The Journal of Phytopharmacology 2023; 12(2):76-81
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2023; 12(2): 76-81
March- April
Received: 10-03-2023
Accepted: 27-04-2023

doi: 10.31254/phyto.2023.12203

V Ramakrishnan
Assistant Professor, Krishi Vigyan Kendra, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Kundrakudi, Sivagangai, Tamil Nadu, India

P Gokul
B.V.Sc. and A.H. Scholar, Veterinary College and Research Institute, Tirunelveli, Tamilnadu, India, Tamilnadu Veterinary and Animal Sciences University (TANUVAS), Kundrakudi, Sivagangai, Tamil Nadu, India

R Hemalatha
B.V.Sc. and A.H. Scholar, Veterinary College and Research Institute, Tirunelveli, Tamilnadu, India, Tamilnadu Veterinary and Animal Sciences University (TANUVAS); Kundrakudi, Sivagangai, Tamil Nadu, India

AT Karthika
B.V.Sc. and A.H. Scholar, Veterinary College and Research Institute, Tirunelveli, Tamilnadu, India, Tamilnadu Veterinary and Animal Sciences University (TANUVAS); Kundrakudi, Sivagangai, Tamil Nadu, India

R Mohan Raj
B.V.Sc. and A.H. Scholar, Veterinary College and Research Institute, Tirunelveli, Tamilnadu, India, Tamilnadu Veterinary and Animal Sciences University (TANUVAS); Kundrakudi, Sivagangai, Tamil Nadu, India

R Shankar
M.V.Sc. Scholar, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Kerala, Kerala Veterinary and Animal Sciences University (KVASU), Kerala Veterinary and Animal Sciences University (KVASU), Pookode, Kerala, India

Correspondence:
Dr. V Ramakrishnan
Assistant Professor, Krishi Vigyan Kendra, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Kundrakudi, Sivagangai, Tamil Nadu, India
Email: ramakrishnanvet@gmail.com

In vitro anthelmintic activity of *Tinospora cordifolia* nanoparticles against strongyle nematode of small ruminants

V Ramakrishnan, P Gokul, R Hemalatha, AT Karthika, R Mohan Raj, R Shankar

ABSTRACT

The age old Ethnoveterinary Medicine has turned into a limelight recently in the treatment of livestock as it is cheaper, easily available, sustainable and environmentally acceptable with nil residues in animal tissues and offals. These pristine herbal medicines can be the better alternatives to conventional anthelmintics with a greater resistance problem. The objective of the present investigation was to evaluate the effectiveness of *Tinospora cordifolia* extract as a reducing agent in the biosynthesis of silver nanoparticles as well as the effectiveness of the biosynthesized silver nanoparticles in inhibiting the hatching of strongyle nematode eggs in small ruminants. Aqueous extract of *T. cordifolia* leaves and stems was prepared and screened for phytochemical constituents. Silver nanoparticles were synthesized using the extract and characterized by using visual appearance, particle size and Zeta potential values. Egg hatch assay (EHA) was carried out according to the techniques and recommendations of the World Association for Advancement of Veterinary Parasitology. Dynamic light scattering and Scanning electron microscope (SEM) were showed that the average particle size was 2.8nm. Zeta potential of synthesized silver nanoparticle was -18.4 mv. The egg hatch inhibition assay revealed a significant inhibition in a dose dependent manner, when compared with aqueous extracts of *T. cordifolia*. Thus, the present study warrants the potential anti- nematode activity of *T. cordifolia* however further studies are required to investigate the active principle responsible for the activity.

Keywords: *Tinospora cordifolia*, Silver nanoparticles, Egg hatch assay, Zeta potential, Dynamic light scattering, Scanning electron microscope.

