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

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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 anthelminthic 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 anthelmintics. 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 anthelminthic (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 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 anthelmintics 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 *Haemonchus contortus* (Pavlina 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 anthelmintic 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 anthelmintic against *Haemonchus 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 *Haemonchus 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 Soxhlet 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 °C-25 °C and it was stored in airtight bottles at 4 °C 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
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$_3$)$_2$·3H$_2$O 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 Anthelmintic 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
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 anthelmintic 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 albendazole 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 anthelmintics and this activity became more pronounced when it was blended with metal nanoparticles. These observations are in conformity with the results of Patekar et al. (2017) who have reported the anthelimthic 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 antibacterial and anthelmintic agent. In addition to silver nanoparticles, copper nanoparticles have also been effectively used as anthelmintics (Shafienejad Jalali et al., 2021). These evidences clearly indicate that metal nanoparticles, either independently or infused with plant extracts, seem to have potent anthelmintic 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.

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

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 anthelmintic drugs. Therefore our study regarding copper and silver nanoparticles infused with *S. nuxvomica* extracts showed potent anthelmintic 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 aqueous 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 anthelmintics 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 *Strychnos nuxvomica* is more effective with the green synthesized copper nanoparticles in comparison with other extracts. Therefore copper nanoparticles blended with *Strychnos nuxvomica* may be suggested as best therapeutic alternative to pharmaceuticals.

**References**


