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Evaluation of Anthelminthic Activity of Nanoparticles of *Strychnos nuxvomica* on *Haemonchus contortus*

Bhuvanala Suresh Kumar* and Sunila Kumari Y.

Department of Zoology, University College for Women, (Osmania University), Koti, Hyderabad , Telangana, India

**Corresponding Author*

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Abstract: *Haemonchus contortus* (Barber's pole worm), is a significant common pathogenic nematode parasite of sheep and goats. This blood-feeding parasite can cause severe anaemia and rapid death in livestock. Though quite a number of synthetic analogues are available to treat the infection, there is overwhelming increase of drug resistance. In this regard, quite a number of plant extracts have been identified and tested for antihelminthic activity. Based on consultation with sheep farmers in Karimnagar District of Telangana state, it was revealed that the ethno medicinal plant extracts specifically *Strychnos nuxvomica* (Visha mushti tree) was used by them effectively to treat gastrointestinal parasites of sheep. Recent studies, suggest that metal nanoparticles infused with plant extracts are quite effective as anthelminthics. In the present study, *Strychnos nuxvomica* leaf extract was used in combination with silver and copper nanoparticles, along with aqueous and alcohol extracts. *In vitro* analysis of all these extracts upon *Haemonchus*, showed a greater antihelminthic (nematicidal activity) with copper nanoparticles. This was followed by silver nanoparticles, alcohol infused leaf extract and lastly the aqueous extracts, suggesting that *Strychnos nuxvomica* as leaf extract is a potent antihelminthic, and its properties were further enhanced when infused with silver and copper nanoparticles. Our study clearly suggests that *Strychnos nuxvomica* is a potent nematicide which may be considered as a natural drug alternative to synthetic analogues in future.

Keywords: *Strychnos nuxvomica*, *Haemonchus contortus*, Silver nanoparticles, Copper nanoparticles

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Introduction

Sheep husbandry is a billion dollar industry globally. However, sheep farming has suffered huge losses due to several gastrointestinal parasites. It has been seen that because of the parasites, the quality of wool, meat, milk and also mortality of the animal are greatly impacted (Gupta, 2006). Among the different helminth parasites, *Haemonchus contortus* tops the list globally (Perry *et al.*, 2002). Though quite a number of synthetic anthelminthics are available, to control *Haemonchus* infections, there are studies and evidences, which suggest the rapid

increase in drug resistance and this being transmitted quickly to upcoming generations specifically in *Heamonchus contortus* (Pavlína *et al.*, 2020). In this scenario of rapid progression of drug resistance against synthetic anhelminthics, traditional veterinary ethno medicinal plants seem as best alternatives, as they are a good source for biologically active compounds having valuable therapeutic and nutritional characteristics (Swargiary *et al.*, 2017). Though natural plants provide a green alternative to synthetic drugs, on the flip side, large quantities of plant materials have to be used to synthesize these drugs. In this regard, plant extracts infused with metal nanoparticles, offer a feasible solution. This provide, a two way advantage as, plant extracts can be used in minute quantities and also when they are infused with metal nanoparticles such as silver and copper they offer greater efficacy. Usability of silver and copper nanoparticles as antimicrobial and anthelminthic agents has also been reported (Jeong *et al.*, 2005; Yilleng, 2020). Practice of using silver nanoparticles infused with plant extracts to control *Haemonchus* infection in sheep has been reported (Spickett *et al.*, 2012; Avinash *et al.*, 2017; Ilavarashi *et al.*, 2019). The present study attempted to determine the efficacy of *Strychnos nuxvomica* leaf as a suitable anthelminthic against *Hemonchus contortus* using crude extract and metal (silver and copper) infused nanoparticles.

Materials and Methods

The *In vitro* investigation was carried out to evaluate the anthelmintic efficiency of Ethnomedicinal plant extract of the Leaf of *S. Nuxvomica* against *Heamonchus contortus*. Albendazole was used as a standard drug (positive control) for this assay. The experiment was conducted at the Laboratory of the Department of Zoology of University College for Women, (Osmania University), Koti, Hyderabad, India.

