# Kidney tissue selenium levels of *Toxocara Canis* infected mice given *Nigella Sativa*


## ABSTRACT

Nigella sativa (NS) has a protective effect on cellular damage caused by oxidative stress. Selenium has an antioxidative effect. *Toxocara canis* is one of the nematodes causing visceral larva migrans. Men infected with this parasite ingesting an embryonic egg. It is more common in children between 1-4 years than adults. From the ingested embryonic egg, the larvae released in the small intestine and they migrate to so many organs such as liver, spleen, kidney, lung, and brain, retina of the eye, pancreas and causing lesions. In particular, it is known to cause intense damage to kidney tissue. In this study mice with *Toxocara canis* infection were administered Nigella sativa in prophylactic and treatment doses (100 and 200 mg/kg body weight) and selenium levels were determined in their kidney tissues. In the healthy control group, kidney selenium levels were 980,46±236,68 ng/g and in mice infected with *Toxocara canis* 1240,15±315,77 ng/g. Kidney tissue Se levels of mice given NS in two different doses for treatment (Treated N100, Treated N200) and prophylaxis (Prophylactic N100 and ProphylacticN200) respectively are 1297,95±354,37; 1361,29±410,46 ng/g; 1148,55±240,28 ng/g and 1465,81±450,25 ng/g. Kidney tissue selenium levels were high in both treatment and prophylaxis dose NS given mice. In conclusion, Nigella sativa can cause increases in kidney tissue selenium levels depends on given doses.


**Keywords:** Nigella sativa, selenium, oxidative stress, kidney, *Toxocara canis*

## INTRODUCTION

Despite the advancement and use of modern medicine, the demand for complementary and alternative medical practices is increasing day by day (Ekor, 2014). Nigella sativa (NS) is an annual herbaceous plant that belongs to the Ranunculaceae family, which grows naturally in the Mediterranean, Eastern European, Southern and South-western Asian countries (Cheikh-Rouhou et al., 2007). The plant, also known as nigella or fennel flower, is characterized by its black seeds and is known to be traditionally used against a variety of diseases (Jakaria et al., 2018; Meral et al., 2001). Nigella sativa is known to act as a major antioxidant and anti-inflammatory agent. It has also been reported to have a wide range of effects such as anti-diabetic, anti-carcinogenic, immunomodulator and bronchodilator effects (Tavakkoli et al., 2017). Proper functioning of the immune system depends on the presence of trace elements that are essential to be taken with nutrients like antioxidants and cofactors (Lukác and Massányi, 2007). Selenium (Se) is a trace element that affects both the natural and acquired immune responses. Selenium acts as an antioxidant system that can prevent tissue and cell damage and can affect enzyme activity in different organs such as the kidneys (Huang et al., 2012).

***How to cite this article***
**Sayıner, S., Altaş, MG., Camkerten, G., Şehirli, AÖ., Paksoy, N., Balkan., BM, Soran GŞ (2021).** Kidney tissue selenium levels of *Toxocara Canis* infected mice given *Nigella Sativa*. *Journal of Advances in VetBio Science and Techniques*, *6*(3), 278-283. https://doi.org/10.31797/vetbio.977962

**Research Article**

**Serkan Sayıner**[1a]
**Mehtap Gül Altaş**[2b]
**Güzin Camkerten**[3c]
**Ahmet Özer Şehirli**[4d]
**Nilgün Paksoy**[5e]
**Burcu Menekşe Balkan**[6f]
**Gülten Senocak Soran**[7g]

[1]Department of Biochemistry, Faculty of Veterinary Medicine, Near East University, Nicosia, Cyprus
[2]Department of Parasitology, Faculty of Veterinary Medicine, Harran University, Sanliurfa, Turkey.
[3]Department of Biochemistry, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Turkey.
[4]Department of Pharmacology, Faculty of Dentistry, Near East University, Nicosia, Cyprus
[5]Department of Biochemistry, Faculty of Veterinary Medicine, Harran University, Sanliurfa, Turkey.
[6]Department of Biochemistry, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur, Turkey
[7]Department of Biology, Faculty of Art and Science, Harran University, Sanliurfa, Turkey

