In vitro Anthelmintic Activity of Mentha longifolia (L.) Leaves Against Ascardia galli

Javid Ahmad, Syed Tanveer and Bilal A. Zargar

Abstract: The in vitro anthelmintic activity of the aqueous and hydroalcoholic extracts of Mentha longifolia against Ascardia galli was evaluated in comparison to Fenbendazole. In folklore medicine, M. longifolia (Lamiaceae) is used in the treatment of various gastrointestinal ailments and other conditions. Worm Motility Inhibition (WMI) assay was used for in vitro assessment that revealed anthelmintic effects of crude hydroalcoholic extract (CHE) and crude aqueous extract (CAE) of M. longifolia on live Ascardia galli worms as evident from their paralysis and/or death at 6 h after exposure. Different concentrations (25 mg ml⁻¹ and 50 mg ml⁻¹) of aqueous and hydroalcoholic extracts were used against A. galli which exhibited concentration and time dependent anthelmintic effects on A. galli. It is concluded that the leaves of M. longifolia possesses significant anthelmintic activity and could be a potential alternative for treating cases of A. galli infections in chickens.

Key words: Mentha longifolia • Anthelmintic • Ascardia galli • In vitro • Kashmir

INTRODUCTION

Domestic fowl (Gallus gallus domesticus) is reared in most parts of the world either in the backyard or commercial production system. It acts as the source of meat, eggs, feathers and organic manure of high fertility. However, parasitism presents a main threat to the indigenous poultry production and cause heavy economic losses in the production of meat and eggs. Helminthosis is considered one of the most common diseases that affect free-range backyard chickens[1,2]. Like in other parts of the world, chickens are reared as the main backyard poultry bird in the households of Kashmir Valley and are known to be infected with different parasitic helminthes [3-6]. A. galli shows the highest prevalence in different poultry production systems [7-9]. It is known to cause droppiness, hemorrhages and diarrhea in heavily infected chickens [10,11]. A. galli not only damages the integrity of intestinal mucosa but also affects utilization of nutrients, which results in reduced weight gain [12,13]. Heavy infection of A. galli may cause blockage of intestinal lumen and death of the host [14,15]. A. galli infections also result in economic losses due to treatment cost, decreased feed efficiency and impaired performance [10, 16]. A. galli acts as a vector for some infectious organisms like Salmonella enterica and Escherichia coli [17,18]. The development of drug resistance by helminthes against chemotherapy products and the associated risks of chemical residues in poultry products have drawn the attention to alternative approaches.

Different approaches have been utilized for the sustainable control of A. galli infections in poultry birds like utilization of genetic resistance [19-21], nutrition of host animal [13], biological control [22] and the use of plants with anti-parasitic properties as well as the use of traditional herbal remedies [23,24]. Because of easy availability, the use of A. galli worms as a suitable model for the screening of anthelmintic drugs had been advocated earlier [25-28]. To our knowledge, no scientific studies are available on the use of M. longifolia extracts against A. galli. Therefore, the present study was carried out to scientifically evaluate the in vitro and in vivo anthelmintic activity of different M. longifolia extracts against the common poultry nematode, A. galli.
MATERIALS AND METHODS

Collection of Plant Material: Mentha longifolia (L.) L. is the scientifically accepted name for Horse Mint, locally known as Vena or Yena (family Lamiaceae). It grows along streamsides, waysides, bogs, irrigation channels and borders of paddy fields throughout the Kashmir Valley. Kashmir valley is a temperate North West Himalayan region of Jammu and Kashmir State in India and lies between 33°20’ and 34°54’N latitudes and 73°55’ and 75°35’E longitudes, covering an area of about 15,948 sq km [29].

The plant material was collected from the stream sides and waysides of Bugam-Chadoora area of District Budgam,Kashmir. The mature plant material at the peak of flowering was collected in polythene bags and processed by standard technique adopted by Kashmir University Herbarium (KASH). The labeled fresh specimen was discussed with local people regarding its uses/abuses. The data were reconfirmed by discussing the plants usage with other populations. The plant was identified and authenticated by a plant taxonomist, Dr. Aijaz Ahmad at the Department of Botany, University of Kashmir, Srinagar, India. A voucher specimen (Voucher no.401) was deposited in KASH.

