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In Vitro Anthelmintic Efficacy of *Trigonella foenum-graecum* Seed Extract on *Haemonchus* sp. (An Abomasal Parasite) in Goats

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Abstract

This study was meant to assess anthelmintic potency (*in vitro*) of methanolic extract of the seeds of *Trigonella foenum-graecum* .The phytochemical screening results revealed the presence of various types of secondary metabolites i.e. alkaloids, terpenoids, flavonoids, tannins, saponins, phenol, anthraquinone and carbohydrates. Moreover, *in vitro* anthelmintic results shown that the methanolic extract of the seeds of *Trigonella foenum-graecum* exhibited significant ($P \leq 0.05$) dose-dependent manner of anthelmintic activity on *Haemonchus* sp. as compared to albendazole. Various modern anthelmintic drugs have been used to eradicate helminths but these drugs are heavily-priced and unapproachable for poor farmers. The present research recommends that the seeds of *Trigonella foenum-graecum* as an alternative to the pharmaceutical industries to develop herbal anthelmintic drugs to control helminthiasis.

Keywords: *Trigonella foenum-graecum, Secondary metabolites, Anthelmintic activity, Haemonchus sp., Helminthiasis*

1. Introduction

Helminthiasis exerts adverse effects on the fitness and yield of the small ruminants. The effects are more diverse, and prominent in goat and sheep as compared with other species of livestock (Iqbal *et al.*, 1993). Among the parasitic infestation, endoparasites have played the most important role to reduce productivity and health. *Haemonchus* sp., a gastrointestinal blood sucking nematode is found to be the most prevalent species in goats and it causes enormous economic losses like loss of appetite, anaemia, damages in gastric function and alterations in internal body metabolism (Vatta *et al.*, 2001). The main prophylactic mode used against this parasite has been chemical anthelmintic treatments. However, the recurrent use of anthelmintic medicines has inexorably led to the development of drug resistance and nowadays it is becoming a worldwide phenomenon (Jackson and Coop, 2000). The emergence of drug resistance, as well as chemical residue and their toxicity, lead the researchers to focus towards the herbal medicine as an alternative to cure haemonchosis. In the ancient times, herbal medicinal plants have been used to treat parasitism without any side effect. Many scientists used different medicinal plants to treat haemonchosis in different parts of the World such as; *Butea frondosa* (Jangde *et al.*, 2001; Swarnkar *et al.*, 2008), *Vernonia amygdalina* and *Annona senegalensis* (Alawa *et al.*, 2003), *Chenopodium album* (Yadav *et al.*, 2010).

Trigonella foenum-graecum (Linn.); commonly known as Methi and Fenugreek, is an annual herb and belongs to the family Leguminosae, (Amri *et al.*, 2009). It is extensively cultivated in many parts of India and the World. Ayurveda and Siddha (Indian traditional systems of medicine) have mentioned that this plant was used to combat different types of diseases viz., dysentery, fever, arthritis, loss of appetite, bronchitis and heart diseases, etc., however, in Unani system it is used to treat aphrodisiac, diuretic (Nadkarni, 1982;

Bahatti *et al.*, 1996). Several previous research disclosed the medicinal efficacy of this plant as an anticancerous (Shabbeer *et al.*, 2009), antibacterial (Premanath *et al.*, 2011, Kumari *et al.*, 2016) and antifungal (Dharajiya *et al.*, 2015), antidiabetic (Bliga *et al.*, 2017). But very few reports are available on the anthelmintic potential of this plant such as anthelmintic activity of this plant on *Pheritima posthuma* (Khadse and Kakde, 2010), on *Hymenolepis nana* (cestode) and *Syphacia obvelata* (nematode) in mice (Ghafgazi *et al.*,1981-82), and on the *Gastrothylax crumenifer* in cattle (Swarnakar *et al.*, 2014). Keeping this in the mind the present study is aimed to carry out the anthelmintic efficiency of *Trigonella foenum-graecum* on *Haemonchus* sp. in goats as an alternative to herbal veterinary medicine in the forthcoming future.

1. Materials and Method

In vitro investigation was carried out to evaluate the anthelmintic efficiency of methanolic extract (ME) of the seeds of *Trigonella foenum-graecum* against *Heamonchus* sp. in goats. Albendazole was used as a standard drug (Positive control) for the assay. The experiment was conducted at the Parasitology and Silkworm Pathology Laboratory of the Department of Zoology (formerly Dept. of Applied Animal Sciences) Babasaheb Bhimrao Ambedkar University (B. B. A. U.), Lucknow, Uttar Pradesh.

2. Collection of plant material

Seeds of *Trigonella foenum-graecum* were bought from the local markets of Lucknow and were validated in the Department of Applied Plant Sciences of the same University.