ORCİD-
[a]0000-0002-3297-5369
[b]0000-0002-0120-9266
[c]0000-0003-2732-9490
[d]0000-0002-5175-9290
[e]0000-0001-6231-5524
[f]0000-0002-0206-6455
[g]0000-0001-8273-1904

***Correspondence***
**Serkan SAYINER**
cemayvazoglu@hotmail.com

***Article info***
*Submission*: 10-09-2021
*Accepted*: 22-12-2021

***e-ISSN:*** 2548-1150
***doi prefix:*** 10.31797/vetbio
• http://dergipark.org.tr/vetbio

Selenium can be stored in many organs and is found in its highest concentrations in the kidney. Thus, the kidney is the most sensitive indicator of the animal's selenium status, and selenium concentrations in this organ can provide valuable diagnostic information (Combs Jr, 2015; Shamberger, 1983).

*Toxocara canis* is a worldwide-distributed nematode that belongs to the Ascaridae family, which can cause various forms of the disease in its hosts. Soils in both rural and urban areas are known to be contaminated by eggs spread by dogs or foxes (Kleine et al., 2017). The parasite completes its life cycle in dogs. Human beings become infected by accidental exposure of parasites as hosts. After the parasite egg is ingested, the larvae penetrate the intestinal wall and then penetrate various tissues, including the kidney. The dominant clinical manifestation associated with *T.canis* is the visceral larva migrans (VLM) syndrome, a multi-systemic disease. Oxidative damage is known to play a role in the pathophysiology of *T.canis* infection, which is a common parasitic infection (Yarsan et al., 2003).

The question of to what extent the changes in selenium intake with nutrients and organ intake can affect the parasitic infections and concomitant immune response is important. Literature information about the effect of Toxocariasis and NS administration on tissue and serum selenium concentration is lacking. Therefore, it was aimed to evaluate the role of selenium, which has endogenous antioxidant and immunomodulatory properties in *T.canis*-infected mice, and the effect of prophylactic and therapeutic NS administration on kidney selenium levels.

## MATERIAL and METHOD

### *Animals*

A total of 50 healthy adult male BALB/c mice weighing 25±2 g was obtained from the Department of Biology, Istanbul University Faculty of Science. The animals were kept in a room at a constant temperature of +22±2°C under 12 hours dark/light cycle conditions. The mice were fed with water and standard pellets ad libitum. The mice were accustomed to laboratory conditions two weeks before the experiment. The animal protocols were pre-approved by the Harran University Animal Experiments Ethics Committee, Sanliurfa, Turkey, before the commencement of the experiment.

### *Collection and preparation of parasite eggs*

The female adult *T.canis* were collected from naturally infected puppies from a shelter in Sanliurfa. The female worms of the obtained *T.canis* were washed in physiological saline, and the eggs were extracted from the vagina and distal third of the uterus. Then, the development of the eggs was realized in 20 days with continuous ventilation at 28ºC (Bowman et al., 1987). The eggs were diluted with 10% gum arabic suspension to achieve 500 embryonated eggs in 1 ml of suspension (Musa et al., 2011).

### *Plant material*

NS seeds were purchased from a local herbal shop in Sanliurfa Turkey, and two different doses of the extract were formulated (100 and 200 mg/kg body weight NS) (Musa et al., 2011). Firstly, the seeds were cleaned, washed and pulverized in an electrical grinder. It was waited on methanol for 24 hours and was filtered. Its extracted matter was obtained with using a rotary evaporator and dried by lyophilisation and suspended with 10% gum arabic solution.

### *Experimental groups*

Fifty albino mice, weighing 18-25 g, were used. Mice were divided into six experimental groups; 7 mice in control and 43 in experimental groups (Table 1). The treatment was given for seven consecutive days to control and experimental groups. At the beginning and the end of the experiment, blood smears were used to determine *T.canis* infection.

