

In vitro* anthelmintic activity of medicinal plant *Tinospora cordifolia* extract on amphistome *Gastrothylax crumenifer

Babita Jogpal, Gayatri Swarnakar*, Hitendra Singh Chouhan and Kiran Roat

Department of Zoology, Govt. P.G. Meera Girls College,
MLS University, Udaipur 313001, India

(Received 30 April, 2021; Accepted 31 May, 2021)

ABSTRACT

In vitro anthelmintic activity of medicinal plant *Tinospora cordifolia* extracts was studied on the amphistome *Gastrothylax crumenifer* parasites. Live amphistome *Gastrothylax crumenifer* were collected from the infected rumen of freshly slaughtered domestic Goat at the local meat market of Chittorgarh (Raj.). The anthelmintic effects of different concentration of albendazole, alcoholic and aqueous stem extract of *T. cordifolia* against *G. crumenifer* were studied. High concentration of albendazole of 100 mg/ml showed 72% mortality rate after 5 hours of exposure on *G. crumenifer*. The aqueous extracts of *T. cordifolia* showed highest mortality rate of 100% at concentration of 100 mg/ml after 5 hours of exposure time. Whereas, the highest mortality rate of 100% was observed with alcoholic extracts at 100 mg/ml of concentration in 4 hours exposure time. The study revealed that alcoholic and aqueous extracts of *T. cordifolia* caused more mortality compared albendazole. The alcoholic extract was highly potent than aqueous extract of *T. cordifolia*. The present study will improve the socio-economic condition of the Goat farmers of Chittorgarh (Raj.) by removing pathogenic amphistome *G. crumenifer* parasites with the treatment of medicinal plants *T. cordifolia*.

Key words : Anthelmintic, Amphistome, *Gastrothylax crumenifer*, *Tinospora cordifolia*

Introduction

The amphistome *Gastrothylax crumenifer* is common in Goats and causes lowered production of meat, milk and wool production. This amphistome parasite is harmful for livestock and caused severe diseases called Paramphistomiasis. To control this trematode parasite depends upon the use of synthetic drug like albendazole which involves economics. However, there is development of resistance in amphistome parasite against synthetic drugs and problem of potential residue of drugs in milk, meat and other animal products (Jabbar *et al.*, 2006). *In vitro* anthelmintic activity of plants have been studied on trematode parasites (Jeyanthilakan

et al., 2012, Swarnakar *et al.*, 2014 and 2015; Anuracpreeda 2017, Rajesh *et al.*, 2017; Damor and swarnakar, 2018; Minsakorn *et al.*, 2019 and Swarnakar *et al.*, 2020).

Tinospora cordifolia is a large, deciduous climbing shrub, glabrous belongsto the family Menispermaceae. *T. cordifolia* has been traditionally used as an anthelmintic agent, syphilis, urinary diseases, skin diseases, bronchitis, hepatoprotective, immune-modulatory, antipyretic, (Warrier *et al.*, 1996; Treadway *et al.*, 1998 and Prince *et al.*, 1999). It has been shown to possess anti allergic, antioxidant, anti-inflammatory (Leyon *et al.*, 2004), antihyperlipidimic properties (Alexander *et al.*, 2010 and Hamsa *et al.*, 2010) and antidiabetic (Singh *et al.*,

2010). Therefore, a study was undertaken to the efficiency of *Tinospora cordifolia* stem extract on *Gastrothylax crumenifer*.

Materials and Methods

Live amphistome *Gastrothylax crumenifer* were collected from the infected rumen of freshly slaughtered domestic Goat at the local meat market of Chittorgarh (Raj.) and kept in 0.9% physiological saline. Amphistome parasites survived 2-4 hours in physiological saline. Fresh *Tinospora cordifolia* were collected from the moist area of Chittorgarh (Rajasthan). Stem of plant were used to prepared extract of plant. Anthelmintic activity was studied by in vitro petri dish method as described by Githiori *et al.*, (2006). The in vitro anthelmintic effect of alcoholic and aqueous *Tinospora cordifolia* stem extract was evaluated in comparison with synthetic drug albendazole. Albendazole was obtained from Veterinary hospital, Chittorgarh. The mortality and motility of amphistome parasite were examined

after 1,2,3,4 and 5 hours. The dead parasites were examined visually and mechanically stimulated using a dissecting needle. Motility was scored using the following criteria:-

- Score 3 – Movement of the whole body,
- Score 2 – Movement of only part of the body,
- Score 1 – Immobile but not dead and
- Score 0 – Dead.

The dead parasites were counted in each experimental set and the percentage of average mortality was calculated according to the following formula:

Average Mortality rate =

$$\frac{\text{Total number of dead flukes}}{\text{Total number of experimental flukes}} \times 100$$

Results

The anthelmintic effects of different concentration of albendazole, alcoholic and aqueous stem extract of *Tinospora cordifolia* against *Gastrothylax crumenifer* shown in the Table 1. Due to effect of synthetic drug

Table 1. *In vitro* anthelmintic effects of albendazole, aqueous and alcoholic *Tinospora cordifolia* extract on *Gastrothylax crumenifer*.