Preparation of Plant Extracts: The whole plants collected were processed for shade drying in a well-ventilated room (Drying Room at Animal House, Development of Zoology, University of Kashmir, Srinagar). The dried leaves were powdered mechanically in electric grinder. The powdered plant material was then stored in an airtight container at 4°C until extraction. Two types of extracts (aqueous and methanolic) of the powdered plant leaves were prepared following standard procedures. The crude aqueous extracts (CAE) of leaves were prepared according to the techniques described by Iqbal et al. [30]. The powdered plant leaves (500 g) were extracted with distilled water (2000 ml) at 90-100°C in a Soxhlet extractor for 8 h. The extracts were filtered and solvent was allowed to evaporate under reduced pressure of 22-26 mmHg in a vacuum rotary evaporator (R-201, Shanghai Shenshen) at 55°C yielding few grams of dry aqueous extracts. Similarly, the powdered leaves (500 g) were extracted with methanol (2,800 ml, Qualigens) at 60°C in a Soxhlet extractor for 8 h, yielding few grams of dry methanolic extracts. These dry extracts were stored at 4°C until their use in in vitro and in vivo experiments.

In vitro Experiment: The in vitro trails for anthelmintic activity of different extracts of M. longifolia were conducted on adult live A. galli worms. Naturally infected chickens were purchased from local market in Srinagar, Jammu and Kashmir State, India. The adult A. galli worms were collected from the intestine of freshly slaughtered birds (Gallus gallus domesticus) and immediately transferred to phosphate buffered saline (PBS). The adult worms of both sexes were randomly distributed to 6 treatment groups. These six groups were concurrently exposed to one of the following treatments at controlled temperature (37 ± 1°C) up to the maximum period of 6 ½ hours:

- Group 1 worms were exposed to 50 mg/ml of CHE
- Group 2 worms were exposed to 25 mg/ml of CHE
- Group 3 worms were exposed to 50 mg/ml of CAE
- Group 4 worms were exposed to 50 mg/ml of CAE
- Group 5 worms were exposed to 0.50 mg/ml of Fenbendazole (Positive Control)
- Group 6 worms were exposed to 0.9 % of PBS (Negative Control)

Twenty A. galli worms were suspended in each of the above concentrations in separate Petri dishes, each containing Fifty milliliter of the formulation. The inhibition of motility of the worms kept in the above treatments (followed by the fading away of their body colour) was used as the criterion for anthelmintic activity [31].

Worm motility was observed at 0, 1, 2, 4 and 6 hour intervals. After 6h all worms were withdrawn, washed and re-suspended in Luke warm fresh PBS for 30 min to observe the revival of the worms. There were five replicates for each treatment, to avoid any observational bias and to minimize other sources of errors.

Percentage worm motility inhibition (%WMI) was determined according to Rabel et al. [32] using the following formula: [(number of mobile worms in negative control Petri dish number of mobile worms in treatment Petri dish) /number of mobile worms in negative control Petri dish] × 100.

The data were statistically analysed using statistical Package for the social Sciences (SPSS) version 11.5.

RESULTS

In vitro assessment of anthelmintic activity of M. longifolia revealed that the effect CAE and CHE was...
concentration dependent. Both CHE @ 50 mg ml⁻¹ and Fenbendazole showed a significant (P < 0.001) overall in vitro anthelmintic activity, with Fenbendazole exerting a greater effect (P < 0.05) than CHE, against adult *A. galli* worms in terms of worm paralysis when compared to the negative control (PBS). Highest mortality (83.00%) of worms was observed for CHE @ 50 mg ml⁻¹ 6 hours (h) post exposure (PE) (Table 1). The CAE of *M. longifolia* @ 25 mg ml⁻¹ resulted in paralysis of *A. galli*; however, their motility was revived after they were placed in PBS for 30 minutes. The CHE and CAE of *M. longifolia* exhibited time-dependent anthelmintic activity against *A. galli* of chickens, as revealed from the inhibition of motility and/or death of worms. The CHE of *M. longifolia* @ 50 mg ml⁻¹ resulted in mean percentage worm motility inhibition (%WMI) of 83.00%, while CAE @ 50 mg ml⁻¹ resulted in mean %WMI of 41.00%, as observed after the worms were put in PBS for 30 min after exposure to different treatments. Hydroalcoholic extracts of *M. longifolia* were thus more potent than the aqueous extracts. These findings suggested the presence of varying degrees of anthelmintic activity in CHE and CAE.

There was 100% mortality of worms in Fenbendazole (used as a reference drug) within 6 h PE. Almost all the worms (98%) that served for the negative control treatment (PBS) survived till 6 h PE.

**DISCUSSIONS**

*In vitro* trials confirmed the potential of *M. longifolia* extracts (CHE and CAE) to control the common poultry nematode *A. galli*. Fenbendazole and *M. longifolia* extracts (CHE and CAE) showed a significant *in vitro* anthelmintic activity against adult *A. galli* worms in terms of worm motility. Fenbendazole extremely paralyzed all worms after 6 h of exposure, while CHE and CAE @ 50 mg ml⁻¹ resulted in 83 % and 41 % mortality respectively after the same interval. Fenbendazole caused a rapid and significant (P < 0.001) decrease in worm motility during the first hour of exposure and at all successive intervals. In contrast, CHE and CAE showed a time-dependent decrease in worm motility. However, Fenbendazole was more effective than CHE @ 50 mg ml⁻¹, the results showed a significant (P < 0.05) *in vitro* anthelmintic activity of CHE @ 50 mg ml⁻¹ against the nematode after 6 h of *in vitro* exposure. *A. galli* worms showed a long time of active motility in PBS which provides a reliable potential for *in vitro* investigations.