INTRODUCTION

Sheep and goats are one of the most affordable animals in the world and can be accommodated in any kind of weather conditions. They are also called “poor man’s cow”. Raising goats and sheep will have a significant economic impact by creating jobs in the area [1]. Small ruminants are important sources of meat and cash for farmers in Southern Districts of Tamil Nadu. The rearing of sheep and goats provide a substantial income and employment to rural people. Within Tamil Nadu, the Tirunelveli district ranks 1st in sheep population and 7th rank in goat population [2]. Despite of this positive progress, the sizeable population of young animals of these species succumbed to the worm infection. To encounter this problem, use of synthetic anthelmintics in combination with grazing management was followed. However, the misuse and poor formulations of these products have led to the development of anthelmintic resistance which is world concern in recent days [3]. The age old Ethnoveterinary Medicine has turned into a limelight recently in the treatment of livestock as it is cheaper, easily available, sustainable, and environmentally acceptable with nil residues in animal tissues and offals. These pristine herbal medicines can be the better alternatives to conventional anthelmintics with resistance problem [4]. Nanotechnology is a fast-evolving science involved in the production and utilization of nano-sized particles. Various nanoparticles can be synthesised from plant extracts, among these, silver nanoparticles have many advantages due to their stability, good conductivity, antimicrobial activity, eco-friendliness, non-pathogenic nature and cost-effectiveness [5].

Tinospora cordifolia (Menispermaceae), a tropical region's native herbaceous vine, is found in India, Myanmar and Sri Lanka. This plant is a glabrous climbing shrub that is common throughout India and prefers dry, deciduous woodlands to grow. It has been demonstrated to have anti-allergic, anti-inflammatory, antioxidant, immuno-modulatory, hepatoprotective, antipyretic, antidiabetic, anti-hyperlipidemic qualities and to be effective against bronchitis, syphilis, urinary tract infections and skin conditions [6]. *T. cordifolia* has long been employed as an anthelmintic. However, the anthelminthic activity of silver nanoparticles biosynthesised using aqueous extract of *T. cordifolia* stem and leaves has not yet been demonstrated experimentally. Thus, the present study was designed to biosynthesise, characterise and evaluate its anti-nematode activity.

MATERIAL AND METHODS

Preparation of the plant extract

The leaves and stems of *Tinospora cordifolia* plant was collected from Erode district of Tamilnadu and authenticated from the Government Siddha Medical College, Palayamkottai, Tirunelveli, Tamilnadu. The plant material was collected, cleaned and rinsed thoroughly with distilled water and shade dried for 15 days. The dried plant material was finely powdered using a pulveriser. To 25 g of the powder 100 mL of sterile autoclaved distilled water was mixed and boiled for 15 minutes. Mixture was filtered through Whatman No.1 filter paper. Aqueous extract thus prepared was maintained at 4ºC until further investigations. The process was depicted in figure 1. Phytochemical screening of the herbal preparation was carried out using standard chemical methods to identify for the presence of various chemical constituents.

Biosynthesis of silver nanoparticles using plant extract

Silver nitrate ($AgNO_3$) was used at a concentration of 0.1 M, which was prepared by dissolving 4.25 g $AgNO_3$ in 250 mL double distilled water. To 10 mL of the aqueous extract of *T. cordifolia*, 240 mL of 0.1M aqueous $AgNO_3$ solution was added at room temperature. Reduction of silver ions takes place within 15 min at room temperature (Figure 2). The aqueous extract acts as reducing and stabilizing agent for 0.1M of $AgNO_3$. The prepared AgNPs were further characterized.

Characterisation of biosynthesised silver nanoparticles

The reduction of silver nitrate to pure Ag+ ions using aqueous extract of *T. cordifolia* stem was characterized by using the visual observation, particle size and Zeta potential values.

Visual observation

The visual evidence is used for the initial confirmation of the biosynthesised silver nanoparticles. The shift in colour slowly from colourless to brown and finally reddish brown, indicates the formation of silver nanoparticles.

Size

Dynamic light scattering and Scanning electron microscope (SEM) were employed to evaluate the particle size of the nanoparticle. Dynamic light scattering (DLS) was used to determine the particle size distribution of the silver nanoparticles, using the particle size analyzer SZ-100 (nano partica HORIBA Scientific). Measurement was carried out in triplicates after diluting the sample to a 1:100 ratio using milli-Q water.

