

Pathological Changes of *Fasciola gigantica* After Treatment with *Nigella sativa* *in Vitro*

Muhammad Hambal^{1*}, Alfifatul Hidayah², Ummu Balqis³, Farida Athaillah¹,
Muttaqien¹, Siti Rani Ayuti⁴, Wahyu Eka Sari⁵, Henni Vanda⁶

¹Laboratory of Parasitology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia

²Student of Study Program of Veterinary Education, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia

³Laboratory of Pathology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia

⁴Laboratory of Biochemistry, Faculty of Veterinary Medicine, Universitas Syiah Kuala Banda Aceh, Indonesia

⁵Laboratory of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia

⁶Laboratory of Pharmacology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia

*Corresponding author. Email: hambal.m@unsyiah.ac.id

ABSTRACT

Black cumin (Nigella sativa) has been traditionally used as medicine for various infectious and non-infectious diseases, such as skin infection, conjunctivitis, hypertension, gastrointestinal problems, diabetes, and diarrhea. This study aimed at finding out the effect of black cumin, *N. sativa* on the survival and pathological changes of liver flukes *Fasciola gigantica in vitro*. The seeds of *N. sativa* were extracted with maceration using methanol. Mature *F. gigantica* flukes were stored in PBS solution and treated with different concentrations of *N. sativa* extract namely 10% (T1), 25% (T2), and 50% (T3). Similar experiment using Albendazole 0.24 mg/ml and 1xPBS was used as positive (C1) and negative (C2) controls, respectively. All treatments were done at room temperature. The dead flukes were further examined for histopathological changes. The results showed that 50% concentration of extract killed all flukes within 15 minutes, much faster than caused by albendazole (30 minutes). In T2 (25% extract), all flukes were died in 31.5 minutes. The extract of *N. sativa* caused severe disruption of tegument, the alteration of internal organs, and the absence of tegument spine. The intestine showed inflammation with severity levels were in line with the extract concentration. There was also disintegration of testicular cells observed in the flukes treated with black cumin. In conclusion, *N. sativa* seed extract has potential medical property against *F. gigantica in vitro*.

Keywords: Herbal anthelmintic property, tegument disruption, *Fasciola gigantica*, *Nigella sativa*

1. INTRODUCTION

Fasciolosis, helminthiasis caused by *F. gigantica* and *Fasciola hepatica (F. hepatica)* is widespread in cattle, sheep, and other ruminants which cost a great deal of economic loss [1-3]. Fasciolosis usually has no significant clinical symptoms, in chronic infection, it will cause severe weight loss, low milk production, infertility [2, 4], and liver cirrhosis [5]. The disease is not only restricted in ruminants, but also reported in human, and a wide population is at high risk [6-8].

The fasciolosis found in Indonesia is caused by *F. gigantica*. Ruminants usually get infected by ingesting metacercaria from intermediate host *Lymnea rubiginosa*. The high fasciolosis case in Indonesia is

related to extensive rice cultivation, lead to large scale paddy field as an ideal habitat for *L. rubiginosa* [9].

Synthetic drugs have been used for centuries as the most effective tool for the treatment, yet the infection rate is still high. Furthermore, synthetic drugs may cause several disadvantages such as drug resistance, adverse side effect, chemical residue, and high cost. All of these problems could be resolved by using common traditional herbs to replace synthetic drugs which would be more acceptable, easier to obtain, and low-cost.

Nigella sativa Linn. has been used as traditional medicine in Middle East and South Asia for over 100 years. This plant has been introduced and utilized in

other parts of the world as one of the most promising treatment as antihypertensive, diuretics, analgesic, antibacterial, anti-diarrheal, immunostimulatory, bronchodilator, liver and gastro protectant, and anthelmintic [3, 10-13].

The present study aims to investigate the efficacy of *N. sativa* seed extract as anthelmintic against a naturally acquired fasciolosis infection in cattle in Indonesia.

2. MATERIALS AND METHODS

2.1. Source of Plant Extract

Nigella sativa Linn. were purchased from local market in Banda Aceh, washed, dried, and ground into powder. The powder was then filtered through gauze cloth and stored at 4°C until further used.

Five hundred grams of grinded seed of *N. sativa* were extracted with maceration using 96% methanol. The plants were soaked in 2 L of methanol for three days at room temperature. The filtrate was then collected and the solvent was removed by rotary evaporator at 40°C. The crude extract was resuspended in phosphate buffer saline (PBS) and diluted to the desired concentration. If necessary, 0.5% of carboxy methyl cellulose (CMC) was added to the solution.

2.2. Collection of Liver Flukes

Mature *F. gigantica* flukes were collected from bile duct of infected cattle slaughtered at Banda Aceh abattoir. The flukes were kept in PBS solution and examined immediately to avoid any disruption. After several washing steps with PBS, the healthiness of flukes was determined based on microscopic method.

