Effects of alcoholic extract of *Curcuma longa* on *Ascaridia* infestation affecting chicken

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*Ascaridia galli*, the common intestinal nematode, remains a major cause of economic loss in the poultry industry in developing countries. Treatments using chemicals are not only expensive but also affect host health. Plant extracts as better alternative is gaining significance. Here, we have studied the effects of alcoholic extract of turmeric, *Curcuma longa* L. (Zingiberaceae) roots, against *A. galli* infection in chicken. Different concentrations of *C. longa* root extract were tested *in vitro* on 5 groups of adults *A. galli* worms and *in vivo* on 6 groups of chicks. The results showed that the turmeric root extract @ 60 mg mL⁻¹ *in vitro* significantly (P <0.001) proved paralytic and fatal against worms (16.80±1.28 h). *In vivo*, chicken groups (G2-G6) were infected with an average of 300±12 embryonated eggs of *A. galli*. The G2 was not given any treatment while G3 was treated with piperazine (@ 200 mg kg⁻¹ body wt.); and Groups 4, 5 and 6 were given turmeric @ 200, 400 and 600 mg kg⁻¹ body wt., respectively. The mean number of worms extracted at the end of the trial in G2 (untreated) was 18.10±2.42, while the G3 treated with piperazine had no worms. Groups 4 and 5 did not show any significant difference compared to G2. However, G6 that had 3.20±1.33 worms was statistically significant. Higher concentrations of turmeric given to infected chickens significantly reduced the length and weight of worms. The study showed that the worm infestation damaged the intestinal villi, and treatment with high concentration of *C. longa* had healing effects and restored the integrity of intestinal mucosa. The results have demonstrated the ameliorating effect of *C. longa* turmeric on *A. galli* infected chickens.

**Keywords:** Gallus gallus domesticus, Intestinal parasites, Nematodes, Piperazine, Poultry, Round worms, Turmeric

In poultry, infestation by intestinal nematode *Ascaridia galli* (Family. Ascaridiidae), leads to reduced growth rate, weight loss, low egg production and poor general condition of the birds. At times they cause serious pathological lesions by puncturing the mucous membrane of the duodenum and result in inflammation and bleeding. The infective eggs containing the L₂ larvae hatch in duodenum of susceptible host and predating period is about 5-6 weeks. Drugs used to treat *A. galli* including piperazine sulfate compounds, flubendazole and levamisole, could be harmful if given in incorrect doses. Many plants species used throughout the world in traditional medicine for the treatment of both veterinary and human diseases but few plants were screened for activity. One of these plant species is *C. longa*. Plant extracts as better alternative has been gaining significance. Kumer *et al.* and Abdelqader *et al.* used the roots of reed *Acorus calamus* and citrus peels ethanolic extracts respectively against worm *A. galli*. *Curcumin*, a powerful antioxidant, is being used for cancer prevention, liver protection, premature aging and anti-inflammatory, easing conditions for bursitis, arthritis and back pain. Turmeric rhizomes are known to contain phyto-constituents viz. alkaloids, saponins, flavonoids, terpenes and steroids. Ethanol extract of the *C. longa* rhizomes has been shown to have anti-protozoa activity against *Entamoebahistolytica*, *Leishmania major* and *Plasmodinn falciparum*. This study explores the effects of alcoholic extract of *Curcuma longa* L. roots against *Ascaridia galli* infecting chickens, both *in vitro* and *in vivo*.

**Material and Methods**

Plant material (rhizomes) of *Curcuma longa*, purchased from local market, were cut into small pieces, oven-dried at 45 °C, powdered and macerated in 70% ethanol (hydro-alcoholic) 200 g L⁻¹ for 48 h using Soxhlet apparatus. The extract was filtered using Wartman No. 1 filter, concentrated to dark yellow residue on a rotary evaporator, and stored at 4 °C in airtight containers.

*In vitro experiment*—Adult worms of *A. galli* were taken out of the gut from infected chickens

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Gallus gallus domesticus L. and distributed randomly into 5 groups of 7 each (n= 7) in Petri dishes comprising either control treatments or different concentrations of turmeric solution in 0.9% phosphate buffered saline (PBS) and maintained at 37±1 °C in an automated glass chambered19. Group 1(G1) had phosphate buffered saline (PBS) only; Group 2 had 20 mg mL⁻¹ of citrate Piperazine (GlaxoSmithKline, India). Group 3-5 had turmeric @ 20, 40 and 60 mg mL⁻¹ of turmeric, respectively.

