Original Research

Anthelmintic Activity of *Curcuma longa* Ethanolic Extract against Benzimidazole Resistant Gastrointestinal Nematodes in Goats

Subhradal Nath*, Susanta Pal, Prabir Kumar Sanyal, Sushovan Roy[1], Sanju Mandal[2] and Yogesh Nayak[3]

Department of Veterinary Parasitology, Veterinary College Anjora, Durg, Chhattisgarh, 491001, INDIA

[1]Department of Veterinary Medicine
[2]Department of Veterinary Physiology & Biochemistry
[3]Department of Veterinary Gynecology & Obstetrics

***Corresponding author:** subhradal@gmail.com

Rec. Date:	Feb 09, 2019 14:04
Accept Date:	Apr 30, 2019 14:10
DOI	10.5455/ijlr.20190209020421

Abstract

This paper describes the in vivo anthelmintic activity of *Curcuma longa hydro alcoholic rhizome* extract *(CHE 50:50) in naturally infected benzimidazole resistant gastrointestinal nematodes of goats maintained in a farm. Thirty non-descript goats, infected with gastrointestinal nematodes were divided into three groups of ten animals each consisting of Group A (untreated controls), Group B (ABZ at 7.5mgkg^{-1} body weight) and Group C (CHE at 500mgkg^{-1} body weight). Prior to conducting faecal egg count reduction test (FECRT), egg hatch assay (EHA) was performed by pooling samples from each group showing ED_{50} value 0.594 indicating high resistance to benzimidazole. Faecal egg count reduction of 64% and 66% on day 10 post-infection was observed in albendazole treated and CHE treated goats, respectively.*

Key words: Albendazole, *Curcuma longa*, EHA, FECRT, Gastrointestinal Nematodes

How to cite: Nath, S., Pal, S., Sanyal, P., Roy, S., Mandal, S., & Nayak, Y. (2019). Anthelminthic Activity of Curcuma Longa Ethanolic Extract against Benzimidazole Resistant Gastrointestinal Nematodes in Goats. International Journal of Livestock Research, 9(6), 117-122. doi: 10.5455/ijlr.20190209020421

Introduction

Helminth infections are a major cause for reducing the productivity in livestock, particularly in poor world (Githiori *et al*., 2006). The control of parasitic helminths in domestic animals relies largely on the use of anthelmintic drugs (Taylor *et al*., 2002). However, increasing reports of parasitic populations that have developed anthelmintic resistance have become increasingly common, and severely threatens the beneficial exploitations of this control strategy (Waller, 1997). The resistance menace particularly poses a great threat in the viability of organic small ruminant production system (Thamsborg and Roepstorff, 2014). In India

Thiabendazole resistance was first reported in sheep from Rishikesh (Uttarakhand) (Varshney and Singh, 1976). Other reports are from Madhya Pradesh (Dixit *et al*., 2017), Assam (Phukan *et al*., 2012) Rajasthan (Maharshi *et al.,* 2011) and Southern India (Easwaran *et al.,* 2009, Manikkavasagan *et al*., 2015). Anthelmintic resistance is widespread in several states of India (Singh and Swarnkar, 2008) resulting in decreasing efficacy of the valuable control option. This has set up a stage for using alternative control options. World Health Organization has now realized that the traditional system of medicine may play an important role in the development of livestock of the third world countries (WHO, 1993). Juice of turmeric (*Curcumma longa*) traditionally has long been traditionally used to cure worm infections in south and Southeast Asia (Nadkarni, 1976). It has been documented that curcumin inhibit thioredoxinreductases (TrxRs) of nematehelminthes, which are essential for cell growth and survival and thereby may play a vital role in alternative medicine (Hudson *et al*., 2010). Therefore, the present study was undertaken to know the efficacy of hydro-alcoholic extract of *Curcuma longa* against benzimidazole resistant nematode parasites of goats.

