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In vitro Anthelmtic Activity of *Nyctanthes arbortristis* Leaves Against *Ascaridia galli*

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ABSTRACT

The present study was undertaken during August 2018 to October 2019 to investigate the anthelmtic activity of crude extracts prepared from leaves of *Nyctanthes arbortristis* in water (AE), ethanol (EE) and hydro-ethanol (HEE) against *Ascaridia galli*. Adult *Ascaridia galli* of nearly equal size were divided into groups of six worms and placed in petri-dishes containing 25ml of phosphate buffer saline solution (PBS). They were exposed to the extracts at the rate of 10 mg ml^{-1} and 50 mg ml^{-1}, respectively and observed for mortality at every 15 m, 30 m, 1 h, 2 h and 4 h of exposure. AE exhibited 100% mortality of the worms after 6 h of exposure irrespective of the concentrations used. In case of EE, 100% mortality was observed after 4 h of exposure at a concentration of 10 mg ml^{-1} while the exposure time was reduced to 2 h at a concentration of 50 mg $^{-1}$ml with same efficacy. Similarly, at a concentration of 10 mg ml^{-1} and 50 mg ml^{-1}, the exposure time was 4 h and 1h, respectively when exposed to HEE. The results suggest that *in vitro* anthelmintic activity of AE of *N. arbortristis* against *Ascaridia galli* was not concentration-dependent, but, time-dependent. On the other hand, anthelmintic activity of EE and HEE was both concentration dependent and time dependent. The hydro-ethanolic extract was found to be comparatively better among all the 3 extracts and was at par with piperazine hydrate when compared.

KEYWORDS: Anthelmintic, *Ascaridia galli*, *Nyctanthes arbortristis*, *Piperazine hydrate*

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Helminthiasis or worm infestation is one of the major problems in both man and animals. It has been estimated that one fourth of the world population is being infested with worms. In regards to animals, it is a major productivity constraint for the farmers, especially in the case of small ruminants in the tropics and subtropics (Thomson and Geary, 1995). Infestation with gastrointestinal helminth parasites has been considered as the most common and economically important among diseases of grazing livestock (Perry et al., 2002). Adulteration of anthelmintics seems to be a common practice (Monteria et al., 1998) leading to poor efficacy of available marketed anthelmintics. In addition, these drugs are relatively expensive. Therefore, pastoralists and small holder farmers have been continuously exploring the use of indigenous medicine as alternative livestock dewormers (Danoe and Bogh, 1999). Research has shown that plants not only contribute to nutrition of animals, but also have anti-parasitic effects (Thomson and Geary, 1995, Danoe and Bogh, 1999).

Nyctanthes arbortristis Linn (Oleaceae), commonly known as Harsinghar or Night Jasmine, is one of the commonly available medicinal plants. It is widely distributed along subtropical, tropical to sub - Himalayan regions in the South East Asia and has been extensively used as a therapeutic agent in the ayurvedic, sidha and unani systems of medicines (Waghorn and McNabb, 2003). Different parts of the plant are also known to be used for various ailments by rural folk mainly tribal people of India (Satyal et al., 2012). Crude extract of the plant earlier showed anti-leishmanial activity (Shukla et al., 2011). Both crude extract and ursolic acid, a triterpenoid isolated from ethyl-acetate fraction of crude extract prepared from leaves of *N. Arbortristis* were reported to have significant micro-filaricidal activity against *Setaria cervi* and *Wuchereria bancrofti* as well as macrofilaricidal activity against *S. cervi* (Saini et al., 2014). In several *in vitro* studies, extract prepared from leaves (Meghashri and Gopal, 2012) and flowers (Hussain and Ramteke, 2012) of *N. Arbortristis* were found to have anti-oxidant activity and the anti-oxidant activity might be due to the presence of high phenolics and flavonoids content apart from its reducing action. Not only that, the crude extract prepared from flowers of the plant exhibited hypoglycaemic and hypolipidemic properties (Rangika et al., 2015). Similarly, administration of fresh leaves of the plant to malaria patients also showed a disease modifying activity with early clinical recovery accompanied with a decline of TNF-α and gradual clearing of malaria parasite (Godse et al., 2016). In addition, crude extract of *N. Arbortristis* leaves showed anti-inflammatory and anti-arthritic activity (Uroos et al., 2017). In yet another study, extract prepared from *N. Arbortristis* flowers showed anti- AML (adult acute myeloid) and anti-CLL (Chronic Lymphocytic Leukaemia) efficacy on apoptosis assay indicating that the flowers of this plant also have anti-cancer activity (Heendenya et al., 2020).

