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Research Article

METHANOLIC LEAF EXTRACTS OF TULSI AND SPINACH AFFECTS MORPHOMETRY AND OXIDATIVE MARKERS IN *HAEMONCHUS CONTORTUS*

P. ANJANEYULU, G. SRIVIDYA*, G. SAI PRATHYUSHA, V. SRI HARSHINI, K.V. VENKATA RAO[1], V. SAMATHA[2], G.S. RAO and K. SRI HARSHA
Dept. of Veterinary Pharmacology & Toxicology, NTR CVSc, Gannavaram,
[1]Dept. of Veterinary Pharmacology & Toxicology, CVSc, Garividi,
[2]Department of Veterinary Pathology, NTR CVSc, Gannavaram,
Sri Venkateswara Veterinary University (Andhra Pradesh)

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ABSTRACT

An attempt was made to determine the anthelmintic activity of methanolic leaf extracts of Tulsi and Spinach against *Haemonchus contortus*. The anthelmintic activity was determined by keeping *H. contortus* live parasite in different concentrations of Tulsi and Spinach for 3 h. After completion of 3 h treatment the worms were homogenized using Triton-X buffer. A control group of parasite was also kept in PBS. The anthelmintic activity was assessed by estimating the oxidative markers like SOD, GSH and total protein. Morphometric alterations and motility tests were also performed. Results of morphometric studies revealed that there was a reduction in the body width and length of vulva in *H. contortus* in albendazole and extracts treatment. The anthelmintic activity of selected plant extracts was compared with albendazole. Results of the motility test revealed that the time required for complete immobilization of parasite in albendazole was 25 minutes, whereas in Tulsi extract immobilization was observed for 6, 7, 8h at 10, 3 and 1mg/mL concentrations, respectively. However, in Spinach, the immobilization time observed was 7, 8, 9h at 10, 3 and 1mg/mL concentrations, respectively. The total protein and SOD levels increased significantly in the treatment groups compared to the control parasites maintained in PBS buffer at $P<0.05$. Results depicts Tulsi and Spinach exhibit anthelmintic activity comparable to that of albendazole *in vitro* based on the parameters tested.

Keywords: Albendazole, Athelmintic activity, *Haemonchus contortus*, Tulsi, Spinach

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Haemonchus contortus, a common gastrointestinal nematode in sheep and goats, is significant for its high reproductive rate and short life cycle, which are key factors contributing to anthelmintic resistance (Rashid and Irshadullah, 2018). While commercial drugs are commonly utilized to evaluate anthelmintic activity against gastrointestinal nematodes, there is limited research on the effectiveness of herbal preparations against *H. contortus* (Kalkal and Vohra, 2023). Due to the rise of drug resistance in gastrointestinal nematodes, alternative control strategies such as rotational grazing, selective breeding, nutritional supplementation, targeted treatments and the use of plants with natural anthelmintic properties are increasingly being applied (Rashid and Irshadullah, 2018). Recent approaches of using phytochemicals as anthelmintic agents due to the presence of bioactive compounds may serve as an alternative to overcome the resistance as they are safe, non-toxic and have a changed site of action (Buchineni *et al.*, 2015).

By keeping this in view the present study was conducted to determine the anthelmintic activity of Tulsi and Spinach extracts against *H. contortus*.

*Corresponding author: vidyaprasad125@gmail.com

MATERIALS AND METHODS

Preparation of plant extracts

The fresh leaves of *Ocimum sanctum* and *Spinacia oleracea* were collected, shade dried, powdered and stored in air tight containers. 10 g of powder was combined in methanol (1:10) in a flask firmly sealed and kept at room temperature for 48 hours on orbital shaker at 120 rpm. The contents in the beaker were filtered by using Whatman's Filter Paper 1. The resulting filtrate was transferred to pre-weighed beaker and kept at room temperature for evaporation of solvent. After complete evaporation of the solvent, the beaker was once again weighed to know the amount of extract (Senthilnathan *et al.*, 2019). The stock solution (100 mg/ml) was prepared in DMSO and was stored in cool dry place until further use. For this study we have used 1, 3, 10 mg/ml concentrations of Tulsi and Spinach leaf extracts.