Collection of Strychnos nuxvomica:

S. nuxvomica is available in the forests of Telangana state in different regions namely forest of Manthani, Mancheryal, Eturunagaram, Manuguru, Saidapur etc. The fresh Leaves of *Strychnos nuxvomica* was collected in June 2022 from the village Saiadapur, District Karimnagar of Telangana, India.

Preparation of plant aqueous extract:

The leaves were washed thoroughly with distilled water and allowed to dry in the shade at room temperature. The dried leaves were crushed using an electric lab blender to make fine powder. and was stored in airtight containers for further analysis. This powder was used for preparation of various extracts. 25g fine powder of plant leaves were dissolved in 250 ml distilled water. The mixture was heated at 80 °C for 3 h with continuous stirring, and the resultant extract was then filtered through Whatman filter paper no. 1. Prepared aqueous extract consist 100 mg/ml concentration and the extract was stored at 4 °C until further use (Venkata Subbaiah *et al.*, 2014; (Pandey *et al.*, 2018a, b). With this various working concentrations, i.e.1.25 mg/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ml were prepared and 20 ml of each concentration was used for the experiment in each petri dish.

Preparation of Ethanol extract:

Ethanol extract was prepared using **S**oxhlet apparatus. 25 g fine leaf powder was dissolved in 250 ml of Ethanol for extracting secondary metabolites of plant parts and then dried at 20 ℃-25 ℃ and it was stored in airtight bottles at 4 ℃ until use **(**Sermakkani and Thangapandian*.,* 2012; Pandey *et al.*, 2018a, b). Various concentrations, i.e., 1.25 mg/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ml were used by taking 20 ml of each concentration.

Green Synthesis of Silver Nanoparticles:

For the green synthesis of silver nanoparticles, we used the crude aqueous extracts of *S. nuxvomica* plant leaves (Fig. 1). Synthesis of silver nanoparticles, was prepared as per the standard protocol suggested by Venkata Subbaiah *et al.* (2014). The formation of the AgNPs was preliminarily detected by the change in colour

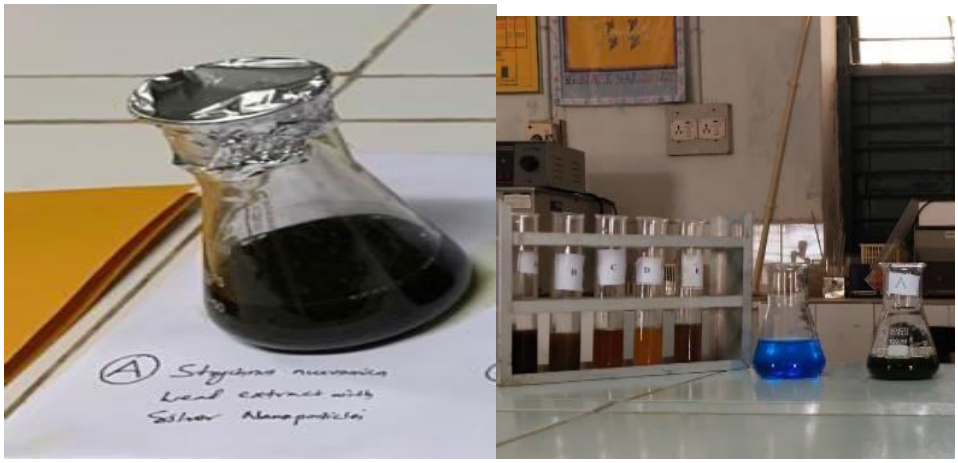

Fig. 1: Extract with Silver NPs., Copper NPs.

from yellow to dark brown. Various concentrations i.e., 1.25 mg/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ml were prepared. After preparation 20 ml of each concentration was used. The green-synthesized nanoparticles were separated using centrifugation at 15,000 rpm for 20 min. This process was repeated thrice to get rid of free silver associated with AgNPs. The final green-synthesized silver nanoparticles (AgNPs), were freeze-dried and then stored at 4 °C until further use.