In regards to its effect on helminth parasite, hydro-ethanolic extract of the leaves of the plant was shown to be effective against earthworm (Shruti et al., 2009). But there is no scientific data about the potential use of the plant against either human or animal parasites. Keeping this view in mind, the present *in-vitro* study was carried out to see the anthelmintic activity of crude extract of the leaves of *N. arbortristis* Linn prepared using different solvent extracts against avian parasite *Ascaridia galli*.

2. MATERIALS AND METHODS

2.1. Collection of Plant material and preparation of extracts

The present work was carried out between August, 2018 and October, 2019. The leaves of *N. arbortristis* were collected from in and around Khanapara, Guwahati, Assam, India. The leaves were dried at room temperature (22–28°C). 3 extracts of the plant were prepared using distilled water (AE), ethanol (EE) and hydro-ethanol (HEE) at the ratio of 1:1, respectively, as solvent in soxhlet apparatus for 8–10 h. The % yield of extracts was 14.56, 17.0 and 27.56 for AE, EE and HEE, respectively and the yield of plant constituents was dependent on extraction based on increasing polarity of solvent. After completion, the extracts were pressure dried and transferred into clean and dried airtight vials.

2.2. Analysis of chemical constituents

The extracts were subjected to qualitative analysis for presence of steroids, alkaloids, phenolic compounds, tannins, flavonoids, glycosides, and tri-terpenes using standard protocol.

2.3. Acute toxicity studies

Male albino wistar rats (150–200 g) were used to study acute toxicity using all three extracts of *N. arbortristis* plant as per Organization of Economic Corporation Development (OECD) guidelines- No. 425. The rats were housed in polypropylene cages with clean bedding material with a 12 h light and dark cycle. They were provided with rodent pellet diet and clean drinking water throughout the experiment. The extracts were given orally to a group of rats (n=3) @ 2g Kg^{-1}. Animals were kept fasting overnight and water was given *ad libitum* prior to the study. They were observed for mortality up to 48 h and for any behavioural abnormalities up to 14 days.

2.4. Worm collection and anthelmtic activity

Ascaridia galli worms were collected from local poultry market in and around Khanapara, Guwahati-781022,

Assam, India. The anthelmintic activity was carried out as per the standard method (Ajayeoba et al, 2001) with minor modifications. For initial evaluation of anthelmintic activity *in-vitro*, earth worms were used because of easy availability and also for their anatomical and physiological resemblance with the intestinal round worm parasite of man and animals. Thereafter, adult *Ascaridia galli* of nearly equal size were used for evaluation of anthelmintic activity of the extracts *in-vitro*. They were divided into groups of 6 worms and placed in petri-dishes containing 25ml of phosphate buffer saline solution (PBS) (composition: NaCl 0.8 g, KCl 0.2 g, Na_2HPO_4 1.42 g and KH_2PO_4 0.24 g in 1000 ml distilled water). 2 concentrations (10 and 50 mg ml^{-1}) of each extracts were used at 15 m, 30 m, 1 h, 2 h, 4 h (6 h for AE) of post exposure time to determine whether the anthelmintic activity was dependent on both the dose as well as exposure time. Thus, 3 groups (n=3) were used for each concentration of the extracts and 1 group of piperazine hydrate as standard was used for comparison.

Time of paralysis was considered as when no movement of any sort was exhibited by the worms. Similarly, time of death of the worms was considered as when no movement of the worms, even after being shaken vigorously or being dipped in warm water (50°C), was observed.

3. RESULTS AND DISCUSSION

3.1. Chemical constituent

The qualitative analysis of all the three extracts carried out in the present study showed presence of steroids (with exception of Salkowski's test), alkaloids (with exception of Wagner's test), phenolic compounds, tannins, flavonoids, glycosides, and tri-terpenes. The aqueous extract was negative for phenolic compounds. Similarly, foam test showed negative for saponins in both hydro-ethanolic and aqueous extract.

3.2. Acute toxicity studies

Acute toxicity studies did not show mortality of any rat administered with AE, EE or HEE of *N. arbortristis* at the rate of 2 g kg^{-1} body weight within 48 h. In addition, the rats also did not show symptoms of either behavioural change or gross abnormality up to 14 days. Therefore, the extracts were considered to be safe up to a maximum dose of 2 g kg^{-1} in the present study.

3.3. Anthelmintic activity

The results of the anthelmintic activity determined by % mortality of the worms against AE, EE and HEE of *Nyctanthes arbortristis* leaves have been presented in the table and in figures (bar diagrams). AE did not show any concentration-dependent increase in % mortality of the worms up to 1 h of exposure as depicted in the Table 1 and Figure 1. However, after exposure from 2 h onward, AE showed some concentration-dependent increase in mortality of the worms. But, 100% mortality of the worms could be achieved only after 6 h of exposure at both 10 mg ml^{-1} and 50 mg ml^{-1}. Thus, even the lowest concentration of AE could exhibit anthelmintic activity when the worms were exposed for longer duration of time.

