometer (SCHUCO, Japan).

Ovarian tissue transplantation

Ovarian tissue transplantation was carried out as previously indicated (Dorsch *et al*., 2004; Mohammed *et al*., 2012; Mohammed, 2018). Briefly, after anesthesia (Mohammed *et al*., 2012, 2018) and hair shaving and disinfection at the right dorsal of the skin, an incision was made to give access to the right ovary. Ovarian tissue transplantation was done through small incision in the fat surrounding the ovarian bursa to uncover the ovarian tissue. The donor's right ovarian tissue was removed and transplanted into recipient female. Then, the slit was closed in the fat surrounding the ovarian bursa, returned to the body cavity, and the incision was closed.

Collections of germinal vesicle oocytes and grading

After four weeks of ovarian transplantation, ten females of each group were injected with 7.5 IU of pregnant serum gonadotropin (PMSG; Folligon, Intervet). The injected females were killed through cervical dislocation 44-48 h after PMSG injection. Ovaries were removed and oocytes of germinal vesicle (GV) stage were released by puncturing of ovarian follicles with 30-G sterile needles under a stereomicroscope. GV-stage oocytes released into HEPES tissue cell culture medium (TCM) 199 supplemented with 5% fetal bovine serum (FBS). Oocytes were collected immediately using glass pipette its tip

diameter larger than the diameter of cumulus-enclosed oocytes and categorized into two class; denuded GV and cumulus-enclosed GV oocytes.

Brilliant cresyl blue staining and measuring oocyte diameter

The collected cumulus-enclosed GV oocytes were washed three times and incubated for 90 minutes at 37˚C in humidified air atmosphere in KSOM medium supplemented with 4% BSA and 26 µM BCB. The oocytes were observed under microscope after the incubation time and classified according to BCB staining into dark blue cytoplasm (BCB+) and colorless cytoplasm (BCB–) (Wu *et al*., 2007). For measuring oocyte diameter, the collected cumulus-enclosed GV oocytes were stripped from cumulus cells using glass pipette its tip diameter smaller than the diameter of cumulus-enclosed oocytes. The diameters of all collected oocytes (denuded GV oocytes and the resulting cumulus-free GV oocytes) were recorded to the nearest micron using 0.01 mm eyepiece for compound biological microscope eyepiece (Beijing).

Reproductive performance

Ten females of each group (G2 and G3 *N. sativa* treated and G1 control groups) were used for evaluating reproductive performance. Females with vaginal plug after insemination were considered pregnant and checked for parturition 4 times/day since day 18 of vaginal plug. The numbers and weight of pops per female were counted and weighted for each group.

Effects of N. sativa *oil on body temperature and blood glucose after general anesthesia*

Values of body temperature and blood glucose after general anesthesia (13.3 mg/kg BW diazepam and 26.6 mg/kg BW xylazine) at 0, 20 min, 40 min, 1 h, 2 h, 3 h and 4 h of G1 control and G2 and G3 *N. sativa* oil groups were recorded as previously indicated in experiment 1.

Statistical analysis

Statistical analysis was done according to general linear model (GLM) of SAS Program (2008). Differences between control and *N. sativa* oil treated groups (1.0 vs. 2.0%) were evaluated in physiological and reproductive characters by one-way ANOVA. Duncan Multiple Range Test was used to test the effect of treatments (Steel and Torrie, 1980). The data were presented as mean ± SEM. Level of significance was set at P<0.05. Statistical model as follow:

$$Y_{ij} = \mu + T_i + E_{ij}$$

Where, Y_{ij} is the experimental observation ij, µ is the overall mean, T_i is the effect due to treatment i and E_{ij} is the experimental error.

Table I.- Effects of *N. sativa* oil on physiological parameters and litter size and weight in mice.