The size and shape of silver nanoparticles were examined using a high-resolution scanning electron microscope (HR-SEM) (Quanta 200 FEG scanning electron microscope). The silver nanoparticle was sprinkled over a double-adhesive tape that was attached to an aluminium stub to create the samples for the SEM analysis. Then, using a gold sputter module in environment scanning electron microscopy (ESEM) mode, the stub was coated with gold while it was enclosed in an argon atmosphere. The SEM was then used to scan the coated samples and take photomicrographs.

Zeta potential

Zeta potential which is the key indicator of stability of particles. It was measured by Malveran zeta analyser. The particle size analyser SZ-100 (nano partica HORIBA Scientific) instrument was used to determine the zeta potential while it was in the electrophoretic light scattering (ELS) mode. The sample that had been dispersed was put in conventional glass cuvette and a helium neon laser with a wavelength of 658 nm was used to detect the zeta potential at 23ºC and a scattering angle of -14.06ºC. To determine the average zeta potential, three 1-minute measurements were made.

Egg hatch assay

Egg hatch assay (EHA) was carried out according to the techniques and recommendations of the World Association for Advancement of Veterinary Parasitology. Briefly, 2 ml of freshly collected eggs were placed in each well of a 24-well plate. Fenbendazole (0.1µL/ml) was used as a positive control. Aqueous extract and biosynthesised silver nanoparticles were added in different wells at 10, 20, 40 and 80 mg/ml concentrations, respectively and incubated for 48hrs. After incubation, two drops of Lugol's iodine were added and atleast 100 death/unhatched, embryonated eggs and hatched larvae were counted. ED_{50} was calculated using log probit analysis [7].

Statistical analysis

The data collected from egg hatch assay was subjected to Duncan's test [8].

RESULTS

Phytochemical screening of the herbal preparation

The phytochemical analysis of the plant extract revealed the presence of cardiac glycosides, terpenoids, flavonoids, hydrolysable tannin, glycosides and saponin.

Characterisation of biosynthesised silver nanoparticles

Visual observation

There was a colour change in the reaction mixture from colourless to reddish brown which indicated that silver nanoparticles were synthesized by using a green method through reduction of silver nitrate ($AgNO_3$) solution by plant extract (Figure 2-B).

Size

The average particle size of silver nanoparticles biosynthesised using *T. cordifolia* extract was found to be 2.8 nm, which confirmed the formation of nanoparticles (Figure 3). Scanning electron microscopic study revealed nanoparticles of irregular size (Figure 4).

Zeta potential

The zeta potential of silver nanoparticles biosynthesised using *T. cordifolia* extract measured was found to be -18.4 mv with peak area of 100% intensity. These values indicate the full stabilization of nanoparticles (Figure 5).

Egg hatch assay

The results of Egg hatch assay were presented in the table 1 and figures 6 and 7. In the present study silver nanoparticles of *T. cordifolia* and aqueous extracts of *T. cordifolia* induced significant

egg hatch inhibition in a dose dependent manner. Silver nanoparticles of *T. cordifolia* induced 60.29, 65.97, 79.76 and 90.82 per cent inhibition at 10, 20 mg/ml, 40mg/ml and 80 mg/ml, respectively. Similarly, the aqueous extracts of *T. cordifolia* induced 76.63 per cent inhibition at 80 mg/ml, which was comparatively less than the silver nanoparticles and the positive control-Fenbendazole (0.1µl/ml) showing 94.51 per cent of inhibition.

DISCUSSION

Endoparasites are pathogens with substantial global impact that pose a serious threat to food security and are a major issue in animal production globally. A few handfuls of synthetic anthelmintic medications are essentially the only means of controlling them. The risks of parasites developing drug resistance, the cost of medications for farmers in developing nations and the ineffectiveness of currently available medications for specific helminths are the major drawbacks of this reliance on chemotherapy. Hence, there is an urgent need for additional and supplementary helminth control methods. Plants and plant-derived phytochemicals offer a promising alternative to synthetic anthelmintic medications for the treatment of gastrointestinal helminth infections [9].