### *Biochemical analyses*

At the end of 7 days, the animals were decapitated, and kidney samples were collected and stored at -70 °C until the time of analysis. Kidney samples were analyzed for selenium by wet ashing and fluorometric detection of the 2,3-diaminophthalene derivative (Koh and Benson, 1983).

### *Statistical analyses*

Statistical analyses were carried out using GraphPad Prism 6.0 (GraphPad Software, San Diego; CA; USA). All data were expressed as mean ± standard deviation (Mean ± SD). The groups were compared with the analysis of variance (ANOVA) and then post hoc Tukey's multi-comparison tests. Significance levels were considered as $P<0.05$.

**Table 1.** Experimental groups and treatments

| Groups | Treatments |
|---|---|
| **Control** | Healthy mice group, 1 ml 10% gum Arabic suspension. |
| ***T.canis* infected** | 500 embryonic eggs of *T.canis* were injected orally using an oesophageal catheter. |
| **Treated N100** | 500 embryonic eggs of *T.canis* were injected orally using an oesophageal catheter and then during 7 days 100mg/kg NS was given. |
| **Treated N200** | 500 embryonic eggs of *T.canis* were injected orally using an oesophageal catheter and then during 7 days 200mg/kg NS was given. |
| **Prophylactic N100** | During 7 days, 100mg/kg NS was given at first then 500 embryonic eggs of *T.canis* were injected orally using an oesophageal catheter. The day after given *T.canis* eggs, NS was given during 7days. |
| **Prophylactic N200** | During 7 days, 200mg/kg NS was given at first then 500 embryonic eggs of *T.canis* were injected orally using an oesophageal catheter. The day after given *T.canis* eggs, NS was given during 7days. |

**Table 2:** Se levels of kidney tissues in all groups (ng/g).

| Groups | n | Mean ± SD |
|---|---|---|
| **Control** | 7 | **980,46±236,68** |
| ***T.canis* infected** | 9 | **1240,15±315,77** |
| **Treated N100** | 9 | **1297,95±354,37** |
| **Treated N200** | 9 | **1361,29±410,46** |
| **Prophylactic N100** | 6 | **1148,55±240,28** |
| **Prophylactic N200** | **10** | **1465,81±450,25** |

## RESULTS

In control group which mice were healthy, kidney selenium levels were 980,46±236,68 ng/g and in mice infected with *T.canis* 1240,15±315,77 ng/g. Kidney tissue Se levels of mice given NS in 100mg/kg doses for treatment (Treated N100) are 1297,95±354,37 and 200mg/kg doses for treatment (Treated N200) are 1361,29±410,46 ng/g. Kidney tissue selenium levels for prophylactic groups were founded 1148,55±240,28 ng/g in Prophylactic N100 group and 1465,81±450,25 ng/g Prophylactic N200 group (Table 2).

## DISCUSSION

Toxocariasis is one of the most common zoonotic infections in humans with a chronic course. Long-term use of anti-parasitic drugs may cause resistance to these drugs (Geerts and Gryseels, 2001; Kappagoda et al., 2011). Today, natural products with antioxidant and anti-inflammatory effects are recommended to replace antiparasitic drugs or co-use of them, including those against *Toxocara* infections. Besides, selenium intake with foods and tissue selenium content may be effective on immune system. In the present study, the efficacy of NS on treatment and prophylactic doses, which has been reported to have antioxidant activity on kidney selenium levels, was evaluated in experimentally infected mice with *T.canis*.