In case of EE, concentration-dependent increase in mortality of the worms was not observed until 30 m of exposure. Beyond 1 h onward of exposure, although there was slight concentration-dependent increase in mortality, 100% mortality was observed after 4 h of exposure with 10 mg ml^{-1} and after 2 h of exposure with 50 mg ml^{-1} of EE, respectively (Figure 2). Therefore, when lower concentration was used, longer duration of exposure time was needed while higher concentration needed shorter duration of exposure time.

Interestingly, HEE showed concentration-dependent mortality after exposure of the worms from 15 m onward.

Table 1: % mortality of A. galli worms after exposure to 10 mg ml^{-1} and 50 mg ml^{-1} of AE, EE, HEE and P, respectively at different time intervals

Drug	Concentration	0 m	15 m	30 m	1 h	2 h	4 h
AE	10 mg ml^{-1}	0	5.6±5.6	5.6±5.6	11.13±5.6	33.3	38.9±5.6
AE	50 mg ml^{-1}	0	5.6±5.6	11.13±5.6	11.13±5.6	55.6±5.6	72.2±5.5
EE	10 mg ml^{-1}	0	5.6±5.6	11.13±5.6	22.2±5.5	38.9±5.6	100
EE	50 mg ml^{-1}	0	11.13±5.6	22.2±9.6	38.9±5.6	100	100
HEE	10 mg ml^{-1}	0	11.13±5.6	16.7±9.6	22.2±5.5	33.3±9.6	100
HEE	50 mg ml^{-1}	0	88.9±5.6	94.4±5.6	100	100	100
P	10 mg ml^{-1}	0	83.3±0	88.9±5.6	88.9±5.6	100	100
P	50 mg ml^{-1}	0	100±0	100	100	100	100

The values represent mean±SEM (n=3 for each group). (AE: Aqueous Extract; EE: Ethanolic extract; HEE: Hydro-ethanolic extract; P: Piperazine)

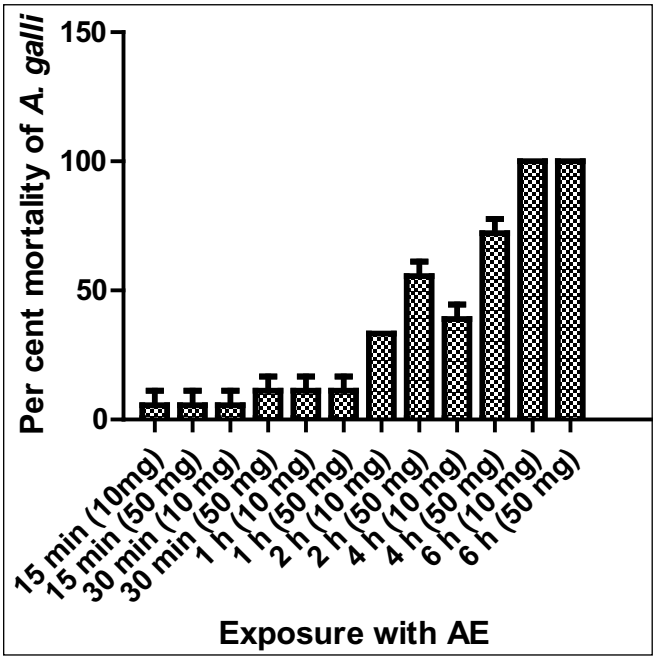

Figure 1: effect of AE (10 mg ml^{-1} and 50 mg ml^{-1}) of *N. arbortristis* Linn on % mortality of *A. galli* worms at different time interval of exposure up to 6 h. Note that there was no concentration-dependent increase in % mortality when the worms were exposed at different time intervals. However, 100% mortality was achieved following 6 h of exposure time irrespective of the concentrations used