Items	G1	G2	G3
Temperature (°C)	37.35 ± 0.05a	37.37 ± 0.03a	37.41 ± 0.04a
Partial pressure of oxygen (mmHg)	87.75 ± 2.01a	88.75 ± 2.72a	90.36 ± 2.26a
Pulse rate (min)	139.91 ± 5.84a	137.41 ± 5.43a	127.75 ± 3.10a
Red blood cells (10^6)	7.10 ± 0.08b	8.09 ± 0.20ab	9.54 ± 0.42a
Packed cell volume (%)	36.08 ± 0.24b	38.33 ± 0.83ab	42.58 ± 0.74a
White blood cells (10^3)	3.10 ± 0.10a	03.25 ± 0.05ab	03.75 ± 0.07a
Glucose (mg/dl)	150.50 ± 2.42a	135.00 ± 1.54b	133.67 ± 1.35b
Total protein (g/dl)	5.93 ± 0.09b	6.53 ± 0.11ab	6.80 ± 0.03a
Litter size (n)	8.30 ± 0.40b	9.70 ± 0.60a	9.80 ± 0.49a
Litter weight (g)	10.02 ± 0.51b	11.62 ± 0.76ab	11.91 ± 0.38a

a and b, values with different superscripts between groups of the same row differed significantly at P < 0.05. G1, control; G2, animals treated with 1.0% *N. sativa* oil/kg diet; G3, animals treated with 2.0% /kg diet.

RESULTS AND DISCUSSION

In the present study, the effects of two levels of dietary *N. sativa* oil supplementation (1.0% vs. 2.0%) to female mice were explored on physiological and reproductive performance. Because the information from our previous studies indicated hyperglycemia, hypothermia and bradycardia in mice and rats due to general anesthesia, therefore, the effects of the two levels of dietary *N. sativa* oil supplementation on values of body temperature and glucose were investigated after general anesthesia using diazepam and xylazine drugs. The result indicated that *N. sativa* oil supplementation resulted in decrease (P > 0.05) in pulse rate (P > 0.05) and glucose (P < 0.05) versus significant increase (P < 0.05) in RBC (millions/µl), packed cell volume (%), WBCs (thousands/µl) and total protein (g/dl) (Table I). Furthermore, the oocytes' quality and reproductive performances were improved due to *N. sativa* oil supplementation (Table II). Upon general anesthesia, the results indicated that *N. sativa* oil supplementation did not improve the transient negative effects of anesthesia concerning hypothermia and hyperglycemia (Tables III, IV).

Table II.- Effect of *N. sativa* oil on oocyte quality after ovarian transplantation in mice.

Items	G1	G2	G3
Number of collected oocytes/two ovaries	10.00 ± 0.73a	10.70 ± 0.66a	11.20 ± 0.29a
Denuded oocytes/two ovaries	4.10 ± 0.43a	4.00 ± 0.25a	3.70 ± 0.15a
Cumulus enclosed oocytes/two ovaries	5.90 ± 0.45b	6.70 ± 0.39ab	7.50 ± 0.26a
+ Brilliant cresyl blue	2.90 ± 0.27b	3.20 ± 0.24ab	3.60 ± 0.16a
- Brilliant cresyl blue	3.00 ± 0.25b	3.50 ± 0.22ab	3.90 ± 0.17a
Diameter (60 µm)	3.00 ± 0.25a	3.00 ± 0.25a	3.20 ± 0.13a
Diameter (70 µm)	3.20 ± 0.29a	3.70 ± 0.15a	3.70 ± 0.15a
Diameter (80 µm)	3.80 ± 0.24a	4.00 ± 0.25a	4.30 ± 0.15a

For statistical details and abbreviations, see Table I.

Table III.- Effect of *N. sativa* oil on body temperature (°C) in general anesthetized mice.

C	G1	G2	G3
0 min	37.34 ± 0.18a	37.56 ± 0.11a	37.44 ± 0.12a
20 min	36.86 ± 0.15a	36.84 ± 0.13a	36.62 ± 0.20a
40 min	32.06 ± 0.29a	32.48 ± 1.34a	32.68 ± 0.21a
1 h	31.96 ± 0.23a	32.08 ± 1.16a	32.44 ± 0.28a
2 h	31.80 ± 0.27a	31.92 ± 0.34a	32.20 ± 0.31a
3 h	32.06 ± 0.20a	32.12 ± 0.30a	32.66 ± 0.31a
4 h	31.26 ± 0.16a	32.22 ± 0.31a	32.86 ± 0.20a

For statistical details and abbreviations, see Table I.