Paralysis & mortality assay

The mature *H. contortus* worms, collected from abomasum of sheep and performed paralysis and mortality assay according to Goel's method (2020). In each petridish, ten worms were taken and exposed to

Table 1. Antioxidant parameters

S.No	Group		Total Protein (mg/mL)	GSH (µm of GSH/mg of protein)	SOD (units/mg of protein)
I	Control		0.32 ± 0.07	2.28 ± 0.05	2.65 ± 0.13
II	Albendazole (10 mg/mL)		0.71 ± 0.03	2.13 ± 0.13	3.22 ± 0.29
III	Tulsi	10 mg	0.71 ± 0.02	2.88 ± 0.70	3.82 ± 0.59**
		3 mg	0.72 ± 0.04	2.99 ± 0.66	3.44 ± 0.27
		1 mg	0.55 ± 0.12	2.04 ± 0.04	2.66 ± 0.09
IV	Spinach	10 mg	0.56 ± 0.10	2.25 ± 0.10	2.94 ± 0.15
		3 mg	0.56 ± 0.03	2.12 ± 0.05	2.27 ± 0.40
		1 mg	0.41 ± 0.10	2.24 ± 0.15	1.92 ± 0.16

Table 2. Morphometric alterations of *Haemonchus contortus*

S.No	Group		Body length (cm)	Body width (µm)	Vulva length (µm)
I	Control		3.23 ± 0.11	31 ± 2.21	59.5 ± 2.17
II	Albendazole		2.17 ± 0.18	24 ± 2.08	43.5 ± 1.97
III	Tulsi	10 mg	2.5 ± 0.14	29 ± 1.94	50 ± 2.36
		3 mg	2.72 ± 0.17	28.5 ± 1.83	49 ± 2.21
		1 mg	2.8 ± 0.15	33 ± 1.53	48.5 ± 3.95
IV	Spinach	10 mg	2.71 ± 0.15	29.3 ± 1.17	47.5 ± 1.86
		3 mg	2.79 ± 0.14	27.5 ± 1.71	45 ± 2.47
		1 mg	2.86 ± 0.16	30.5 ± 1.89	49 ± 2.56

Table 3. Paralysis and death time analysis of adult *Haemonchus contortus* worms.

Time of Paralysis

Time of exposure	Control	Alb (10 mg/mL)	Tulsi			Spinach		
			10 mg/mL	3 mg/mL	1 mg/mL	10 mg/mL	3 mg/mL	1 mg/mL
0 h	-	-	-	-	-	-	-	-
0.5 h	-	8.5 ± 0.5	-	-	-	-	-	-
1 h	-	1 ± 0	-	-	-	-	-	-
2 h	-	-	-	-	-	-	-	-
4 h	-	-	0.5 ± 0	-	-	-	-	-
6 h	-	-	9 ± 0.5	5 ± 0.5	4 ± 2	5.5 ± 1	4 ± 0.5	1 ± 1
8 h	-	-	1 ± 0	5 ± 0.5	5.5 ± 2	4 ± 1	6 ± 0.5	8.5 ± 1
12 h	7 ± 1	-	-	-	-	-	-	-

Time of Death

Time of exposure	Control	Alb (10 mg/mL)	Tulsi			Spinach		
			10 mg/mL	3 mg/mL	1 mg/mL	10 mg/mL	3 mg/mL	1 mg/mL
0 h	-	-	-	-	-	-	-	-
0.5 h	-	3.5 ± 1	-	-	-	-	-	-
1 h	-	6 ± 1	-	-	-	-	-	-
2 h	-	-	-	-	-	-	-	-
4 h	-	-	0 ± 0		-	-	-	-
6 h	-	-	3 ± 2	3 ± 0.5	3.5 ± 2	3 ± 1	2.5 ± 1	1 ± 0.5
8 h	-	-	6 ± 2	7 ± 0.5	6 ± 2	7 ± 1	7 ± 1	8 ± 1
12 h	5 ± 0.5	-	-	-	-	-	-	0.5 ± 0.5

methanolic extracts of Tulsi and Spinach at three different concentrations (10; 3; 1 mg/ml). Albendazole sulfoxide (10 mg/ml, positive control) and PBS (negative control).