Production of infective eggs of A. galli—A. galli female worms were collected from the small intestines of naturally infected 40 chicks by necropsy. The eggs were recovered from the gravid uteri of worms under microscope and incubated at ~20 °C in 4% potassium dichromate (K₂Cr₂O₇) solution for 2 wk. When the eggs became infective, they were stored at ~4 °C until inoculation20.

In vivo experiment—Experiments were conducted in civil fields, north of the Babylon province (southern Baghdad) from 2nd January to 4th April in 2013. Sixty chicks of hens Lohman brown breed were divided into 6 groups (n=10) as follows: Group 1, control non infected; Group 2, infected without treatment; Group 3, infected+piperazine @ 200 mg kg⁻¹ body wt.; Group 4-6, infected+turmeric @ 200, 400 and 600 mg kg⁻¹ body wt., respectively. All birds were fed with starter diet during the first 3 weeks and divided into 5 groups of 7 each (n= 7) in Petri dish es as the heavily infected chicken show signs of droopiness, hemorrhages, diarrhea and intestinal mucosal damage affecting weight gain24.

In vitro experiment—In vitro experiment using piperazine and different concentrations of alcoholic extract of turmeric exhibited their effect against A. galli (Table 1). The piperazine @ 20 mg mL⁻¹ made the worms paralytic in 6.06±0.39 h and killed them by 6.90±0.75 h. It was significant compared to G1 (PBS) where death occurred at 41.8±1.28 h (t = 46.586, P < 0.001). Treatments with turmeric also proved useful. Time taken for paralysis and death of worms were proportional to the concentrations of turmeric. The ability of piperazine citrate causing flaccid paralysis of worm muscles by blocking the response to acetylcholine, eventually leads to expulsion of worms25-27. The group (G2) took less time for paralysis and death of worms when compared with lower concentrations of turmeric groups. The concentration of 60 mg mL⁻¹ of alcoholic extract of turmeric (G5) caused paralysis in 16.8±1.28 h and death in 17.85±1.23 h, comparatively faster than the groups 3 and 4. It could be explained that the biologically active substances present in curcumin like alkaloids, flavonoids (diferuloylmethane), saponins, steroids, polyphenol, zingeribone and triterpenoids16,28.

Results and Discussion
The study demonstrated the nematocidal activity of Curcuma longa on adult Ascaridia galli, one of the most prevalent nematode parasites found in different poultry intestine. A. galli has attracted much attention as the heavily infected chicken show signs of dropiness, hemorrhages, diarrhea and intestinal mucosal damage affecting weight gain24.

Table 1—Effects of Curcuma longa extract on Ascaridia galli in vitro

<table>
<thead>
<tr>
<th>Groups</th>
<th>Conc. (mg mL⁻¹)</th>
<th>Paralysis time (h) Mean±SE</th>
<th>Death time (h) Mean±SE</th>
<th>Student’s t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (PBS)</td>
<td>0</td>
<td>41.8±1.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2 (piperazine)</td>
<td>20</td>
<td>6.06±0.39*</td>
<td>6.90±0.75*</td>
<td>46.586</td>
</tr>
<tr>
<td>G3</td>
<td>20</td>
<td>34.87±0.85*</td>
<td>37.16±0.96*</td>
<td>5.914</td>
</tr>
<tr>
<td>G4</td>
<td>40</td>
<td>26.72±0.91*</td>
<td>28.01±1.19*</td>
<td>17.300</td>
</tr>
<tr>
<td>G5</td>
<td>60</td>
<td>16.80±1.28*</td>
<td>17.85±1.23*</td>
<td>45.264</td>
</tr>
</tbody>
</table>

Number of worms per group (n = 7). Piperazine served as test control. (*) results significantly compared with PBS P < 0.001.
act at higher concentrations. Perez-Arriaga et al reported similar growth inhibition of *Giardia lamblia* trophozoites *in vitro* with a 30 μM of curcumin up to 72 h and of 100 μM longer than 48 h.29