The present study showed that mortality of adult *A. galli* worms was a time and concentration-dependent variable when used as a parameter to evaluate the anthelmintic activity of *M. logifolia* extracts. Higher concentrations of CHE and CAE of *M. logifolia* with long exposure periods may result in higher anthelmintic activity against *A. galli*. Similar observations were confirmed in different medicinal plants [33-37].

Based on the findings of earlier studies, the active principles in medicinal plants responsible for the anthelmintic activity are non-polar compounds, therefore, water extracts of medicinal plants have shown lower anthelmintic activity than less polar solvent extracts (ethanol, methanol, hydro-alcohol, acetone) [35,38-41]. Eguale et al. (2007) [39] attributed the greater anthelmintic activity of ethanolic extract of *Coriandrum sativum* against *H. contortus* to its lipid soluble nature which enhances rapid transcuticular absorption of active ingredient into the body of the worms. In addition, different components of the crude extracts may work together in a synergistic fashion. However, the merits and demerits of the existing methodologies used to determine the acting of antiparasitic plants are to be given due consideration [42]. Furthermore, the active principles in plants are diverse and stable natural compounds with low molecular weight which can prevent the occurrence of anthelmintic resistance [35].

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**Table 1:** *In vitro* anthelmintic efficacy of CAEs and CHEs of *M. longifolia* on *A. galli* of Chickens

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conc. (mg/ml)</th>
<th>0 h</th>
<th>1h</th>
<th>2 h</th>
<th>4h</th>
<th>6 h</th>
<th>Fresh PBS for 30 Min²</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHE</td>
<td>50</td>
<td>20±0.00</td>
<td>14.4±0.50 (28)</td>
<td>11.4±0.50 (43)</td>
<td>7.0±0.31 (65)</td>
<td>3.6±0.24 (82)</td>
<td>3.4±0.24 (83)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>20±0.00</td>
<td>17.0±0.31 (15)</td>
<td>15.6±0.24 (22)</td>
<td>11.6±0.40 (42)</td>
<td>7.2±0.37 (64)</td>
<td>7.2±0.37 (64)</td>
</tr>
<tr>
<td>CAE</td>
<td>50</td>
<td>20±0.00</td>
<td>18.8±0.20 (6)</td>
<td>16.6±0.40 (17)</td>
<td>14.4±0.24 (28)</td>
<td>10.8±0.37 (46)</td>
<td>11.6±0.37 (41)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>20±0.00</td>
<td>19.4±0.24 (3)</td>
<td>17.6±0.40 (12)</td>
<td>15.2±0.20 (24)</td>
<td>14.8±0.20 (26)</td>
<td>16.4±0.24 (18)</td>
</tr>
<tr>
<td>Fenbendazole</td>
<td>0.50</td>
<td>20±0.00</td>
<td>8.2±0.37 (59)</td>
<td>3.6±0.40 (82)</td>
<td>0.8±0.20 (96)</td>
<td>0±0.00 (100)</td>
<td>0±0.00 (100)</td>
</tr>
<tr>
<td>PBS</td>
<td>0.9%</td>
<td>20±0.00</td>
<td>20±0.00</td>
<td>20±0.00</td>
<td>20±0.00</td>
<td>19.8±0.20</td>
<td>19.8±0.20</td>
</tr>
</tbody>
</table>

CAE, Crude aqueous extract; CHE, crude hydroalcoholic extract; h, hour; SEM, standard error of mean.

a= Worms were exposed to phosphate-buffered saline for 30 min after exposure to the different treatments to confirm their mortality.

b =PBS (phosphate-buffered saline) indicates negative control.
Anthelmintic property of *M. logifolia* represents a promising alternative to chemical treatments. This would be of great importance for poultry production in free-range or organic farming systems which will contribute in sustainable agriculture.

**CONCLUSION**

In conclusion, the results of the present study on *M. logifolia* against *A. galli* suggest a potential alternative to commercially available anthelmintics for the treatment of gastrointestinal nematodiasis in chickens. There is a need to undertake detailed phytochemical, pharmacological and anthelmintic studies of *M. logifolia* to determine the active principles, their mode of action and proper doses. Research is recommended for evaluating anthelmintic activity of *M. logifolia* against other nematode infections in vitro and in vivo.

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**REFERENCES**