Materials and Methods

Raw turmeric tubers were purchased from the local market and were identified and authenticated at the Department of Botany, Government V.Y.T. Post Graduate Autonomous College, Durg, Chhattisgarh. These rhizomes were cut into small pieces and sun dried till they became brittle or easily breakable. These were powdered and extraction was done by using Soxhlet apparatus with 50% ethanol and 50% distilled water (hydro-alcoholic) as solvent. The extract was yellowish-brown, while its consistency was semiliquid to solid and stored at 4^0C in air tight containers for further studies. Thirty non-descript goats of either sex, naturally infected with gastrointestinal nematodes were selected from Goat Farm of College of Veterinary Science & A.H., Anjora, Durg, Chhattigarh, India. These animals were divided in three groups, of 10 animals each. Group A animals served as untreated controls while Group B animals were subjected to oral administration of albendazole (ABZ) at 7.5mgkg^{-1} body weight and Group C animals with oral administration of *Curcuma longa* hydro ethanolic (CHE) extract (50:50) at 500mgkg^{-1} body weight. The particular dose and concentration of hydro-ethanol for preparation of CHE extract is as per Pande, (2011). From each group of animal fresh faecal sample were collected per rectum, pooled and subjected to egg hatch assay (EHA). The pooled sample was mixed very well and around 60-70 gm faecal sample was put in a 500 ml beaker and water added in the beaker. It was kept for 20-30 minutes so that faecal matter gets soaked and loosened and then mixed thoroughly with stirrer. After mixing faecal slurry was strained 2-3 times using tea strainer to remove the faecal debris. Strained faecal slurry was put in centrifuge tubes and centrifuged at 2000 rpm for 5 minutes. After centrifugation supernatant was thrown from all centrifuge tubes. Sediment was well agitated and saturated sodium chloride solution was poured up to top in each

 DOI 10.5455/ijlr.20190209020421

centrifuge tube for floatation of parasitic eggs. Microscopic cover glasses were put on each centrifuge tube for adhering of eggs. After 10 min cover glass were held with forcep and dipped in a beaker containing distilled water. *In vitro* egg hatch assay (EHA) was performed on five replicates of eggs separated from pooled faeces collected on day 0, using pure thiabendazole (TBZ; Sigma, St. Louis, Missouri, USA) in a two-fold dilution series of 0.0625, 0.125, 0.25, 0.50 and 1.0 µg TBZ ml^{-1} (Coles *et al*., 1992, 2006). Eggs (n=100) per well, along with varying concentrations of TBZ, were incubated in 24-multiwell plates for 24h at 28^0C in a BOD incubator, followed by addition of a drop of Lugol's iodine to each well. Unhatched eggs and larvae (L1) were counted to determine the proportion of eggs hatched. Data were analysed by Probit Analysis to obtain ED_{50} values for egg hatch. An ED_{50} value in excess of 0.1µg TBZ ml^{-1} was considered to indicate the emergence of benzimidazole resistance.

In vivo faecal egg count reduction test (FECRT) was performed using faecal samples collected *per rectum* from each animal on day 0 (pre-treatment) and day 10 (post-treatment) for Group A, B and C (Coles *et al*., 1992, 2006). The intensity of infection was determined by modified McMaster technique (Skerman and Hillard, 1966). The left over faecal samples were pooled on both pre-treatment and post-treatment and subjected to larval culture at room temperature for 7 days. Infective larvae were harvested and identified (Hansen and Perry, 1990). The data were analysed for faecal egg count reduction (%FECR) using RESO calculator (Martin and Wursthorn, 1991).

Result and Discussion

Observations on egg hatch assay (EHA) to know the status of benzimidazole resistance in gastrointestinal nematodes of goats revealed ED_{50} for egg hatch was 0.594 (Table 1), which is indicative of high degree of resistance towards benzimidazole in goats prevailed in the farm.

Table 1: Status of benzimidazole resistance in gastrointestinal nematodes of goats maintained in farm by egg hatch assay (EHA)

***In vitro* EHA (For Benzimidazole only)**	
Parameters	**Values**
Slope	1.819
Variance of Slope	5.30E-02
Intercept	5.411
LN_{50}	-0.226
ED_{50}	**0.594**
Lower Limit of ED_{50}	0.406
Upper Limit of ED_{50}	0.909
Result	**Resistant**

Earlier, Sanyal *et al*. (2014) observed ED_{50} value of 0.150 in the same farm. The results of the faecal egg count reduction test revealed 64%and 66% reduction in faecal egg counts for ABZ and CHE, respectively

DOI 10.5455/ijlr.20190209020421

(Table 2). There is significant reduction in faecal egg counts in group B and C in comparison to group A on tenth day whereas there was non-significant difference between animals of Group B and C (Table 2). Various workers from India and abroad had shown variable efficacy for ABZ showing resistance for the drug which is dependent on many factors like frequency of use, abuse or extensive use, genetic changes in the worm etc. Efficacy of CHE extract is not upto the mark as per the recommendation of World Association for Advancement of Veterinary Parasitology (WAAVP) against benzimidazole resistant nematode parasites of goats but certainly this work provide vital information regarding alternative medicine for sustainable control of internal parasites.