**In vivo experiment**—Investigation of chicken groups (G2-G6) infected with *A. galli* showed various results revealing the potential of turmeric as better alternative (Table 2). At the end of experiment, the mean number of worms were 18.10±2.42 in G2 (untreated), while the treated group with piperazine (G3) no worms has been found. There were no significant differences among G2, G4 and G5 groups. However, mean number of worms in G6, was 3.20±1.33, statistically significant compared to the untreated. These results showed that higher concentrations of turmeric (600 mg/kg) *in vivo* had significant effect on worms which might be due to the presence of substances in turmeric that acted at higher concentration. Earlier works have also shown such effects of curcumin against *Schistosoma mansoni* infected mice30,31. Morais *et al.* demonstrated that incubation of *S. mansoni* with concentrations of turmeric affects viability of worms and reduction in eggs production32. Curcumin is known for its antiprotozoal activity against *Trypanosoma brucei*, *Leishmania donovani* and *Giardia lamblia*33-35. Curcumin component exhibits strong antioxidant (Bisdemethoxy curcumin) activity about eight times more potent than vitamin E in lipid peroxidation, and three times more powerful than vitamin C in neutralizing free radicals14. This study showed that the percentage of female worms (Table 2) in all groups ranged between (54.14 to 58.92%) which was not statistically significant. while Gabrashanska *et al.* and Dehlawi found that males were more stable than the females36,37. High concentrations of turmeric caused reduction in the weight and length of worms (Table 2). That might be due to presence of active substances in turmeric that affect the growth of worms or cause damage to them as observed by Lalchhandama in *A. galli* on administration of the *Acacia oxyphylla* extract25.

**Histopathology**—Effect of the extract on the intestinal villi as observed by the height and width are shown in Table 3 and Fig. 1. The treated groups (G2-G5) exhibited significant changes compared to

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Worms</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male worm Number</td>
<td>0</td>
<td>8.3±2.10</td>
<td>0</td>
<td>6.9±1.92</td>
<td>7.0±1.61</td>
<td>1.4±0.66*</td>
<td></td>
</tr>
<tr>
<td>weight (mg) Mean±SE</td>
<td>-</td>
<td>0.041±0.01</td>
<td>-</td>
<td>0.038±0.01</td>
<td>0.034±0.01</td>
<td>0.032±0.01*</td>
<td></td>
</tr>
<tr>
<td>length (cm) Mean±SE</td>
<td>-</td>
<td>4.39±0.58</td>
<td>-</td>
<td>4.10±0.70</td>
<td>3.77±0.66*</td>
<td>3.19±0.53*</td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>0</td>
<td>9.8±1.66</td>
<td>0</td>
<td>9.9±1.57</td>
<td>9.5±2.61</td>
<td>1.8±1.16*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(54.1%)*</td>
<td></td>
<td>(58.90%)</td>
<td>(57.6%)</td>
<td>(56.3%)</td>
<td></td>
</tr>
<tr>
<td>Female worm weight (mg) Mean±SE</td>
<td>-</td>
<td>0.073±0.01</td>
<td>-</td>
<td>0.072±0.01</td>
<td>0.066±0.01</td>
<td>0.054±0.02*</td>
<td></td>
</tr>
<tr>
<td>length (cm) Mean±SE</td>
<td>-</td>
<td>5.88±1.13</td>
<td>-</td>
<td>5.55±0.76</td>
<td>5.08±0.72</td>
<td>4.83±0.43*</td>
<td></td>
</tr>
</tbody>
</table>

Piperazine served as test control. *Results statistically significant P <0.05. *Percentage of female worm number in each group.

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Fig. 1—Photomicrographs of histological section (20X) [H & E] of infected *Ascaridia galli*. (A) Section of intestinal wall & villi of a healthy bird (G1); (B, C & D) infected of *A. galli* but no treatment (G2); (E) treatment with piperazine @ 200 mg/kg body wt. (G3); and (F) turmeric @ 600 mg/kg.
the non-infected group (G1). However, group 6 (infected+turmeric @ 600 mg kg⁻¹) did not show any significant difference with the control group (G1).

Photomicrograph of histological sections showed that the damage in villi were evident in (G2-G5) with the presence of inflammatory cells, while in (G6) restoration of the normal pattern of villi was seen (Fig. 1F) which was not evident even in piperazine presence of inflammatory cells, while in (G6) the damage in villi were evident in (G2-G5) with the significant difference with the control group (G1).

Level of significance: $P<0.05$  
[*$P<0.001$; **$P<0.01$; ***$P<0.05$]

In conclusion, the treatment of Ascaridia galli infestation with Curcuma longa was effective and may take a role in therapy of A. galli. Higher concentrations of turmeric were significant in limiting the infestation as well as effectively restoring normal mucosal pattern.

References