Tinospora cordifolia is an extensively used medicinal plant as a general tonic, antiperiodic, antispasmodic, anti-inflammatory, antipyretic, anti-arthritic, anti-lepritic, anti-allergic and anti-diabetic characteristics [6]. Reddy *et al.* [9] reported the anthelminthic activity against *Pheretima posthuma*, an Indian earthworm. Thus, the present study was targeted to evaluate the anthelminthic activity of aqueous extract of stem and leaves of *T. cordifolia* and the silver nanoparticles biosynthesised using the extract.

Phytochemical screening of plant extract

The aqueous extract of stem and leaves of *T. cordifolia* was subjected to phytochemical screening while revealed the presence of cardiac glycosides, terpenoids, flavonoids, hydrolysable tannin, glycosides and saponin, which was in accordance with the previous report of Sakthi Priya *et al.* [10]. Pradhan *et al.* [11] reported the presence of similar phytochemicals in the aqueous extract of stem of *T. cordifolia* except the absence of tannin in contrary to the present study, which may be due to the variation in the geographical location.

Characterisation of biosynthesised silver nanoparticles

Visual observation

The formation of silver nanoparticles was evinced by the colour change from colourless to reddish brown. The reddish-brown colouration was due to surface plasma resonance excitation [12] and reduction of silver (Ag^+) [13]. Prajwala *et al.* [14] reported a similar colour change into a dark brown colour while synthesising the silver nanoparticles using the aqueous extract of the leaves of *T. cordifolia.*

Size

Dynamic light scattering analysis of the silver nanoparticle revealed a particle size of 2.8 nm which confirmed the nanoparticle formation. Sakthi Priya *et al.* [10] reported size of a silver nanoparticle biosynthesised using aqueous extract of stem of *T. cordifolia* using DLS as 0.4 nm, which was in accordance with the present study. Singh *et al.* [13] reported a particle size of 35.4 nm of silver nanoparticles biosynthesised using whole plant extract of *T. cordifolia.* Scanning electron microscopic analysis revealed nanoparticles of irregular shape.

Zeta potential

The stability of nanoparticles in suspension and the initial adsorption of nanoparticles onto the cell membrane are both largely influenced by the zeta potential, which is dependent on the surface charge. The endocytic absorption rate after adsorption is influenced by particle size [15]. According to the zeta potential analysis, the silver nanoparticles biosynthesised were of appropriate stability. Sakthi Priya *et al.* [10] reported zeta potential of biosynthesised silver nanoparticles from aqueous extract of stem of *T. cordifolia* as -13.4mv with peak area of 100% intensity, which was comparable with the present study.

Egg hatch assay

The present study revealed that stem and leaves of *T. cordifolia* possesses anthelminthic activity. There was a significant dose-dependent inhibition of egg hatching in eggs treated with aqueous extract of *T. cordifolia*. The inhibition was higher at same doses of silver nanoparticles biosynthesised using aqueous extract of *T. cordifolia*, which was comparable to that of positive control. The reduced activity of the plant extract may be presumably due to small concentrations of the active ingredient in the plant extract. Moussouni *et al.* [16] evaluated the anthelminthic activity of aqueous and ethanolic extracts of *Marrubium vulgare* leaves against Bovine digestive Strongyles and found that both possessed ovicidal and embryotoxic activity.