2.3. Experimental Design

The effectiveness of *N. sativa* extract against *F. gigantica* was studied in vitro according to the protocol described by Jeyathilakan *et al.* [14].

Using albendazole 0.24 mg/ml as positive control (C1), and PBS as negative control (C2), the flukes treated with different concentration *N. sativa* seed extract namely 10% (T1), 25% (T2), and 50% (T3) at room temperature. The observation was conducted until all flukes died.

2.4. Histopathological Examination

The death flukes were further processed for histopathological examination. The flukes were fixed in 10% formalin for 24 hours, dehydrated in ascending concentration of ethanol, and cleared in xylol. After embedding and blocking processes in paraffin,

samples were dissected longitudinally at 3-5 μ m thickness, and stained with hematoxylin and eosin. The observation was done using a light microscope (Olympus, Tokyo, Japan) with 40x and 100x magnifications and photographed [14].

3. RESULTS AND DISCUSSION

3.1 Mortality Time of *F. gigantica*

Nigella sativa with 50% concentration could kill all flukes within 15 minutes of incubation, much faster than that found in control group, where all flukes died in 30 minutes incubation with albendazole. In the group given 25% extract, all flukes died in 31.5 minutes, whilst in 10% extract group, the time required to kill all flukes was longer, 44.4 minutes. All the flukes in control group (PBS) could survive until 411 minutes. The average mortality time of *F. gigantica* after treatment with *N. sativa* extract is showed in Figure 1.

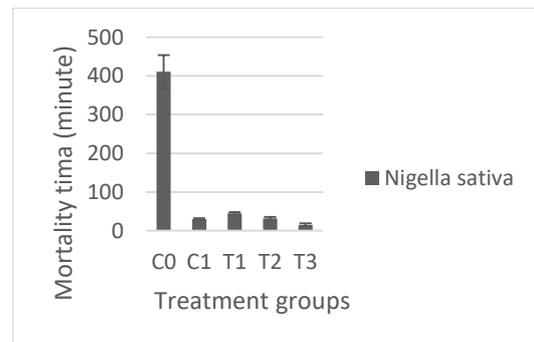


Figure 1 Mortality time of *F. gigantica* treated with *N. sativa* seed extracts and control. C0: negative control (PBS); C1: positive control (albendazole); T1-T3: *N. sativa* extract of 10%, 25%. and 50%.

3.2 Mortality Time of *F. gigantica* Histopathological Finding

Histopathological observation found some alterations in *F. gigantica* tissues. In both *N. sativa* treatments and positive control, the tegument showed disruption and separation from its parenchyma. The severity of changes found among groups was different. The higher concentration, the more severe impact for flukes was (Figure 2).

The flukes in the group given 50% *N. sativa* extract showed more severe disruption of tegument compared to other treatments. However, the alteration effect caused by the extract in the internal organs is less distinct compared to those found in tegument. In negative control, the spine was still present and tegument was intact.

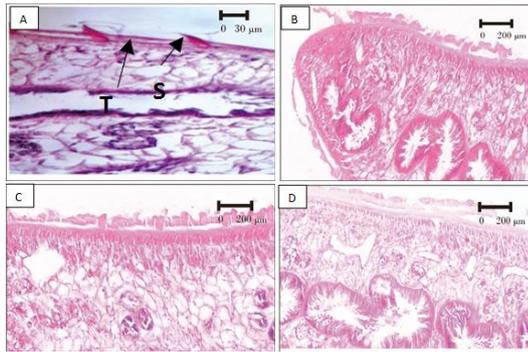


Figure 2 Histopathology of tegument (T) and spine (S) of *F. gigantica*. A= Negative control (PBS). B= *Nigella sativa* extract 10%. C= *N. sativa* extract 25%. D= *N. sativa* extract 50%.

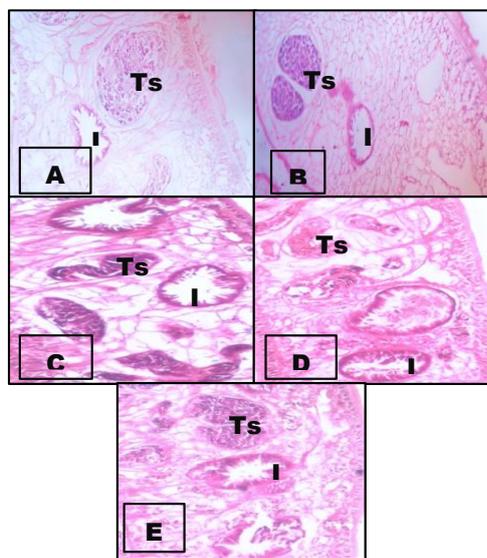


Figure 3. Histopathology of intestine (I) and testes (Ts) of *F. gigantica*. A= Negative control (PBS). B= Positive control (*Albendazole*). C= 10% of *N. sativa* extract. D= 25% of *N. sativa* extract. E= 50% of *N. sativa* extract.