Green Synthesis of Copper Nanoparticles:

For the green synthesis of Copper nanoparticles (Fig. 1), 50 ml of plant aqueous extract was mixed with 200 ml of 0.2 M $Cu(NO_3)_2{\cdot}3H_2O$ solution (1 : 4) slowly drop wise with constant stirring. The mixture was incubated at room temperature for 24 h. The colour change was checked periodically (after 30 min and 60 min). The change in colour from blue to light brownish indicated the formation of CuNPs. Then, the solution was centrifuged for 15 min at 10000 rpm. The obtained Cu NPs were washed by deionized water and ethanol to remove any impurities. Thereafter, the NPs were allowed to dry and ground so as to be used for further analysis. Various concentrations, i.e., 1.25 mg/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ml to were used. 20 ml of each concentration was used.

Collection of Gastro intestinal parasites from infected Sheep:

The Gastro intestinal parasites were collected from the slaughtered sheep. The stomach and intestine of the sheep were brought in to the laboratory and dissected to observe the parasites, After tracing the *Haemonchus contortus*, they were separated from the gut with the help of brush and transferred to the sterilized petri dishes (Fig. 2). After that motile worms were collected and cleaned with lukewarm water and normal saline (0.9 %). The cleaned worms were transferred into Petri dishes containing PBS at room temperature.

In vitro Antihelminthic Assay:

Five actively motile and equal sized worms were selected and subjected in petri dishes having 1.25 mg/ml, 2.5 mg/ml, 5 mg/ml and 10 mg/ ml concentration of 20 ml volume of aqueous extract, ethanol extract, green synthesized silver and copper nanoparticles serially. Each concentration was set up as three replicates. Parasites were observed for the time taken for paralysis and finally, the death of the individual worms at 1, 2, 3, 4, 5, 6, 7, and 12 h and readings were recorded in minutes. The paralyzed worms were suspended in phosphate buffer saline for 30 min after each interval of time, for attainment the possible rescue of the parasite motility. After the completion of the assay the alive and dead worms were observed under a dissecting microscope for

Fig. 2: Isolated *Haemonchus contortus* parasite from abomasum of sheep.

the confirmation of death. The paralysis time was analysed on the basis of the behaviour of the parasites i.e. no retrieval in motility even after placing in PBS where as death was determined on the basis of the complete loss of motility with discoloration in body colour as suggested by Iqbal *et al*. (2001). After 24 h, the test solution was washed away and the parasite was again suspended in PBS for about 30 min for possible recovery of parasite motility (Hounzangbe-Adote *et. al*. 2005).

Results and Discussion

The results obtained regarding the Nematocidal efficacy of *in vitro* assay with the four tested extracts, ethanol, aqueous extracts and infused with Silver and Copper nanoparticles of *S. nuxvomica* leaf are presented in Table 1.

In the present study the results of *in vitro* anthelminthic assay was assessed as the time consumed to paralyse and kill the worms in all the tested concentrations of the *S. nuxvomica* leaf extracts (aqueous, ethanol extract, leaf extract with silver and copper nanoparticles). All the extracts showed the concentration dependent nematicidal efficacy against the *Haemonchus* sp. Antihelminthic activity was tested using Albendazole as positive control. The plant extracts were tested for mortality of *H. contortus* using four different concentrations 1.25, 2.5, 5, and 10 mg/ml, respectively. Each concentration was used as a set for three replicates.

In the present study, nematicidal activity of *Strychnus nuxvomica* upon *Haemonchus* was evaluated *in vitro.* Four different extracts (aqueous, alcohol, silver nanoparticles, and copper nanoparticles) using *S. nuxvomica* leaves were prepared and tested using albandezole as the standard or positive control. Our results showed maximum nematicidal activity when leaves of *S. nuxvomica* were infused with copper nanoparticles, followed by silver nanoparticles, alcoholic extract and aqueous extract suggesting that *S. nuxvomica* leaves are potent anthelminthics and this activity became more pronounced when it was blended with metal nanoparticles. These observationsare in conformity with the results of Patekar *et al.* (2017) who have reported the antheminthic activity of *Strychnos colubrine* L. and in their study revealed the presence of two major alkaloids namely brucine and strychnine, which seem to be responsible for the major medicinal properties. A similar kind of observation was reported by Ahn *et al.* (2014). In this study silver nanoparticles effectively killed nematodes. Another study by Kumar *et al*. (2023) reported that silver nanoparticles infused with plant (*Viscum orientale*) extracts can be potent anti-bacterial and anthelminthic agent. In addition to silver nanoparticles, copper nanoparticles have also been effectively used as anthelminthics (Shafienejad Jalali *et al.*, 2021). These evidences, clearly indicate that metal nanoparticles, either independently or infused with plant extracts, seem to have potent anthelminthic properties,

Table 1: *In vitro* anthelmintic assay of Leaf Aqueous Extract, Ethanol Extracts, Ag.N.Ps . and Cu.N.Ps of *Strychnos nuxvomica* against *Haemonchus* spp.