Table 1: Percent Nematode egg hatch inhibition

Treatment Groups	Egg hatch inhibition (%)
Positive control	94.51^{f} ±0.80
AETC at 10 mg/ml	57.63^{a} ±0.63
AETC at 20 mg/ml	65.14^{b} ±1.84
AETC at 40 mg/ml	73.56^{c} ±0.61
AETC at 80 mg/ml	76.63^{c} ±1.17
SNTC at 10 mg/ml	60.29^{a} ±0.72
SNTC at 20 mg/ml	65.97^{b} ±1.54
SNTC at 40 mg/ml	76.76^{d} ±0.84
SNTC at 80 mg/ml	90.82^{e} ±0.82

Values are expressed as Mean ± SEM; n = 6; AETC: Aqueous extract of *T. cordifolia*; SNTC: Silver nanoparticles biosynthesised using aqueous extract of *T. cordifolia;* Means carrying different superscripts (from a-f) differs significantly.

Figure 1: Preparation of aqueous extract of *Tinospora cordifolia.* A- Dried plant material of *T. cordifolia;* B- Pulverised plant material; C- Extraction of plant material.

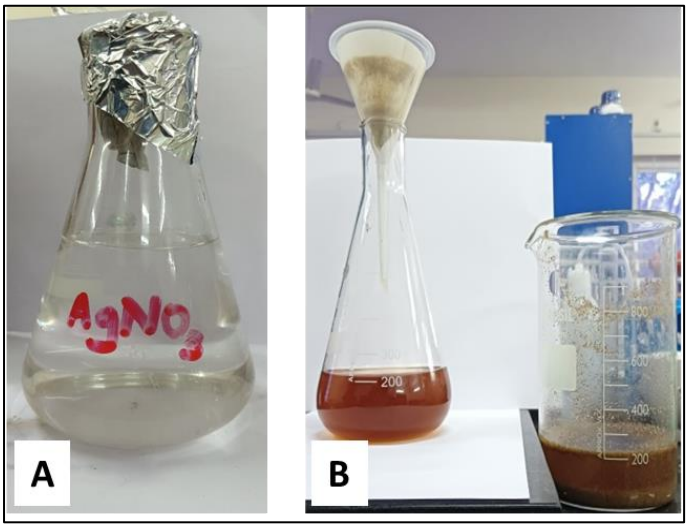

Figure 2: Synthesis of silver nanoparticles using aqueous extract of *Tinospora cordifolia.* A- Silver nitrate solution; B- Colour change from colourless to dark brown/ reddish brown indicating formation of silver nanoparticles.

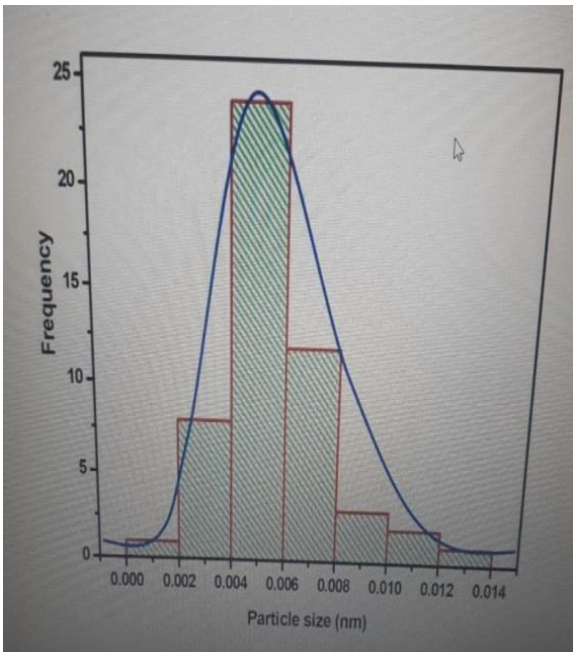

Figure 3: Particle size distribution of *Tinospora cordifolia* silver nanoparticles.