In a study, 100 and 200 mg/kg NS administration was shown to decrease the total *T.canis* larvae burden and the inflammation degree (Musa et al., 2011). In another study, the effectiveness of NS treatment on serum cytokine levels was demonstrated and its use in treatment was suggested (El-Refai et al., 2017). The anti-Toxocara effects of NS may depend on many factors. NS has been suggested to have a direct lethal effect on Toxocara larvae in vivo (El-Refai et al., 2017). In addition, NS has been found to cause vacuolisation and irregularity in the adult parasite cuticle (Shalaby and El-Moghazy, 2013). Nigella sativa is thought to play a role against the changes caused by parasites due to the stimulating effect on the immune system and some degree of antioxidant effect in parasitic infections (Mahmoud et al., 2002; Majdalawieh and Fayyad, 2015). In addition, NS has anti-inflammatory properties by inhibiting the formation of eicosanoids, the inflammatory promoters (Darakhshan et al., 2015). In our study, the effect of NS on selenium levels, which is known to have antioxidant, anti-inflammatory and immunomodulatory effects, which is an important component of the antioxidant system in the kidneys, was investigated in mice infected with *T.canis*.

Kidney tissue has the highest selenium concentration, and normal selenium levels are greater than 1000 ng/g. A decrease of about half indicates marginal selenium deficiency (Shamberger, 1983). Antioxidants are substances that function to protect cells from damage caused by unstable free radicals and reactive oxygen species (ROS) (Lobo et al., 2010). NS has been used in the Middle East for centuries to treat disease processes. Various studies with NS, which is known to be a free radical scavenger, have shown its hypotensive, analgesic, diuretic, choleretic, antidiabetic, anti-inflammatory, antiviral, anthelmintic, antibacterial, antitumoral, antihistamine, immunomodulatory, antioxidant and hepatoprotective effects (Tavakkoli et al., 2017).

In in vitro studies, NS has been found to reduce lipid peroxidation, protein oxidation and has shown a protective effect in various cell types (Tülüce et al., 2009; Khaldi et al., 2018). Despite these high numbers of studies with NS, its effects on endogenous selenium are not fully known. Unlike NS, selenium is found in almost all human tissues and is classified as a trace element. Selenium has the ability to change cells by acting as an antioxidant, regulating the redox status and immunomodulators (Tapiero et al., 2003; Tinggi, 2008). Selenium deficiency inhibits both cellular and humoral responses (Sun et al., 2018). Studies have shown that the immune system is negatively affected by different types of parasitic diseases due to selenium deficiency (Pilarczyk et al., 2012). However, the effect of NS use on endogenous selenium in *T.canis* infected animals has not been investigated. In our study, it was found that kidney tissue selenium concentration increased in all experimental groups, but these increases were not statistically significant compared to the control group. NS may increase Se accumulation in kidney tissues in two different ways; first one is Se is involved in NS extract (Naz, 2011) and the second one NS increases Se intake to tissues. To reveal these further studies should be carried

out to reveal. *T.canis* is known to induce oxidative metabolism (Mahadappa and Dey, 2018). Nigella sativa has a positive antioxidative effect (Darakhshan et al., 2015). Other increases in Treated N200 and Prophylactic N200 groups may be related to the antioxidative effect of NS depending on the given doses.

## CONCLUSION

In this study, an attempt was made to evaluate the effect of selenium intake on *T.canis* infected mice. In conclusion, it is thought that NS may cause an increase in selenium concentration in kidney tissue depending on the administered doses, which may be protective against *T.canis* infections by contributing to the antioxidant system or used as a supportive factor together with treatment.

## ACKNOWLEDGMENT

This study is presented at scientific meeting. (International Symposium on Selenium in Health and Disease, 12-13 October 2006, Ankara)

Ethical approval: Ethical approval of the present study was acquired from the Harran University Animal Experiments Ethics Committee, Sanliurfa, Turkey, before the commencement of the experiment (11.07.2005).

Conflict of interest: The authors declare that they have no competing interests.