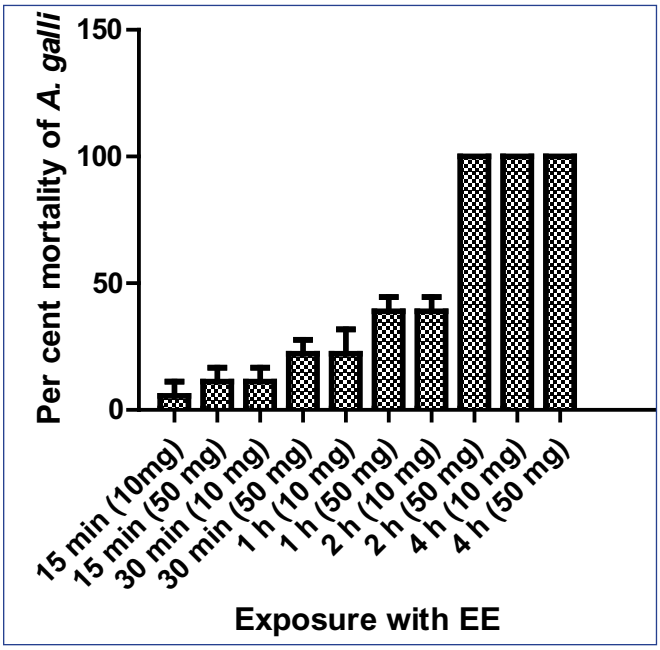

Figure 2: effect of EE (10 mg ml^{-1} and 50 mg ml^{-1}) of *N. arbortristis* Linn on % mortality of *A. galli* worms at different time interval of exposure up to 4 h. Note that 100% mortality was achieved with 10 mg ml^{-1} after a long exposure of 4 h while higher concentration of 50 mg ml^{-1} yielded the same result at a shorter duration of 2 h

The lower concentration showed a similar pattern of mortality with that of EE up to 2 h of post exposure time and 100% mortality was achieved after 4 h. On the other hand, higher concentration of HEE (50 mg ml^{-1}) exhibited higher percentage of mortality from 15 m onward of exposure time unlike EE and 100% mortality of the worms was observed immediately after 1 h of exposure (Figure 3). Thus, when higher concentration of HEE was used, approximately 90% mortality was observed following 15 m of exposure. The effect of HEE was comparable to standard drug piperazine.

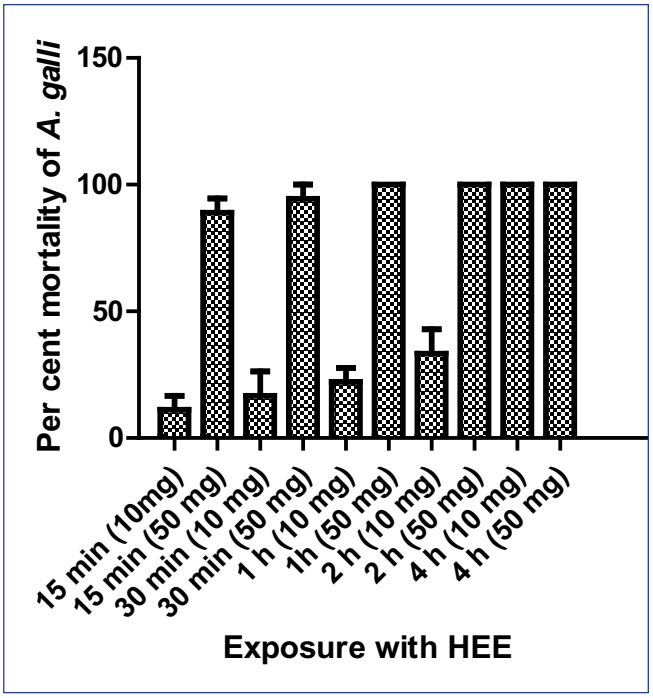

Figure 3: Effect of HEE (10 mg ml^{-1} and 50 mg ml^{-1}) of *N. arbortristis* Linn on % mortality of *A. galli* worms at different time interval of exposure up to 4 h. Note that 100% mortality was achieved with 10 mg ml^{-1} after a long exposure of 4 h while higher concentration of 50 mg ml^{-1} yielded the same result at a much shorter duration of 1 h

Higher efficacy (100%) of plant extract was earlier observed with alcoholic extract rather than with aqueous extract against gastrointestinal nematode in goat. This might be due to the fact that the active plant constituents are more extractable in alcoholic solvent than in aqueous solvent. In our study, we have found that hydro-alcoholic extract showed better results than alcoholic extract alone.

In the present study, it was observed that exposure of the *A. galli* worms to *N. arbortristis* extracts caused paralysis followed by death of the worms. Piperazine is known to cause death of the worms by increasing chloride ion conductance of muscular membrane of worm and thereby reducing excitability and producing hyper-polarization and flaccid paralysis. However, the present study cannot

explain whether death of the parasites caused by exposure of *N. Arbortristis* extracts was due to increase in chloride ion conductance in a way similar to piperazine. One possible explanation of paralysis and death caused by *N. arbortristis* might be due to the presence of tannins in the extracts which bind to glycoprotein on the cuticle of the worms causing death (Shruti et al., 2009).

4. CONCLUSION

Crude extracts of *N. arbortristis* leaves prepared in water, ethanol and hydro-ethanol exhibited promising anthelmintic activity against *A. galli* in *in-vitro* study. The anthelmintic activity of aqueous extract was time-dependent while hydro-ethanolic and ethanolic extracts were both concentration-dependent and time-dependent. The hydro-ethanolic extract was better than ethanolic extract alone and was equivalent with that of standard drug piperazine. Further studies are required to identify the phyto-constituents responsible for anthelmintic action as well as to determine its efficacy using *in-vivo* model.

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