S. No.	Test Substance	concentration	Concentration in petri dish (20ml)	Exp 1	Exp 2	Exp 3	Time in Min for the death of *Haemonchus*
1	**Control**						-
2	**Standarad (Albendazole)**	10 mg/ml	200 mg/20 ml	67	66	67	66.6
3	***S. nuxvomica* - Leaf aqeous extract**	1.25 mg/ml	25 mg/ml	70	65	58	64.3
		2.5 mg/ml	50 mg/ml	60	58	63	60.3
		5 mg/ml	100 mg/ml	58	55	53	56.6
		10 mg/ml	200 mg/20ml	52	49	50	50.3
4	**Ethanol extract**	1.25 mg/ml	25 mg/ml	55	50	55	53.3
		2.5 mg/ml	50 mg/ml	46	47	47	46.6
		5 mg/ml	100 mg/ml	44	42	39	41.6
		10 mg/ml	200 mg/20ml	42	41	39	40.6
5	**Silver Nanoparticles AgNPs)**	1.25 mg/ml	25 mg/ml	52	50	50	50.6
		2.5 mg/ml	50 mg/ml	46	44	45	45
		5 mg/ml	100 mg/ml	40	41	40	40.3
		10 mg/ml	200 mg/20ml	38	37	35	36.6
6	**Copper Nano Particles (CuNPs)**	1.25 mg/ml	25 mg/ml	50	49	45	48
		2.5 mg/ml	50 mg/ml	38	36	37	37
		5 mg/ml	100 mg/ml	34	30	32	32
		10 mg/ml	200 mg/20 ml	31	29	27	29

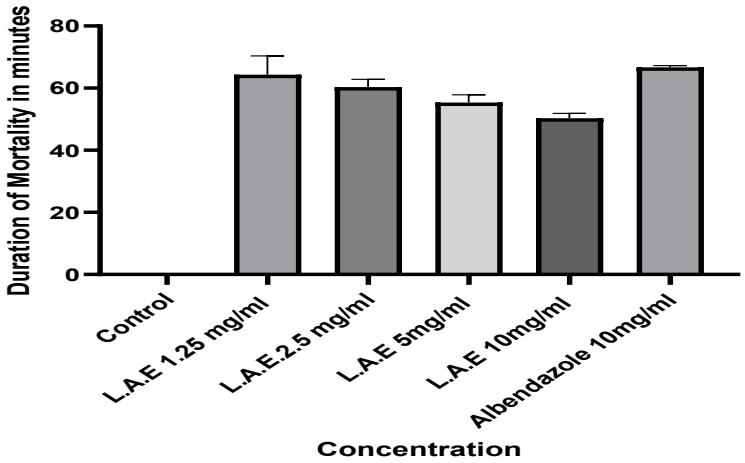

Fig. 3: Illustrates that *S. nuxvomica* leaf aqueous extract on *H. contortus* the average time taken for parasite mortality is 64.3, 60.30, 56.6, 50.3 min, respectively with reference to standard .

thereby making them the most sought after green alternatives to synthetic anthelminthic drugs. Therefore our study regarding copper and silver nanoparticles infused with *S. nuxvomica* extracts showed potent anthelminthic activity on *Haemonchus*, confirming and agreeing with the previous studies. In summary it may be said that *Strychonus nuxvomica* infused with silver