Exposure time in Hrs	Extracts in mg/ml	Albendazole extracts	<i>T. cordifolia</i> Aqueous extracts	<i>T. cordifolia</i> Alcoholic
1 hour	20 mg/ml	0%	16%	44%
	40 mg/ml	4%	32%	48%
	60 mg/ml	8%	40%	52%
	80 mg/ml	12%	48%	60%
	100 mg/ml	16.00%	56.00%	68.00%
2 hour	20 mg/ml	8%	44%	56%
	40 mg/ml	12%	52%	60%
	60 mg/ml	16%	60%	68%
	80 mg/ml	24%	68%	76%
	100 mg/ml	28.00%	72.00%	88.00%
3 hour	20 mg/ml	24%	64%	64%
	40 mg/ml	28%	68%	72%
	60 mg/ml	32%	72%	80%
	80 mg/ml	40%	76%	88%
	100 mg/ml	44.00%	84.00%	96.00%
4 hour	20 mg/ml	32%	72%	84%
	40 mg/ml	36%	76%	88%
	60 mg/ml	44%	80%	92%
	80 mg/ml	52%	88%	96%
	100 mg/ml	56.00%	96.00%	100.00%
5 hour	20 mg/ml	48%	84%	100%
	40 mg/ml	52%	88%	100%
	60 mg/ml	56%	92%	100%
	80 mg/ml	64%	96%	100%
	100 mg/ml	72.00%	100.00%	100.00%

and stem extract amphistome parasites were paralyzed. Mortality was assessed by visually and mechanically stimulated parasites using a dissecting needle. High concentration of albendazole of 100 mg/ml showed 72% mortality rate after 5 hours of exposure on *Gastrothylax crumenifer*. The aqueous extracts of *Tinospora cordifolia* showed highest mortality rate of 100% at concentration of 100 mg/ml after 5 hours of exposure time. Whereas, the highest mortality rate of 100% was observed with alcoholic extracts at 100 mg/ml of concentration in 4 hours exposure time. Same highest mortality rate were also found on 20 to 100 mg/ml concentration at 5 hours exposure time.

Discussion

The present *in vitro* studies on the effect of *Tinospora cordifolia* against *Gastrothylax crumenifer* elucidated the anthelmintic potential from the visual and physical touch observations on the mortality of parasite; it was found that *T. cordifolia* was effective against *G. crumenifer*. The study exhibited that aqueous and alcoholic fruit extracts of *T. cordifolia* caused more mortality than synthetic drug albendazole and higher concentration are more effective than lower concentration. It was also evident that aqueous extract was less potent than alcoholic extracts. The anthelmintic activity of *C. colocynthis* on *Orthocoelium scoliocoelium* (Swarnakar *et al.*, 2015) and *Fasciola gigantica* (Damor and Swarnakar, 2018) was reported. The *in vitro* antihelminthic activity of *Trigonella foenum-graecum*, *Allium sativum* and *Piper longum* evaluated in comparison with albendazole and Niclosamides against *Cotylophoron cotylophorum* and *Gigantocotyle explanatum* showed remarkable paralysis in these parasites (Singh *et al.*, 2008, Nahla *et al.*, 2012 and Swarnakar *et al.*, 2014) as was also observed in the present study.

This study suggests that the alcoholic extract of stem of *T. cordifolia* could offer a suitable and cheaper alternative anthelmintic as comparison to aqueous extract and synthetic drugs. Consequently, it might help to reduce the occurrence of parasite in the host environment after treatments. The results of present study would be supportive in preparing ecofriendly, less costly anthelmintic veterinary drug, improve the socio-economic condition of cattle farmers. This study suggests that the alcoholic extract of stem of *Tinospora cordifolia* could be evaluated *in vivo* for its safety and efficacy.

Conclusion

The present study observed that aqueous and alcoholic fruit extracts of *T. cordifolia* caused more mortality than synthetic drug albendazole and higher concentration are more effective than lower concentration. Present result also found that aqueous extract was less effective than alcoholic extracts. The present study will improve the socio-economic condition of the farmers by removing pathogenic amphistome *G. crumenifer* parasites with the treatment of medicinal plants *T. cordifolia*.

Acknowledgment

Mr. Hitendra Singh Chouhan is grateful to CSIR New Delhi (File No. 09/172 (0107)2019-EMR I (Dated: 15.11.2019) for providing financial assistance to carry out the present research work and also thankful to Dr. Aparna Kumawat, associate professor, and Miss Hemlata Sen, research scholar, Department of Zoology, Government P.G. Meera Girls College, Udaipur for their valuable support.