Table IV.- Effect of *N. sativa* oil on glucose level (mg/dl) in general anesthetized mice.

C	G1	G2	G3
0 min	154.4 ± 8.58b	135.8 ± 3.78ab	130.6 ± 5.76a
20 min	238.0 ± 13.93a	247.8 ± 8.97a	245.2 ± 12.76a
40 min	246.4 ± 6.78a	249.0 ± 15.13a	255.4 ± 15.52a
1 h	264.2 ± 9.24a	270.8 ± 15.73a	276.2 ± 13.04a
2 h	262.4 ± 13.7a	283.8 ± 13.04a	269.8 ± 12.60a
3 h	247.8 ± 9.95a	249.8 ± 13.62a	259.6 ± 6.31a
4 h	229.6 ± 20.1a	225.6 ± 17.91a	238.4 ± 5.05a

For statistical details and abbreviations, see Table I.

There are a wide range of studies, which proved the safety of *N. sativa* upon administration in animals and humans (Babazadeh *et al*., 2012; Dollah *et al*., 2013). In this study, the two levels (1.0 and 2.0%) of *N. sativa* oil improved body's condition through blood profiles [RBC (millions/µl), packed cell volume (%), WBCs (thousands/µl)] as in earlier study in addition to plasma total protein (g/dl) (Justine and Oluwatosin, 2008; Zeweil *et al*., 2008; Zanouny *et al*., 2013). It is recorded in several studies that *N. sativa* significantly increased feed intake and feed conversion in addition to the significant increase of body growth (Mohamed, 2007; Abd El-Rahman *et al*., 2011; Mohammed and Farghally, 2018). *N. sativa seeds* stimulate appetite and increase peristaltic action of the stomach and bowels (Inamul, 2004; DerMarderosian *et al*., 2005). Therefore, the improvement of blood profile in *N. sativa* groups might be attributed to the significant increase of feed intake, feed conversion and body weight. In addition, increased activity of hepatic functions is suggested when *N. sativa* seeds were fed (Tousson *et al*., 2011), which resulted in higher concentration of total protein as in this study.

The two levels (1.0 and 2.0%) of *N. sativa* oil in this study decreased the values of pulse rate ($P > 0.05$) and glucose ($P < 0.05$). It is indicated in earlier study that *N. sativa* treatment decreased the elevated heart rate and glucose concentration of Cd-treated rats (Demir *et al*., 2006). Other studies in humans suggested that *N. sativa* seed might be useful in the treatment of hypertension (Dehkordi *et al*., 2008) and diabetes (Bamosa *et al*., 2010).

The potential roles of *N. sativa* oil on female reproduction in this study were evaluated through oocyte quality after ovarian transplantation (cumulus enclosed, brilliant cresyl staining and oocyte diameter) and offspring number and weight, which were significantly improved (Table II). Follicle loss after ovarian tissue transplantation has been confirmed (Gavish *et al*., 2017). Several trials have been carried out to improve the efficiency of ovarian transplantation including the use of antioxidants such as melatonin and vitamin E (Friedman *et al*., 2012). Because *N. sativa* has immunomodulatory (Haq *et al*., 1999; Islam *et al*., 2004) and antioxidant activity (Badary *et al*., 2003; El-Far *et al*., 2014), it was used in this study to increase efficiency of ovarian transplantation. The result indicated the significant increase of total oocytes collected of transplanted ovaries in addition to the significant increase of oocyte's quality concerning cumulus enclosed, brilliant cresyl stain and oocytes' diameter. The improvement of oocyte quality in *N. sativa* oil groups compared to control one might be related to high levels of β-carotene and

elevation of plasma metabolites and hormones levels. Short-term beta-carotene supplementation in goat affects positively on serum insulin concentrations and ovarian activity (Meza-Herrera *et al*., 2013). Several studies suggest a direct role for insulin action on female reproduction. In a recent study, Ahmad *et al*. (2013) found estrogen-like activity of *N. sativa* extract in immature female rats, which was assumed to stimulate follicular development and corpora lutea formation. The females treated with *N. sativa* extract exhibited increase in the concentration of serum total protein and progesterone hormone.