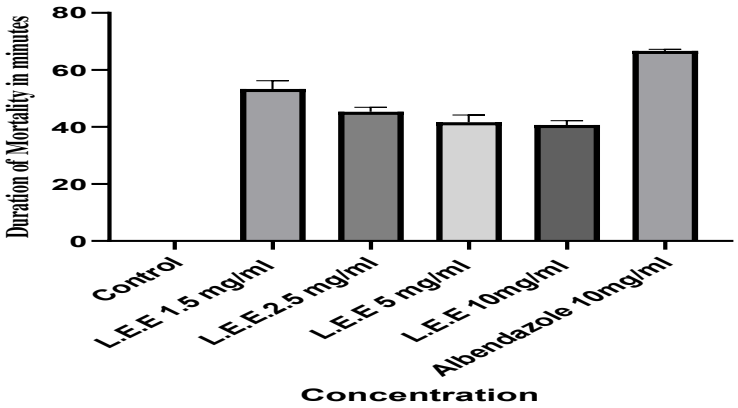

Fig. 4: Illustrates that the Anthelminthic efficacy of *S. nuxvomica* leaf Ethanol extract on *H. contortus* was assessed at three different concentrations 1.25, 2.5, 5,10 mg/ml, the average time taken for their mortality exhibited as 53.3, 46.6, 41.6, 40.6 min respectively, with reference to standard Albendazole is 66.6 min.

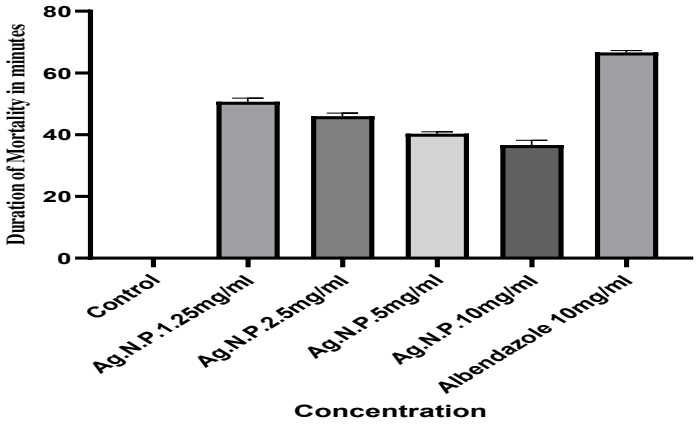

Fig. 5: In case of *S. nuxvomica* - Leaf aqueous extract with Silver nanoparticles on *H. contortus*it is 50.6, 45, 40.3 and 36.6 min to respective concentration.

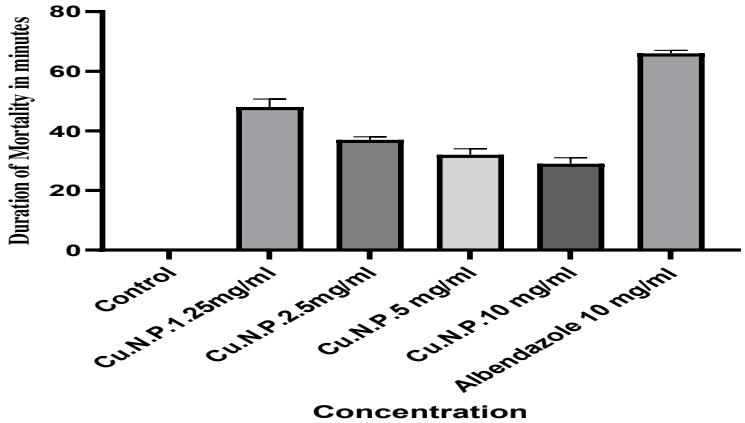

Fig. 6: The Nematicidal efficacy of *S .nuxvomica* Leaf aqeous extract with Copper nanoparticles (CuNPs) on *H. contortus* was assessed at three different concentrations 1.25,2.5, 5,and 10 mg/ml the time taken for parasite mortality is 48, 37, 32 and 29 min, respectively with reference to standard.

and copper nanoparticles seem to be effective as anthelminthics against *Haemonchus* and specifically copper nanoparticles, blended with nuxvomica leaf extract was more effective. Further *in vivo* studies are recommended in this direction before it can be approved as best suited green alternative to synthetic antihelmithic.

Conclusion

The present study revealed that the efficacy of nematicidal activity of *Strychnus nuxvomica* is more effective with the green synthesized copper nano particles in comparison with other extracts. Therefore copper nanoparticles blended with *Strychnos nuxvomica* may be suggested as best therapeutic alternative to pharmaceuticals.

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