Figure 3 showed the changes of testicular and intestinal cells of *F. gigantica* due to treatment of *N. sativa* extracts. The intestine exhibited villi desquamation with severity level was in line with the extract concentration. There was also disintegration of testicular cells observed in the flukes. The damage of epithelial cells both in testes and intestine was correspond to the concentrations of the extract.

Several researches have proved the effectiveness of *N. sativa* as anthelmintic. A report by El-Far *et al.* [15] stated that *N. sativa* seeds was effective in treating nematodes infection in crossbreed ewes. Shalaby and El-Moghazy [16] showed that *N. sativa* oil was potential in treating *Toxocara vitulorum* infection *in vitro*. Ullah *et al.* [3] reported the promising activity of thymoquinone, a bioactive compound of *N. sativa* seeds, as anthelmintic against

F. gigantica. Shalaby *et al.* [17] also reported that a combination of *N. sativa* oil and ivermectin had a destructive effect on the tegument surfaces of adult *F. gigantica in vitro*.

The main components found in *N. sativa* seeds are thymoquinone (30-48%), thymohydroquinone, dithymoquinone, and p-cymene (7-15%), carvacrol (6-12%), 4-terpineol (2-7%), t-anethol (1-4%), sesquiterpene longifolene (1-8%) α -pinene and thymol [18], linoleic acid (50.3-49.2%), oleic acid (25-23.7%), palmitic acid (17.2-18.4%). Other compounds like myristic, myristoleic, palmitoleic, margaric, margaroleic, stearic, linolenic, arachidic, eicosenoic, behenic and lignoceric acids are also detected [19].

Another plant which has anthelmintic potential against *F. gigantica in vitro* is *Curcuma aeruginosa* Robx [20]. The methanolic extract of this rhizome also could impact on tegument, reproductive organs, and gastrointestinal tract, all of which similar to the finding in our experiment. Since these organs are essential for the flukes survival, the destruction of these organs will cause death.

4. CONCLUSION

This study revealed that methanolic extract of *N. sativa* had the ability to cause severe damage on substantial organs of *F. gigantica*. Therefore, it has potential as anthelmintic on trematodes. Further research is necessary to study the effect of this extract on *F. gigantica in vivo*.

AUTHORS' CONTRIBUTIONS

The research was designed by MH, FA, and UB. The field work was carried out by AH and M. The manuscript was written by MH, HV, WES, and SRA.

ACKNOWLEDGMENT

Authors wish to thank all technical staff in the Laboratory of Parasitology, Laboratory of Pharmacology, and Laboratory of Research, Faculty of Veterinary Medicine, USK, for their assistance. Authors are thankful the Banda Aceh Municipality Abattoir for supporting this research.

REFERENCES

- [1] E.O. Mungube, S.M. Bauni, B.A. Tenhagen, L.W. Wamae, J.M. Nginyi, J.M. Mugambi, The prevalence and economic significance of *Fasciola gigantica* and *Stilesia hepatica* in slaughtered animals in the semi-arid coastal Kenya, Trop.