The effects of *N. sativa* on reproductive performances of females have been reported concerning pregnancy and offspring (Mohammed and Farghally, 2018) and gynecologic disorders. Parhizkar *et al*. (2016) investigated the effect of *N. sativa* on menopausal parameters of ovariectomized rats. The finding indicated the possible beneficial role of *N. sativa* in the treatment of postmenopausal symptoms and possibility of using *N. sativa* as an alternative to hormone replacement therapy for post menopause in humans. The improvement of number of litter size and weight might be attributed also to the beneficial effects of *N. sativa* seed on body health and ovarian function as previously indicated.

In the second part of this study, *N. sativa* oil groups versus control one were tested after general anesthesia (xylazine and diazepam) for levels of body temperature and glucose (Tables III, IV). The results indicated that *N. sativa* oil supplementation did not rescue the transient negative side effects of general anesthesia. On the contrary, comparable hypothermia was found in G2 and G3 *N. sativa* oil groups compared to G1 control at all tested times after general anesthesia. Although *N. sativa* have been shown to reduce elevated blood pressure and decrease blood glucose level (Demir *et al*., 2006), it did not rescue the negative effects of anesthesia in such parameters. To decrease hypertension and hyperglycemia upon general anesthesia, higher levels of *N. sativa* might be required and needed to administer.

CONCLUSION

Supplementation of *N. sativa* oil improved body health and reproductive performances of female mice through the decrease in values of pulse rate and glucose and the increase in values of blood cells, packed cell volume, total protein, oocyte quality after ovarian transplantation and litter size and weight. *N. sativa* oil supplementation did not improve the transient negative effects of general anesthesia in case of hypothermia and hyperglycemia. It could be concluded that 1.0 and 2.0% dietary supplementation of *N. sativa* oil could benefit animals' health and reproduction through the improvement of physiological parameters and oocyte quality after ovarian transplantation. Further studies are still required upon *N. sativa* oil supplementation to investigate *in vitro* the developmental competence of oocytes and the resulting embryos after ovarian transplantation.

ACKNOWLEDGMENTS

This work was supported by the Deanship of Scientific Research of King Faisal University, Saudi Arabia (Grant No. 160072).

Statement of conflict of interest

No conflict of interest for author to declare.

REFERENCES

Abd El-Rahman, H.H., Abedo, A.A., Salman, F.M., Mohamed, M.I. and Shoukry, M.M., 2011. Partial substitution of cumin seed meal by Jatropha meal as a potential protein source for feed. *Afr. J. Biotechnol.,* **10**: 15456-15461. https://doi.org/10.5897/AJB11.1595

Ahmad, N., Rahman, Z.U., Akhtar, N., Ali, S., Ahmad, M. and Ahmad, I., 2013. Effects of *Medicago sativa* on some serum biochemical metabolites in rats. *Int. J. Agric. Biol.,* **15**: 297-300.

Babazadeh, B,. Sadeghnia, H.R., Safarpour Kapurchal, E., Parsaee, H., Nasri, S. and Tayarani-Najaran, Z., 2012. Protective effect of Nigella sativa and thymoquinone on serum/glucose deprivation induced DNA damage in PC12 cells. *Avicenna J. Phytomed.*, **2**: 125-132.

Badary, O.A., Taha, R.A., Gamal-el-Din, A.M. and Abdel-Wahab, M.H., 2003. Thymoquinone is a potent superoxide anion scavenger. *Drug Chem. Toxicol.*, **26**: 87-98. https://doi.org/10.1081/DCT-120020404

Bamosa, A.O., Kaatabi, H., Lebdaa, F.M., Elq, A.M. and Al-Sultanb, A., 2010. Effect of *Nigella sativa* seeds on the glycemic control of patients with type 2 diabetes mellitus. *Ind. J. Physiol. Pharmacol*., **54**: 344-354.