## KAYNAKLAR

**Bowman, D.D., Mika-Grieve, M., Grieve, R.B. (1987).** Circulating excretory-secretory antigen levels and specific antibody responses in mice infected with Toxocara canis. *American Journal of Tropical Medicine and Hygiene, 36*,75–82. https://doi.org/10.4269/ajtmh.1987.36.75

**Cheikh-Rouhou, S., Besbes, S., Hentati, B., Blekcer, C., Deroanne, C., Attia, H. (2007).** Nigella sativa L.: Chemical composition and physicochemical characteristics of lipid fraction. *Food Chemistry, 101*, 673–681. https://doi.org/10.1016/j.foodchem.2006.02.022

**Combs, Jr G.F. (2015).** Biomarkers of selenium status. *Nutrients, 7*, 2209–2236. https://doi.org/10.3390/nu7042209

**Darakhshan, S., Bidmeshki Pour, A., Hosseinzadeh Colagar, A., Sisakhtnezhad, S. (2015).** Thymoquinone and its therapeutic potentials. *Pharmacological Research, 95–96*, 138–158. https://doi.org/10.1016/j.phrs.2015.03.011

**Ekor, M. (2014).** The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology,4* JAN,1–10. https://doi.org/10.3389/fphar.2013.00177

**El-Refai, S.A., El-Aswad, B.E.D.W., Mahmoud, S.F. (2017).** Parasitological, pathological and immunological effects of Nigella sativa oil in experimentally Toxocara canis infected mice. *Tropical Biomedicine, 34*, 781–794.

**Geerts, S., Gryseels, B. (2001).** Anthelmintic resistance in human helminths: a review. *Tropical Medicine & International Health, 6*, 915–921. https://doi.org/10.1046/j.1365-3156.2001.00774.x

**Huang, Z., Rose, A.H., Hoffmann, P.R. (2012).** The role of selenium in inflammation and immunity: from molecular mechanisms to therapeutic opportunities. *Antioxidants & Redox Signaling, 16*, 705–743. https://doi.org/10.1089/ars.2011.4145

**Jakaria, M., Cho, D.Y., Haque, M.E., Karthivashan, G., Kim, I.S., Ganesan, P., Choi, D.K. (2018).** Neuropharmacological potential and delivery prospects of thymoquinone for neurological disorders. *Oxidative Medicine and Cellular Longevity, 4*, 1-17. https://doi.org/10.1155/2018/1209801

**Kappagoda, S., Singh, U., Blackburn, B.G. (2011).** Antiparasitic therapy. *Mayo Clinic Proceedings, 86* (6), 561–583. https://doi.org/10.4065/mcp.2011.0203

**Khaldi, T., Chekchaki, N., Boumendjel, M., Taibi, F., Abdellaoui, M., Boumendjel, A. (2018).** Ameliorating effects of Nigella sativa oil on aggravation of inflammation, oxidative stress and cytotoxicity induced by smokeless tobacco extract in an allergic asthma model in Wistar rats. *Allergologia et Immunopathologia, 46*, 472–481. https://doi.org/10.1016/j.aller.2018.02.005

**Kleine, A., Springer, A., Strube, C. (2017).** Seasonal variation in the prevalence of Toxocara eggs on children's playgrounds in the city of Hanover, Germany. *Parasites and Vectors, 10*, 6–13. https://doi.org/10.1186/s13071-017-2193-6

**Koh, T.S., Benson, T.H. (1983).** Critical re-appraisal of fluorometric method for determination of selenium in biological materials. *Journal - Association of Official Analytical Chemists, 66*, 918–926.

**Lobo, V., Patil, A., Phatak, A., Chandra, N. (2010).** Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews, 4*, 118–126. https://doi.org/10.4103/0973-7847.70902

**Lukác, N., Massányi, P. (2007).** Effects of trace elements on the immune system. *Epidemiologie, mikrobiologie, imunologie, 56*, 3–9.