Processing of Tissue:

A 10% (w/v) homogenate of parasite tissue was prepared for biochemical parameter estimation, following the method of Das *et al.* (2004). Antioxidant assays-

a. Total protein:

Total protein content in *H. contortus* tissue homogenate was estimated according to Lowry's method (1951) using bovine serum albumin (BSA) as a standard.

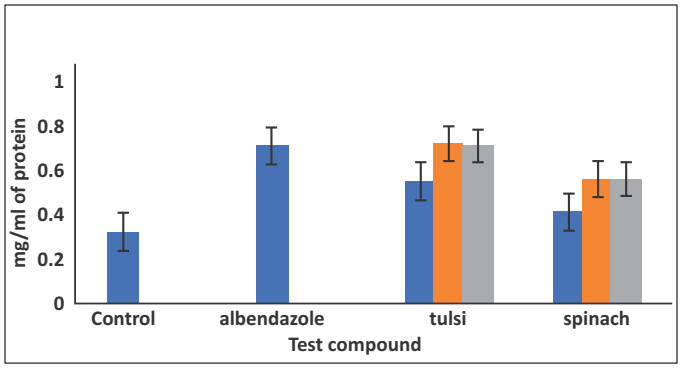


Fig. 1. Total protein levels in *H. contortus* exposed to albendazole, Tulsi and Spinach.

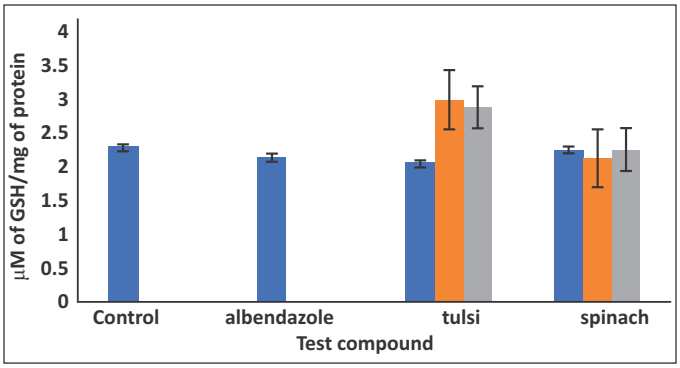


Fig. 2. GSH levels in *H. contortus* after exposure to albendazole, Tulsi and Spinach

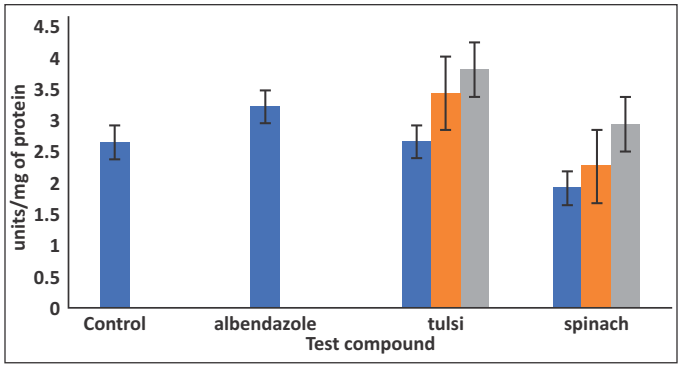


Fig. 3. SOD levels in *H. contortus* exposed to albendazole, Tulsi and Spinach.