References

- Alexander, C.P., Kirubakaran, C.J.W. and Michael, R.D. 2010. Water soluble fraction of *Tinospora cordifolia* leaves enhanced the non-specific immune mechanisms and disease resistance in *Oreochromis mossambicus*. *Fish & Shellfish Immunol.* 29: 765-772.
- Anurapreeda, P., Chawengkirttikul, R., Ngamniyom, A., Panyarachun, B., Puttark, P. and Koedrih, P. 2017. The *in vitro* anthelmintic activity of the ethanol leaf extracts of *Terminaliacatappa* L. on *Fasciola gigantica*. *Parasitol.* 144(14): 1931-1942.
- Damor, R. and Swarnakar, G. 2018. *In vitro* anthelmintic effects of fruit extracts of *Citrullus colocynthis* on liver fluke *Fasciola gigantica* in buffaloes. *Int J. Innov. Res. Review.* 6(1) : 1-11.
- Githiori, B., Athanasiadou, S. and Thamsborg, M. 2006. Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis of small ruminants. *Vet. Parasitol.* 139 : 308-320.
- Hamsa, T.P. and Kuttan, G. 2010. *Tinospora cordifolia* ameliorates urotoxic effect of cyclophosphamide by modulating GSH and cytokine levels. *Exp. Toxicol. Pathol.* 1 : 1-8.
- Jabbar, A., Raza, M.A., Iqbal, Z. and Khan, M.N. 2006. An inventory of the ethno botanicals used as anthelmintic in the southern Punjab (Pakistan). *J. Ethnopharmacol.* 108 (1) : 152-154.
- Jeyanthilakan, N., Murali, K., Anandaraj, A. and Abdul,

- B.S. 2012. *In vitro* evaluation of anthelmintic property of ethano-veterinary plant extracts against the liver fluke *Fasciola gigantica*. *J. Parasit. Dis.* 36 (1) : 26-30.
- Leyon, P.V. and Kuttsn, G. 2004. Effect of *Tinospora cordifolia* on the cytokine profile of angiogenesis-induced animals. *Inter. Immunopharmacol.* 4 : 1569-1575.
- Minsakorn, S., Nuplod, K., Puttarak, P., Chwengkirttikul, R., Panyarachun, B., Ngamniyom, A., Charoenkul, T., Aad, M., Panyaranchun, P. and Anuracpreeda, P. 2019. The anthelmintic effects of medicinal plants extracts against paramphistome parasites, *Carmyerius spatiosus*. *Acta. Parasitol.* 64(3) : 566-574.
- Nahla, A., Radwan, Amal, I., Khalil, Amera E. and Wahdan 2012. *In vitro* evaluation of anthelmintic activity of *Allium sativum* against adult *Cotylophoron cotylophorum* (Paramphistomidae). *P.U.J.* 5(2) : 135-146.
- Prince, P.S.M. and Menon, V.P. 1999. Antioxidant activity of *Tinospora cordifolia* roots in experimental diabetes. *Journal of Ethnopharmacology.* 65 : 277-281.
- Rajesh, K.D., Subramani, V., Annamalai, P. and Durai, R. 2017. *Gastrothylax crumenifer*: Ultrastructure and histopathological study of *in vitro* trematocidal action of *Marattia fraxinea* (Sm.). *Clin. Phytosci.* 3 : 3.
- Singh, J., Bagla, A. and Pahal, V. 2010. Hepatoprotective activity of herbal extracts of carbon tetrachloride intoxicated albino rats by measuring anti-oxidant enzymes. *Inter. J. Pharm. Tech. Res.* 2 (3) : 2112-2115.
- Swarnakar, G., Kumawat, A. and Swarnakar, P. 2015. Effect of aqueous and alcoholic fruit extract of *Citrullus colocynthis* on amphistome *Orthocoelium scoliocoelium*. *Int. J. Adv. Pharma. Sci.* 6 (2) : 2790-2796.
- Swarnakar, G., Roat, K., Sanger, B. and Kumawat, A. 2014. Anthelmintic effect of *Trigonella foenum-graecum* on tegument of *Gastrothylax crumenifer* in cattle of Udaipur. *Int. J. Curr. Microbiol. App. Sci.* 3(5) : 599-606.
- Swarnakar, G., Menaria, K., Kumawat, A., Roat, K. and Damor, R.N. 2020. *In vitro* anthelmintic effect of *Citrullus colocynthis* extract on *Cotylophoron cotylophorum*. *I. Vet. J.* 97(05) : 9-11.
- Treadway, S. 1998. Exploring the universe of ayurvedic botanicals to manage bacterial infections. *Cli. Nut. Insi.* 6 (17): 1-3.
- Warrier, P.K., Nambiar, V.P.K., Ramankutty, C. and Nair, R.V. 1996. Indian medicinal plants: a compendium of 500 species. 5 : 283-285.
-