Dehkordi, F.R. and Kamkhah, A.F., 2008. Antihypertensive effect of *Nigella sativa* seed extract in patients with mild hypertension. *Fundam. Clin. Pharmacol.,* **22**: 447-452. https://doi.org/10.1111/j.1472-8206.2008.00607.x

Demir, H., Kanter, M., Coskun, O., Uz, Y.H., Koc, A. and Yildiz, A., 2006. Effect of black cumin (*Nigella sativa*) on heart rate, some hematological values, and pancreatic beta-cell damage in cadmium-

treated rats. *Biol. Trace. Elem. Res.,* **110**: 151-162. https://doi.org/10.1385/BTER:110:2:151

DerMarderosian, A., Liberti, L., Beutler, J.A., Grauds, C., Tatro, D.S., Cirigliano, M. and DeSilva, Jr. D., 2005. *Alfalfa. Review of natural products: Facts and comparisons*. Wolters Kluwer Health, Alphen aan den Rijn, the Netherlands.

Dollah, M.A., Parhizkar, S., Latiff, L.A. and Bin Hassan, M.H., 2013. Toxicity effect of *Nigella sativa* on the liver function of rats. *Adv. Pharm. Bull.,* **3**: 97-102.

Dorsch, M., Wedekind, D., Kamino, K. and Hedrich, H.J., 2004. Orthotopic transplantation of rat ovaries as a tool for strain rescue. *Lab. Anim.,* **38**: 307-312. https://doi.org/10.1258/002367704323133691

El-Far, A.H., Bazh, E.K. and Moharam, M.S., 2014. Antioxidant and antinematodal effects of *Nigella sativa* and *Zingiber officinale* supplementations in ewes. *Int. J. Pharm. Sci. Rev. Res.,* **26**: 222-227.

El-Harairy, M.A,, Gabr, M.G., El-Ayouty, S.A., Gabr, A.A. and ElGohary, E.S., 2006. Effect of feeding level and replacement of *Nigella sativa* meal in diet of Rahmani ewe lambs on: 2. Onset of puberty, oestrous activity and conception rate. *Egypt J. Sheep Goat Desert. Anim. Sci.*, **1**: 171-186.

Forouzanfar, F., Bazzaz, B.S.F. and Hosseinzadeh, H., 2014. Black cumin (*Nigella sativa*) and its constituent (Thymoquinone): A review on antimicrobial effects. *Iran. J. Basic med. Sci.,* **17**: 929-938.

Friedman, O., Orvieto, R., Fisch, B., Felz, C., Freud, E., BenHaroush, A. and Abir, R., 2012. Possible improvements in human ovarian grafting by various host and graft treatments. *Hum. Reprod.,* **27**: 474-482. https://doi.org/10.1093/humrep/der385

Gavish, Z., Spector, I., Peer, G., Schlatt, S., Wistuba, J., Roness, H. and Meirow, D., 2017. Follicle activation is a significant and immediate cause of follicle loss after ovarian tissue transplantation. *J. Assist. Reprod. Genet.,* **35**: 1-9.

Haq, A., Lobo, P.I., Al-Tufail, M., Rama, N.R. and Al-Sedairy, S.T., 1999. Immunomodulatory effect of *Nigella sativa* proteins fractionated by ion exchange chromatography. *Int. J. Immunopharmacol.,* **21**: 283-295. https://doi.org/10.1016/S0192-0561(99)00010-7

Hoff, J., 2000. Methods of blood collection in the mouse. *Lab. Anim.*, **29**: 47-53.

Inamul, H., 2004. Safety of medicinal plants. *Pak. J. med. Res.*, **43**: 1-8.

Islam, S.N., Begum, P., Ahsan, T., Huque, S. and Ahsan, M., 2004. Immunosuppressive and cytotoxic properties of *Nigella sativa*. *Phytother. Res.,* **18**: 395-398. https://doi.org/10.1002/ptr.1449

Justine, T.E. and Oluwatosin, K.Y., 2008. Some biochemical and haematological effects of black seed (*Nigella sativa*) oil on *Trypanosoma brucei* infected rats. *Afr. J. Biotechol.,* **7**: 153-157.