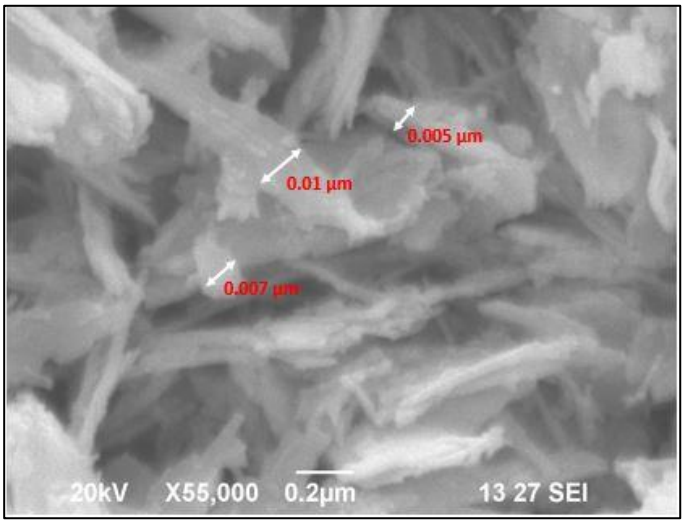

Figure 4: Characterisation of silver nanoparticles using scanning electron microscope.

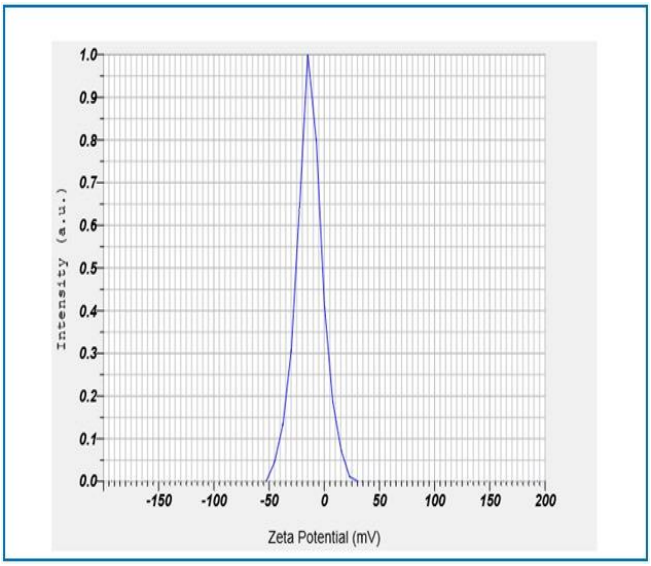

Figure 5: Zeta potential distribution of *Tinospora cordifolia* silver nanoparticles.