Table 2: Faecal egg count reduction following treatment with albendazole 7.5mgkg^{-1} body weight and *Curcuma longa* hydro ethanolic (CHE) extract (50:50) at 500mgkg^{-1} body weight

***In vivo* Faecal Egg Count Reduction Test**			
Parameter	**Group A(Control)**	**Group B(ABZ)**	**Group C (CHE)**
No. of Animals	10	10	10
Mean EPG (Day 0)	900	730	1683
Mean EPG (Day 10)	900	200	275
FECR%	-	**64**	**66**
Result		**Resistant**	

Afrin *et al.* (2016) obtained lowered faecal egg counts and ant-inflammatory effect in Black Bengal goats using 0.2% and 0.3% curcumin for 60 days in the ration. Ullah *et al*. (2013) found anthelmintic effect of *C. longa* due to cucurbitacin as secondary metabolite. Nasai *et al*. (2016) observed an increase in the number of dead larvae of *Haemonchus contortus* over time as turmeric concentrations exhibited the highest antihelmintic activity in a dose-dependent way with the maximum effect observed at the highest dose of turmeric extract (200 mg/ml), where 78% of the worms died 24 h post-exposure. Bazh and El-Bahy (2013) screened the *in vitro* and *in vivo* activity of ginger and curcumin on *Ascaridia galli*. Contrary to the available published results CHE at 500mgkg^{-1} body weight had non-appreciable anthelmintic effect in benzimidazole resistant nematode parasites of goats and needs further investigations on its dose-dependent efficacy.

In these days where an increased resistance to conventional drugs is one of major causes of concern to livestock industry and also is the major drivers of drug discovery (Anthony *et al*., 2005). Recent advance and an increased understanding of phytotherapeutics together with the availability of the structural and functional information of the parasite molecules and the plant products further crusade the search for a novel anti-parasitic agent which may be affordable and sustainable.

Conclusion

Parasitic diseases are a global problem and considered as a major obstacle in the health and product performance of livestock. Chemotherapeutics agents are the first choice for effective control but due to drug

DOI 10.5455/ijlr.20190209020421

resistance problem there is urgent need for alternative therapy. At present time many medicinal plants have been studied in traditional medicine including Ayurveda and Chinese medicine. Curcumin extracted from turmeric have anti-parasitic effects against many endo-parasites. However, the efficacy is not up to the mark but there is always scope for future research work to achieve the ultimate outcome of getting product with minimal problem of drug resistance, economical and sustainable. This present study not only validates our ancient knowledge about using herbal medicines as anthelmintic but also open up new areas for sustainable control of gastrointestinal nematodes.

References

1. Anthony, J. P., Fyfe, L., & Smith, H. (2005). Plant active components–a resource for antiparasitic agents? *Trends in Parasitology*, *21*(10), 462-468.
2. Afrin, F., Chowdhury, M. M. R., Saha, S. S., Mohammad, A. R., Mohammad, A. A. & Mohammad, K. I. (2016). Efficacy of curcumin against gastro-intestinal parasites in goat. *European Journal of Pharmaceutical and Medical Research*, 3, 158-165.
3. Bazh, E. K., & El-Bahy, N. M. (2013). In vitro and in vivo screening of anthelmintic activity of ginger and curcumin on Ascaridia galli. *Parasitology Research*, *112*(11), 3679-3686.
4. Coles, G. C., Bauer, C., Borgsteede, F. H. M., Geerts, S., Klei, T. R., Taylor, M. A. & Waller, P. J. (1992). World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology*, *44*(1-2), 35-44.
5. Coles, G. C., Jackson, F., Pomroy, W. E., Prichard, R. K., von Samson-Himmelstjerna, G., Silvestre, A. & Vercruysse, J. (2006). The detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology*, *136*(3-4), 167-185.
6. Dixit, A. K., Das, G., Dixit, P., Singh, A. P., Kumbhakar, N. K., Sankar, M. & Sharma, R. L. (2017). An assessment of benzimidazole resistance against caprine nematodes in Central India. *Tropical Animal Health and Production*, *49* (7), 1471–1478.
7. Easwaran, C., Harikrishnan, T. J. & Raman, M. (2009). Multiple anthelmintic resistance in gastrointestinal nematodes of sheep in Southern India. *Veterinary Archive*, *79*, 611-620.
8. Githiori, J. B., Athanasiadou, S. & Thamsborg, S. M. (2006). Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. *Veterinary Parasitology*, *139*(4), 308-320.
9. Hansen, J. & Perry, B. D. (1990). The epidemiology, diagnosis and control of gastro-intestinal parasites of ruminants in Africa: A handbook. ILRI (aka ILCA and ILRAD).
10. Hudson, A. L., Sotirchos, I. M. & Davey, M. W. (2010). Substrate specificity of the mitochondrial thioredoxin reductase of the parasitic nematode *Haemonchus contortus. Parasitology Research*, *107*(2), 487-493.
11. Maharshi, A.K., Swarnkar, C.P., Singh, D. & Mahonar, G.S. (2011). Correlation between status of benzimidazole resistance in *Haemonchus contortus* on bio and molecular assays. *Indian Journal of Animal Science*. *81*:110-115.
12. Manikkavasagan, I., Binosundar, S. T. & Raman, M. (2015). Survey on anthelmintic resistance to gastrointestinal nematodes in unorganized goat farms of Tamil Nadu. *Journal of Parasitic Diseases*, *39*(2) 258-261.
13. Martin, P. J. & Wursthorn, L. (1991). RESO faecal egg count reduction test calculator. Melbourne, Australia, Council of Scientific and Industrial Research Organization, Division of Animal Health.
14. Nadkarni, K.M. (1976). *Indian Materia Medica*. Popular Prakashan, Bombay.