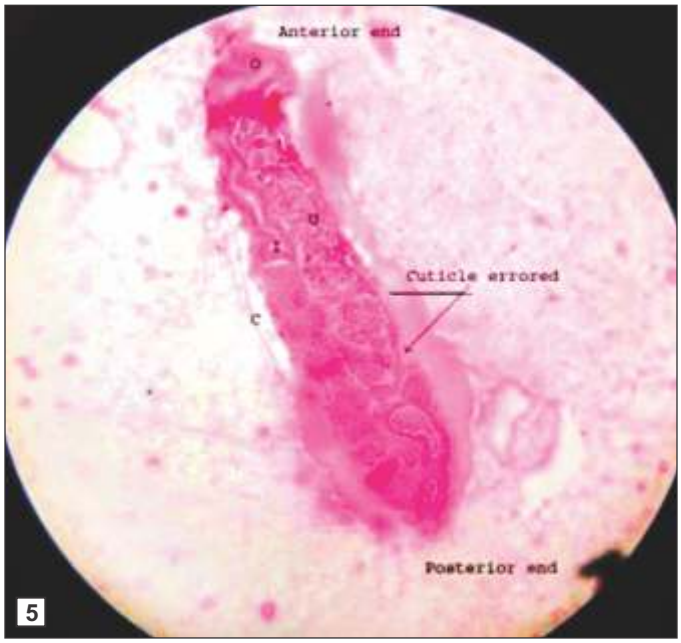


Fig. 5. Albendazole treated *H. contortus*: Cuticle (C) is intact at left side only upto an extent and in remaining areas the cuticle is completely eroded or dissolved indicating the action of the compound. Constriction of muscle cells can be clearly seen that resulted in compaction of internal organs viz., intestines (I) and Uterus (U). Anterior end of the worm is folded and inward dragging of esophagus (0) could be seen in a way to evade from the compound.

Fig. 6. Spinach treated *H. contortus*: Only small longitudinal middle section of the worm is presented in the section. Cuticle (C) is intact without etching or peeling throughout the section. However, Intestines (I) are very much constricted and Uterus is tightly coiled around the intestines indicating serious contractions in the muscle cells of these particular organs.

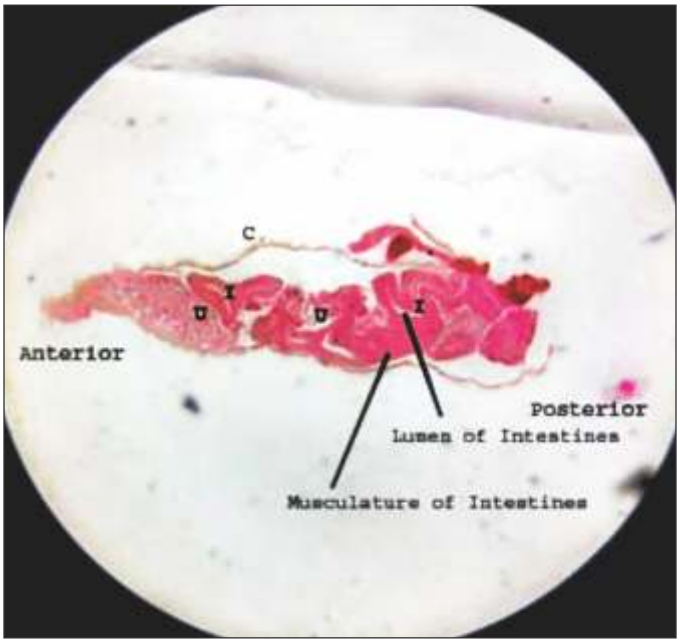
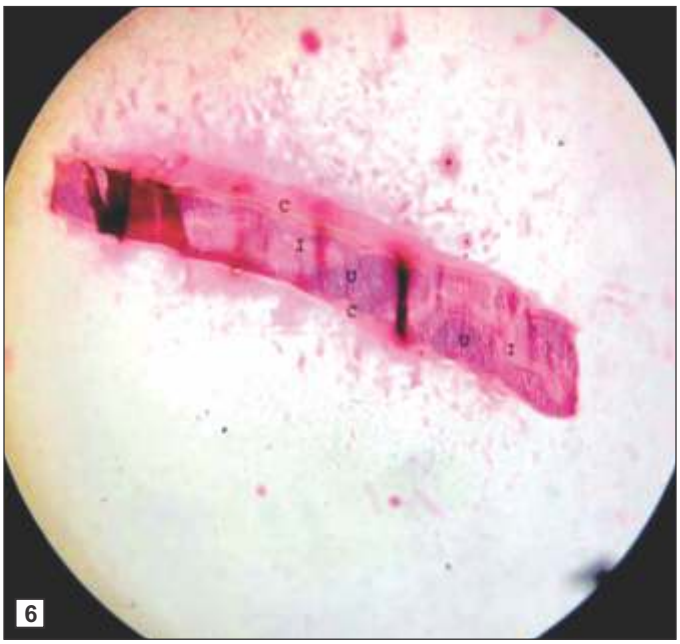