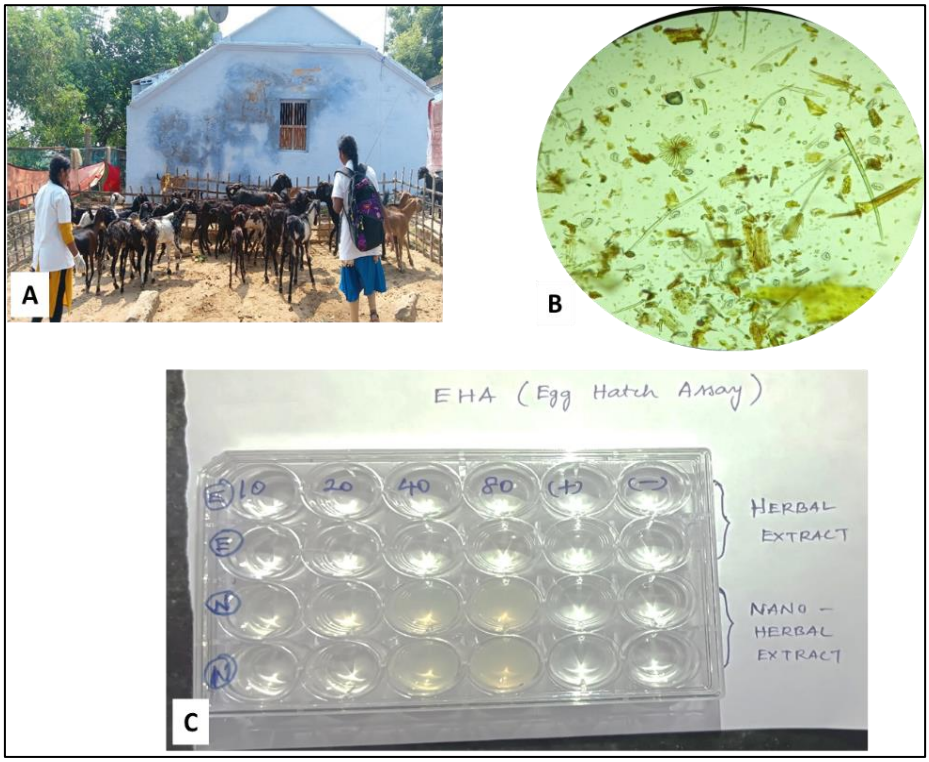

Figure 6: Egg hatch assay. A- Collection of faecal samples from small ruminants; B- Microscopic examination of faecal sample; C- 24-well plate, egg hatch assay.

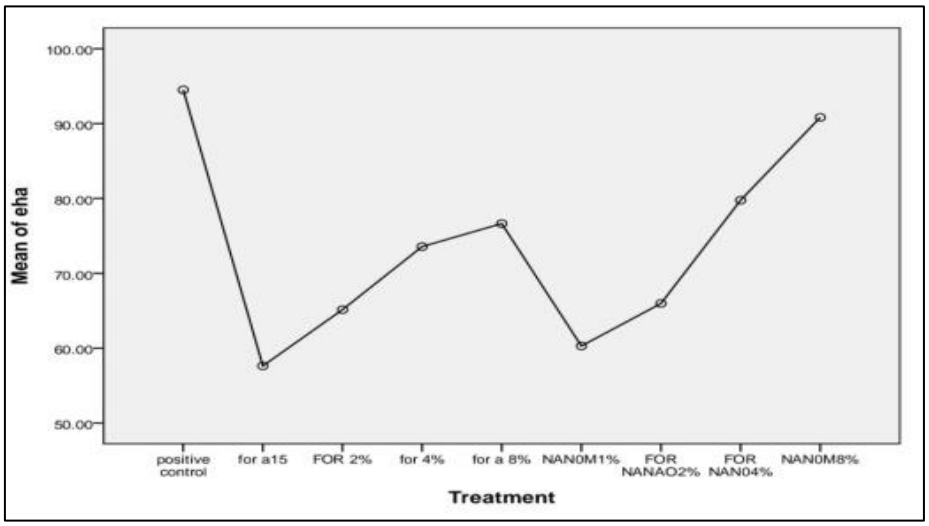

Figure 7: Effect of aqueous extract of *Tinospora cordifolia* and silver nanoparticles biosynthesised using the extract on hatching of nematode eggs.

CONCLUSION

The present study demonstrates biosynthesis of silver nanoparticles using aqueous extract of *Tinospora cordifolia* as a potential reducing and stabilizing agent. However, further investigations on the *in vivo* activity are warranted. So, it can be concluded that *T. cordifolia* has the potential to be established as an anti- nematode activity. But, the molecular mechanism along with the isolation of phytochemical constituents responsible for its anti- nematode activity has to be carried out in further studies.

Acknowledgments

The authors are thankful to Tamilnadu Veterinary and Animal Sciences University for providing the necessary facilities for the smooth conduct of the research work.

Conflict of Interests

The authors declare that there was no conflict of interests.