DOI 10.5455/ijlr.20190209020421

15. Nasai, N. B., Abba, Y., Abdullah, F. F. J., Marimuthu, M., Tijjani, A., Sadiq, M. A., Mohammed, K., Chung, E.L.T. & Omar, M. A. B. (2016). In vitro larvicidal effects of ethanolic extract of *Curcuma longa Linn.* on Haemonchus larval stage. *Veterinary World*, *9*(4), 417.
16. Pande, G. (2011). Active principles and median lethal dose of *Curcuma longa Linn*. *International Research Journal of Pharmacy 2*(5) 239-241.
17. Phukan, S.C., Sarkar. M., Prasad, A., Zahid, A.K., Kakati, P. & Choudhary, D. (2012). Detection of benzimidazole resistance of *H. contortus* by allele- specific PCR. XXIII, N.C.V.P., College of Veterinary Science, AAU, Khanapara, Guwahati, Dec. 12-14, pp 155.
18. Sanyal, P. K., Kerketta, D., Pal, S., Baghel, K. R. & Bisen, S. (2014). Emergence of anthelmintic resistance in ruminants of Chhattisgarh. *Journal of Veterinary Parasitology*, *28*(1), 40-43.
19. Singh, D. & Swarnkar, C. P. (2008). Role of refugia in management of anthelmintic resistance in nematodes of small ruminants–a review. *Indian Journal of Small Ruminants*, *14*(2), 141-180.
20. Skerman, K.D. & Hillard, J. J. (1966). A handbook for studies of helminth parasites of ruminants. Near East Animal Health Institute, Iran. FAO, Rome.
21. Taylor, M. A., Hunt, K. R. & Goodyear, K. L. (2002). Anthelmintic resistance detection methods. *Veterinary Parasitology*, *103*(3), 183-194.
22. Thamsborg, S. M. & Roepstorff, A. (2014). Parasites and Organic Livestock Parasite Problems in Organic Livestock Production Systems and Options for Control, Running Head: The Royal Veterinary and Agricultural University, Copenhagen, Denmark.
23. Ullah, S., Khan, M. N., Sajid, M. S. & Muhammad, G. (2013). Comparative anthelmintic efficacy of Curcuma longa, Citrullus colocynthis and Peganum harmala. *Global Veteranaria*, *11*(5), 560-567.
24. Varshney, T.R. & Singh, Y.P. (1976). A note on development of resistance of worms against phenothiazine and thiabendazole in sheep. *Indian Journal of Animal Science .46*, 666-668.
25. Waller, P. J. (1997). Sustainable helminth control of ruminants in developing countries. *Veterinary Parasitology*, *71*(2-3), 195-207.
26. WHO. (1993). Summary of WHO guidelines for assessment of Herbal Medicines. *Herbal Gram*, *28*, 13-14.

DOI 10.5455/ijlr.20190209020421