Fig. 4. *H. contortus* (control)



b. Superoxide dismutase (SOD) activity:

SOD activity in the *H. contortus* tissue homogenate was determined spectrophotometrically as described by Madesh and Balasubramanian (1998).

c. GSH:

GSH content in the liver was measured spectrophotometrically following the method described by Sedlak and Lindsay (1968).

Histopathological examination of *H. contortus*

Histomorphological changes in the parasite tissue, resulting from various treatments, were examined based on the method of Luna (1968).

Statistical analysis

The results were expressed as Mean ± SEM. The level of significance was determined at $p < 0.05$. in all the treatment groups were compared with control group using Graph Pad InStat.

RESULTS AND DISCUSSION

The results in the present study revealed that total protein in the control group and exposure to Tulsi at three different concentrations i.e., 10, 3, 1 mg/ml causes dose dependent increase in the total protein values whereas exposed to spinach extract altered the protein levels significantly with the range of 0.41 to 0.56 at $p<0.05$. There is no significant difference between 3 and 10 mg/ml dose of Tulsi and Spinach which were presented in table 1.

Albendazole sulfoxide which was taken as positive control to compare the anthelmintic activity of selected plant extracts showed the total protein value is 0.71 ± 0.03 which was similar to that of 3, 10 mg/ml of Tulsi.

The Antioxidant enzymes SOD and GSH serve as the markers of oxidative stress. The SOD levels has been increased in the both the treatment groups when compared to control. The levels of SOD were significantly increased in the Tulsi (10 mg/ml) group. The levels of GSH in albendazole sulfoxide and spinach were similar.

The findings of this study demonstrated that when *H. contortus* worms were exposed to different concentrations of Tulsi and spinach experienced early paralysis and death. The paralysis and mortality time of plant extract treated worms recorded were dose-dependent when compared to control. Of all the test extracts, Tulsi (10 mg/ml) showed the better anthelmintic effect.

Helminth infections usually occur due to lack of proper management practices and deworming schedule. Usually, benzimidazole group of drugs are used to control haemonchosis (Goel *et al.*, 2023). Due to indiscriminate use of these drugs parasites may develop resistance and deworming might become ineffective. Nowadays plant extracts are being extensively used as anthelmintics to overcome anthelmintic resistance as alternatives. Tulsi and spinach were being used at present as they are rich source of polyphenolic compounds. Because of the inclusion of alkaloids, tannins, and flavonoids in the methanolic extract, it was shown to be more effective as an anthelmintic agent. Extracts of *Justicia adhatoda*, *Vernonia amygdalina*, *Mikania micrantha*, *Momordica charantia*, *Lippia javanica*, *Newbouldia laevis* and *Zanthoxylum zanthoxyloides* possese considerable anthelminthic action (Goel *et al.*, 2023).

We observed that tulsi treatment generates more ROS than albendazole treatment. Tulsi has been found to quench free oxygen radicals on lipid peroxidation of various substances and to increase the activity of endogenous antioxidant enzymes and causes oxidative stress in *H. contortus*, resulting in altered metabolism, physical damage, and death of worms. Histological examination of *H. contortus* worms treated with various test compounds were depicted in figure 4, 5 & 6.

CONCLUSIONS

Based on the above findings it is revealed that Tulsi and Spinach showed anthelmintic activity compared to that of albendazole. Further studies may be explored to determine the bioactive compound responsible for anthelmintic activity and safety evaluation of selected plant extracts like Tulsi and Spinach.

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