3. Preparation of extract

The plant seeds were washed cautiously in running water, dried in the oven at 45^0c for 2 to 3 days till the seeds are completely dry, and ground to a fine powder with the help of electric grinder. 100 g of fine powder of the seeds were subjected to extraction using 500 ml methanol as a solvent in a Soxhlet apparatus, for 2 days at room temperature. After that concentrated by evaporation in the water bath, then dried at 20 ^{0}c – 25 ^{0}c and finally, stored in airtight bottles at 4 ^{0}c (Sermakkani *et al.*, 2010; Khyade *et al.*, 2012).

4. Preliminary phytochemical analysis

Phytochemical analysis was carried out on the seed extract of *Trigonella foenum-graecum* by following the standard methods (Harborne, 1973; Trease and Evans, 1989; Sofowora, 1993; Wang *et al.*, 2010; Hegde and Joshi, 2010; Sawant and Godghate, 2013).

5. Collection of test parasites

Freshly slaughtered gastrointestinal (GI) tracts of goats were collected from the slaughter houses in the study area and were quickly transported to the Parasitology Laboratory of the Department of Zoology, B.B.A.U., Lucknow, Uttar Pradesh. The tracts were dissected by following the standard procedure and examined for the presence of *Haemonchus* sp. Adult worms were collected, washed with normal saline (0.9%) and kept in phosphate buffered saline (PBS, pH- 7.5-8.0) until further use in the anthelmintic assays.

6. *In vitro* anthelmintic assay

The anthelmintic assay of the methanolic extract (ME) of *Trigonella foenum-graecum* was performed by following the standard protocol (Dash *et al.*, 2002; Eguale *et al.*, 2007; Ullah *et al.*, 2013) with certain modifications. Extract concentrations and drug solution were freshly prepared before starting the assay. Ten actively motile, same sized worms were chosen and placed in petri dishes having 1mg/ml, 2.5mg/ml, 5mg/ml and 10 mg/ ml concentration of methanolic extract (ME) of the *Trigonella foenum-graecum* in PBS. PBS was taken as the negative control and standard drug albendazole was used as positive control. Each concentration was set for three replicates. Parasites were observed for the time taken for paralysis and finally, the death of the individual worms at 1, 2, 3, 4, 5, 6, 7, and 12 hours of the time interval and readings were recorded in the minutes. The paralyzed worms were placed in PBS for 30 minutes after each interval of time, for attainment the possible rescue of the parasite motility. After completion of the assay (after 12 hours), alive and dead worms were counted for each of the experimental groups under a dissecting microscope and recorded. The paralysis time was analyzed on the basis of the behaviour of the parasites i.e. no retrieval in motility even after

placing in PBS whereas death was determined on the basis of the complete loss of motility with discoloration in body color (Iqbal *et al.*, 2001; Dash *et al.*, 2002; Ghosh *et al.*, 2005).

7. Statistical Analysis

All the data are expressed as Mean ± S.E.M of 10 worms for each concentration. Data analysis is done by using one way ANOVA followed by Tukey- post hoc test with the help of statistics software SPSS version 20.00. The difference in the value at P≤0.05 was set as statistically significant.

8. Results

In the current study, the phytochemical screening of the methanolic extract (ME) of the seeds of *Trigonella foenum-graecum*, showed the presence different groups of the phytochemical compound, namely tannins, flavonoids, saponins, terpenoids, phenol, anthraquinone and, carbohydrates as tabulated in Table 1. Anthelmintic efficacy (*In vitro)* of the methanolic extract of the seeds of *Trigonella foenum-graecum* was evaluated against adult nematode parasites (*Haemonchus* sp.), in goats, and the results are shown in Table 2 and Figure 1. The results revealed that the methanolic extract (ME) of the plant exhibited significant ($P \leq 0.05$) dose-dependent efficacy, causing paralysis as well as the death of the worms under all the tested concentrations as compared to standard drug; Albendazole (Table 2, Figure 1). The lower concentration of Albendazole (positive control) i.e. 1 mg/ml, was very effective to cause paralysis at 32.0±2.3 min and death at 57.3±5.5 min, while in the control (PBS) group (negative control), no paralysis or death occurred within 12 hours of the study period. Worms were paralyzed and died in a duration of 97.3±6.3 min and 148±1.1 min, respectively; whereas, 88.0±4.7 min was taken for paralysis and 126.7±2.0 min for death was taken by the parasites at 2.5mg/ml concentration, However, at the 5mg/ml of concentration, worms were paralyzed at 72.0±1.7 min while, death occurred at 107.7±1.4 min. 10mg/ml concentration showed the most effective anthelmintic activity which is evident in the least amount of time since paralysis and finally, the death of the worms occurred at 48.3±2.0 min and 90.3±2.6 min respectively.

Table 1: Phytochemical screening (qualitative) of methanolic extract (ME) of the seeds of *Trigonella foenum-graecum*

Phytochemical compound	Methanolic extract (ME)
Alkaloids	Present
Flavonoids	Present
Glycosides	Absent
Terpenoids	Present
Tannins	Present
Saponins	Present
Phenol	Present
Carbohydrates	Present
Anthraquinone	Present

Table 2: *In vitro* anthelmintic efficacy of methanolic extract (ME) of seeds of *Trigonella foenum- graecum* against *Haemonchus* sp.