ORCID ID

V Ramakrishnan: https://orcid.org/0000-0002-4480-0144

P Gokul: https://orcid.org/0009-0000-6386-8518

R Hemalatha: https://orcid.org/0009-0005-8792-2271

AT Karthika: https://orcid.org/0009-0006-8815-4080

R Mohan Raj: https://orcid.org/0009-0004-3929-4816

R Shankar: https://orcid.org/0000-0002-2220-2645

REFERENCES

1. Shivakumara C, Kiran S. Economics of sheep and goat rearing under extensive, semi-intensive and intensive methods of rearing. Economic Affairs. 2019; 64(3):553-561.
2. Seetha A, Pandian AS, Thirunavukkarasu M, Senthilkumar S, Kumaravelu N. Constraints perceived by the small ruminant farmers of Tamil Nadu: The socio-economic determinants. Journal of Entomology and Zoology Studies. 2021; 9:953-956.
3. Varadharajan A, Vijayalakshmi R. Prevalence and seasonal occurrence of gastrointestinal parasites in small ruminants of coastal areas of TamilNadu. Journal of Veterinary Parasitology. 2009; 17:159-160.
4. Zirintunda G, Biryomumaisho S, Kasozi KI, Batiha GE, Kateregga J, Vudriko P, et al. Emerging anthelmintic resistance in poultry: can ethnopharmacological approaches offer a solution?. Frontiers in pharmacology. 2022; 12:3722.
5. Keerthika V, Nisha AR, Nair SN, John P, Ramnath V, Shankar R, Arya M. Phytochemical analysis and biosynthesis of silver nanoparticles from aqueous extract of seeds of Sesamum indicum. Journal of Veterinary and Animal Sciences. 2022; 53(2):262-268.
6. Joshi G, Kaur R. *Tinospora cordifolia:* a phytopharmacological review. International journal of Pharmaceutical sciences and research. 2016; 7(3):890-897.
7. Coles GC, Jackson F, Pomroy WE, Prichard RK, von Samson-Himmelstjerna G, Silvestre A, et al. The detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary parasitology. 2006; 136(3-4):167-185.
8. Snedecor GW, Cochran WG. Statistical methods 6th edition. The Iowa State University. 1967.
9. Reddy MR, Reddy KT, Vedamurthy AB, Krishna V, Hoskeri HJ. A study on anthelminthic activity of *Tinospora cordifolia* extracts. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(5):78-80.
10. Sakthi Priya M, Sarathchandra G, Jagadeeswaran A, Preetha SP, Partiban S. Synthesis, characterisation and pharmacological assessment of nanoparticles of *Tinospora cordifolia* for its cytotoxic activity. Journal of Pharmacognosy and Phytochemistry. 2020; 9(3):1901-1906.
11. Pradhan D, Ojha V, Pandey AK. Phytochemical analysis of *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms stem of varied thickness. International Journal of Pharmaceutical Sciences and Research. 2013; 4(8):3051-3056.
12. Vanaja M, Annadurai G. *Coleus aromaticus* leaf extract mediated synthesis of silver nanoparticles and its bactericidal activity. Applied nanoscience. 2013; 3:217-223.
13. Singh K, Panghal M, Kadyan S, Chaudhary U, Yadav JP. Antibacterial activity of synthesized silver nanoparticles from *Tinospora cordifolia* against multi drug resistant strains of *Pseudomonas aeruginosa* isolated from burn patients. Journal of Nanomedicine & Nanotechnology. 2014; 5(2):1-6.
14. Prajwala B, Gopenath TS, Prasad N, Raviraja S, Basalingappa MK. Green synthesis of silver nanoparticle by using *Tinospora cordifolia* leaf extract and its antimicrobial property. International Journal of Pharmaceutical Sciences and Research. 2021; 12(3):1881-1886.
15. Rasmussen MK, Pedersen JN, Marie R. Size and surface charge characterization of nanoparticles with a salt gradient. Nature communications. 2020; 11(1):1-8.
16. Moussouni L, Benhanifia M, Ayad A. *In vitro* anthelmintic effects of aqueous and ethanolic extracts of *Marrubium vulgare* leaves against bovine digestive strongyles. Türkiye Parazitolojii Dergisi. 2018; 42(4):262-267.

HOW TO CITE THIS ARTICLE
Ramakrishnan V, Gokul P, Hemalatha R, Karthika AT, Raj RM, Shankar R. *In vitro* anthelmintic activity of *Tinospora cordifolia* nanoparticles against strongyle nematode of small ruminants. J Phytopharmacol 2023; 12(2):76-81. doi: 10.31254/phyto.2023.12203