Khare, C.P., 2004. *Encyclopedia of Indian medicinal plants*. Springes-Verlag, Berlin, Heidelberg, New York.

Khattab, H.M., El-Basiony, A.Z., Hamdy, S.M. and Marwan, A.A., 2011. Immune response and productive performance of dairy buffaloes and their offspring supplemented with black seed oil. *Iran. J. appl. Anim. Sci.*, **1**: 227-234.

Meza-Herrera, C.A., Vargas-Beltran, F., Tena-Sempere, M., González-Bulnes, A., Macias-Cruz, U. and Veliz-Deras, F.G., 2013. Short-term beta-carotene-supplementation positively affects ovarian activity and serum insulin concentrations in a goat model. *J. Endocrinol. Invest.,* **36**: 185-189.

Mohamed, I.M., 2007. Evaluation of growth performance for growing Maghraby camel fed on un-conventional feed. *Int. J. Agric. Biol.,* **9**: 18-21.

Mohammed, A.A. and Al-Suwaiegh, S.B., 2016. Effects of *Nigella sativa* on mammals' health and production. *Adv. Anim. Vet. Sci.*, **4**: 630-636.

Mohammed, A.A. and Farghally, M.M., 2018. Effects of *Nigella sativa* seeds on oocyte maturation and embryo development in mice. *Egypt. J. Anim. Prod.*, **55**: 195-201.

Mohammed, A.A., Abdelnabi, M.A. and Modlinski, J.A., 2012. Evaluation of anesthesia and reproductive performance upon diazepam and xylazine injection in rats. *Anim. Sci. Pap. Rep.,* **30**: 285-292.

Mohammed, A.A., Al-Hozab, A. and Al-Shahin, T., 2018. Effects of diazepam and xylazine on changes of blood oxygen and glucose levels in mice. *Adv. Anim. Vet. Sci.,* **6**: 121-127.

Mohammed, A.A., 2018. Ovarian tissue transplantation in mice and rats: Comparison of ovaries age. *Pakistan J. Zool.,* **50**: 481-486. https://doi.org/10.17582/journal.pjz/2018.50.2.481.486

Parhizkar, S., Latiff, L.A. and Parsa, A., 2016. Effect of *Nigella sativa* on reproductive system in experimental menopause rat model. *Avicenna J. Phytomed.*, **6**: 95-103.

SAS, 2008. *Statistical analysis system, Guide release, 2008*. SAS Institute Inc., Cary, NC, USA.

Steel, R.G. and Torrie, J.H., 1980. *Principles and procedures of statistics, a biometrical approach*, 2nd ed. Mc Grow-Hill Book Co., New York.

Tousson, E., El-Moghazy, M. and El-Atrsh, E., 2011. The possible effect of diets containing

Nigella sativa and *Thymus vulgaris* on blood parameters and some organs structure in rabbit. *Toxicol. Ind. Hlth.,* **27**: 107-116. https://doi.org/10.1177/0748233710381891

Wu, Y.G., Liu, Y., Zhou, P., Lan, G.C., Han, D., Miao, D.Q. and Tan, J.H., 2007. Selection of oocytes for *in vitro* maturation by brilliant cresyl blue staining: a study using the mouse model. *Cell Res.,* **17**: 722-731. https://doi.org/10.1038/cr.2007.66

Zanouny, A.I., Abd-El moty, A.K.I., El-Barody, M.A.A., Sallam, M.T. and Abd El-Hakeam, A.A., 2013. Effect of supplementation with *Nigella sativa* seeds on some blood metabolites and reproductive performance of Ossimi male lambs. *Egypt. J. Sheep Goats*, **8**: 47-56. https://doi.org/10.12816/0005023

Zeweil, H.S., Ahmed, M.H., El-Adawy, M.M. and Zaki, B., 2008. *Evaluation of substituting nigella seed meal as a source of protein for soybean meal in diets of New Zealand white rabbits*. In: 9th World Rabbit Congress, 10-13 June, Verona, Italy, pp. 863-868.