Treatment	Concentration (mg/ml)	Paralysis time (min) (Mean±SEM)	Death time (min) (Mean ±SEM)
Trigonella foenum-graecum (ME)	1	97.3±6.3*	148.0±1.1*
	2.5	88.0±4.7*	126.7±2.0*
	5	72.0±1.7*	107.7±1.4*
	10	48.3±2.0*	90.3.±2.6*
Albendazole	1	32±2.3*	57.3±5.5*
Control	-	0.0	0.0

Values are Mean ± SEM, (N= 10) analysed by one-way ANOVA followed by Tukey post hoc test, *P ≤ 0.05 as compared to control group.

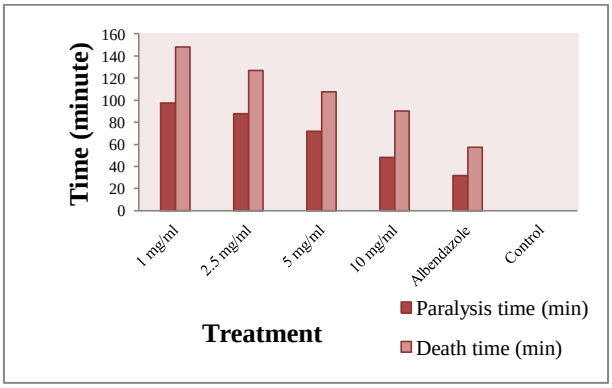

Figure 1: Anthelmintic efficacy of methanolic extract (ME) of seeds of *Trigonella foenum-graecum* as compared to standard drug (Albendazole)

9. Discussion

Synthetic anthelmintic medicines remove the parasites by creating a paralytic stage by interfering with the metabolism of worms, by rupturing cuticle wall, and finally eviction of the parasite by the immune system (Aisawanya *et al.*, 2010). Albendazole gets rid of the worms by knocking down the cytoskeletal structure, putting hindrance

in chloride ion conductance which leads to impaired glucose uptake, and in that way, causing paralysis and finally death of the worm (Nikesh *et al.*, 2011; Parvathy *et al.*, 2012).

Traditional veterinary plants are the best source for numerous types of the biologically active compound having important medicinal and nutritional characteristics (Swargiary *et al.*, 2017). The present study validated that *in vitro* analysis of the methanolic extract of the seeds of *Trigonella foenum-graecum* has significant ($P \leq 0.05$) anthelmintic efficacy against *Haemonchus* sp. Previous studies have exposed that the anthelmintic potential of the plant is because of their biological active constituents' viz., Phenolic group (tannins, Phenols, and flavonoids), saponins, and alkaloids (Anthnasiadou *et al.*, 2000; Wang *et al.*, 2010). Phenolic compounds may be interfering with the energy generation metabolism of parasites by uncoupling oxidative phosphorylation leads to death (Martin, 1997). Saponin leads to loosening and rupture of helminths cuticular surface (Roy *et al.*, 2010). Alkaloids work as anti-oxidating compound. It interferes with nitrate metabolism and simultaneously alters the protein metabolism of the parasites. It also hinders the path of sucrose transportation that affects the glucose uptake in the helminths which leads to degeneration in CNS, causing paralysis (Srivastav *et al.*, 2018).

The current research reported that the methanolic extract showed the presence of Phenolic compound (i.e., tannins flavonoids and phenol), saponins, alkaloids, terpenoids, anthraquinone, and carbohydrates. Similar results were reported by the study conducted by Kumari *et al.*, 2016 and Mishra *et al.*, 2016. The results also disclosed that the methanolic extracts of the seeds of *Trigonella foenum-graecum* showed significant dose-dependent efficacy towards the paralysis and death of the worms, as compared to the standard drug albendazole (positive control). The results are at par with the study conducted by Khadse and Kakde, 2010, who also worked using the same plant on *Pheritima posthuma*. Similar results were reported by other researchers using different medicinal plants on *Pheritima posthuma* (Kane *et al.*, 2009) and *Ascaridia galli* (Sujith *et al.*, 2014).

The potency of anthelmintic efficacy of this plant is may be due to the presence of biologically active compounds, i.e. tannins, saponins, phenols, and alkaloids. Ultimately, the results of this research must be validated through the *in vivo* trials to assess the authenticity of the anthelmintic efficacy of this plant.

10. Conclusion

The Phytochemical results indicated the presence of various types of biochemical constituents. *In vitro*, anthelmintic analysis reports mention that the seeds of *Trigonella foenum-graecum* have significant dose-dependent anthelmintic potency with respect to standard drug Albendazole. The plant hence can be further explored to isolate the active compound which is found to be responsible for anthelmintic efficacy and which can be used as an alternative against synthetic anthelmintics to treat different types of parasitic diseases in the imminent future.

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Conflict of interest

The authors have stated that this article has no conflict of interest.

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