**Mahadappa, P., Dey, S. (2018).** Effects of Toxocara canis Infection and Albendazole Treatment on Oxidative/Nitrosative Stress and Trace Element Status in Dogs. *International Journal of Livestock Research, 8,* 144–153. https://doi.org/10.5455/ijlr.20170720111145

**Mahmoud, M.R., El-Abhar, H.S., Saleh, S. (2002).** The effect of Nigella sativa oil against the liver damage induced by Schistosoma mansoni infection in mice. *Journal of Ethnopharmacology, 79,* 1–11. https://doi.org/10.1016/s0378-8741(01)00310-5

**Majdalawieh, A.F., Fayyad, M.W. (2015). I**mmunomodulatory and anti-inflammatory action of Nigella sativa and thymoquinone: A comprehensive review. *International Immunopharmacology, 28,* 295–304. https://doi.org/10.1016/j.intimp.2015.06.023

**Meral, I., Yener, Z., Kahraman, T., Mert, N. (2001).** Effect of Nigella sativa on Glucose Concentration, Lipid Peroxidation, Anti-Oxidant Defence System and Liver Damage in Experimentally-Induced Diabetic Rabbits. *Journal of Veterinary Medicine. A, Physiology, Pathology, Clinical Medicine, 48,* 593–599. https://doi.org/10.1046/j.1439-0442.2001.00393.x

**Musa, D., Senocak, G., Borazan, G., Altaş, M., Ozgonul, A., Sogut, O., Güldür, M.E. (2011).** Effects of Nigella sativa and albendazole alone and in combination in Toxocara canis infected mice. *Journal of Pakistan Medical Association, 61,* 866–870.

**Naz, H. (2011).** Nigella sativa: the miraculous herb. *Journal of Biochemistry and Molecular Biology, 44,* 44–48.

**Pilarczyk, B., Jankowiak, D., Tomza-Marciniak, A., Pilarczyk, R., Sablik, P., Drozd, R., Tylkowska, A., Skólmowska, M. (2012).** Selenium concentration and glutathione peroxidase (GSH-Px) activity in serum of cows at different stages of lactation. *Biological Trace Element Research, 147,* 91–96. https://doi.org/10.1007/s12011-011-9271-y

**Shalaby, H.A., El-Moghazy, F.M. (2013).** In vitro effect of Nigella sativa oil on adult Toxocara vitulorum. *Pakistan Journal of Biological Sciences, 16,* 1557–1562. https://doi.org/10.3923/pjbs.2013.1557.1562

**Shamberger, R.J. (1983).** *Biochemistry of Selenium*. New York: Plenum Publishing.

S**un, Z., Xu, Z., Wang, D., Yao, H., Li, S. (2018).** Selenium deficiency inhibits differentiation and immune function and imbalances the Th1/Th2 of dendritic cells. *Metallomics, 10,* 759–767. https://doi.org/10.1039/c8mt00039e

**Tapiero, H., Townsend, D.M., Tew, K.D. (2003).** The antioxidant role of selenium and seleno-compounds. *Biomedicine & Pharmacotherapy, 57,* 134–144. https://doi.org/10.1016/s0753-3322(03)00035-0

**Tavakkoli, A., Ahmadi, A., Razavi, B.M., Hosseinzadeh H. (2017).** Black Seed (Nigella Sativa) and its Constituent Thymoquinone as an Antidote or a Protective Agent Against Natural or Chemical Toxicities. *Iranian Journal of Pharmaceutical Research, 16,* 2–23.

**Tinggi, U. (2008).** Selenium: its role as antioxidant in human health. *Environmental Health and Preventive Medicine, 13,* 102–108. https://doi.org/10.1007/s12199-007-0019-4

**Tülüce, Y., Özkol, H., Söğüt, B., Çelik, I. (2009).** Effects of Nigella sativa L. on lipid peroxidation and reduced glutathione levels in erythrocytes of broiler chickens. *Cell Membranes and Free Radical Research, 1,* 95–99.

**Yarsan, E., Altinsaat, Ç., Ayçiçek, H., Şahindokuyucu, F., Kalkan, F. (2003).** Effects of Albendazole Treatment on Haematological and Biochemical Parameters in Healthy and Toxocara canis Infected Mice.*Turkish Journal Of Veterinary and Animal Sciences, 27*,1057–1063.