- Anim. Health Prod. 38(6) (2006) 475-483. DOI: <https://doi.org/10.1007/s11250-006-4394-4>.
- [2] M. Hambal, R. Ayuni, H. Vanda, A. Amiruddin, F. Athaillah, Occurrence of *Fasciola gigantica* and *Paramphistomum* spp Infection in Aceh Cattle, vol. 151, In E3S Web of Conferences, EDP Sciences, 2020, p. 01025. DOI: <https://doi.org/10.1051/e3sconf/202015101025>
- [3] R. Ullah, A. Rehman, M.F. Zafeer, L. Rehman, Y.A. Khan, M.H. Khan, S.N. Khan, A.U. Khan, S.M.A. Abidi, Anthelmintic potential of thymoquinone and curcumin on *Fasciola gigantica*. PloS one, 12(2) (2017) p.e0171267. DOI: 10.1371/journal.pone.0171267
- [4] J. Charlier, L. Duchateau, E. Claerebout, D. Williams, J. Vercruyse, Associations between anti-*Fasciola hepatica* antibody levels in bulk-tank milk samples and production parameters in dairy herds. Prev. Vet. Med. 78(1) (2007) 57-66. DOI: 10.1016/j.prevetmed.2006.09.010
- [5] L.A. Marcos, P. Yi, A. Machicado, R. Andrade, S. Samalvides, J. Sánchez, Hepatic fibrosis and *Fasciola hepatica* infection in cattle. J. Helminthol. 81(4) (2007) 381-386. DOI:10.1017/S0022149X07850231
- [6] M.S. Mas-Coma, M.D. Bargues, M.A. Valero, Fascioliasis and other plant-borne trematode zoonoses, Int. J. Parasitol. 35(11-12) (2005) 1255-1278. DOI: 10.1016/j.ijpara.2005.07.010
- [7] K. Cwiklinski, S.M. O'Neill, S. Donnelly, J.P. Dalton, A prospective view of animal and human Fasciolosis. Parasit. Immunol. 38(9) (2016) 558-568. DOI: <https://doi.org/10.1111/pim.12343>.
- [8] O. Figueroa-Santiago, A.M. Espino, *Fasciola hepatica* fatty acid binding protein induces the alternative activation of human macrophages. Infect. Immun. 82(12) (2014) 5005-5012. DOI: 10.1128/IAI.02541-14
- [9] M. Hambal, A. Sayuti, A. Darmawan, Tingkat kerentanan *Fasciola gigantica* pada sapi dan kerbau di Kecamatan Lhoong Kabupaten Aceh Besar. J. Med. Vet, 7(1) (2013) 49-53. DOI: 10.21157/j.med.vet.v7i1.2921
- [10] K.A. Abdel-Sater, Gastroprotective effects of *Nigella Sativa* oil on the formation of stress gastritis in hypothyroidal rats, Int. J. Physiol. Pathophysiol. Pharmacol. 1(2) (2009) 143. DOI: 10.15171/jhp.2019.02
- [11] M.H. Boskabady, N. Mohsenpoor, L. Takaloo, Antiasthmatic effect of *Nigella sativa* in airways of asthmatic patients, Phytomedicine, 17(10) (2010) 707-713. DOI: <https://doi.org/10.1016/j.phymed.2010.01.002>
- [12] A.O. Abdel-Zaher, M.S. Abdel-Rahman, F.M. Elwasei, Protective effect of *Nigella sativa* oil against tramadol-induced tolerance and dependence in mice: role of nitric oxide and oxidative stress, Neurotoxicology 32(6) (2011) 725-733. DOI: <https://doi.org/10.1016/j.neuro.2011.08.001>
- [13] A. Selvaraju, S.K.D.K.M Dhanraj, Phytochemical analysis and anthelmintic potential of *Nigella sativa* against the trematode, *Cotylophoron cotylophorum*, J. Pharmacog. Phytochem. 8(3) (2019) 3161-3166. DOI: 10.11648/J.AJLS.20150305.14
- [14] N. Jeyathilakan, K. Murali, A. Anandaraj, B.R. Latha, S.A. Basith, Anthelmintic activity of essential oils of *Cymbopogon nardus* and *Azadirachta indica* on *Fasciola gigantica*. J. Vet. Anim. Sci. 6(5) (2010) 204-209.
- [15] A.H. El-Far, E.K. Bazh, M.S. Moharam, Antioxidant and antinematodal effects of *Nigella sativa* and *Zingiber officinale* supplementations in ewes. Int. J. Pharm. Sci. Rev. Res. 26(1) (2014) 222-227.
- [16] H.A. Shalaby, F.M. El-Moghazy, In vitro effect of *Nigella sativa* oil on adult *Toxocara vitulorum*. Pakistan J. Biol. Sci. 16(22) (2013) 1557. DOI: <https://doi.org/10.3923/pjbs.2013.1557.1562>.
- [17] H.A. Shalaby, N.M.T. Abu El Ezz, T.K. Farag, H.A.A. Abou-Zeina, In vitro efficacy of a combination of ivermectin and *Nigella sativa* oil against helminth parasites, Global Veterinaria 9(4) (2012) 465-473.
- [18] M.S. Al-Jassir, Chemical composition and microflora of black cumin (*Nigella sativa* L.) seeds growing in Saudi Arabia. Food Chem. 45(4) (1992) 239-242. DOI: [https://doi.org/10.1016/0308-8146\(92\)90153-S](https://doi.org/10.1016/0308-8146(92)90153-S).
- [19] S. Cheikh-Rouhou, S. Besbes, B. Hentati, C. Blecker, C. Deroanne, H. Attia, *Nigella sativa* L.: Chemical composition and physicochemical characteristics of lipid fraction, Food chem. 101(2) (2007) 673-681. DOI: <https://doi.org/10.1016/j.foodchem.2006.02.022>.
- [20] H. Vanda, R. Parindra, M. Hambal, F. Athaillah, Anthelmintic activity of *Curcuma Aeruginosa* Roxb extract on *Fasciola gigantica in vitro*. In E3S Web of Conferences, 151 (2020) 01046, EDP Sciences. DOI: <https://doi.org/10.1051/e3sconf/202015101046..>