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Investigation of In-Vitro Anthelmintic Activity of *Rumex hastatus* D.Don Stem and Root

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ABSTRACT

The anthelmintic potential of ethanolic and aqueous extracts of stem and roots of *Rumex hastatus* D.Don on adult Indian earthworm *Eisenia fetida*. Different concentrations (25, 50, 75 and 100 mg/ml) of ethanolic and aqueous extracts were tested for anthelmintic activity by observing the time required for paralysis and death of worms. The reference standard used was Albendazole (20 mg/ml) and 1% acacia in normal saline water as a control group. In the present study, the ethanolic and aqueous extracts of *Rumex hastatus* stem and roots at the concentrations of 25, 50, 75 and 100 mg/ml have shown anthelmintic activity. The most significant anthelmintic activity was shown by the ethanolic extract of roots and the least significant activity was shown by aqueous extract of the stem. The preliminary phytochemical screening showed the presence of various phytoconstituents in all the tested extracts.

Keywords: *Rumex hastatus* , Anthelmintic activity, Albendazole, *Eisenia fetida*

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INTRODUCTION

Rumex hastatus D.Don (Polygonaceae), commonly known as 'khatimal' is a perennial shrub, widely distributed in India in Jammu and Kashmir, Himachal Pradesh, Uttaranchal and Kumaun [1]. Traditionally, the decoction of the roots of the plant is used in asthma, backache and rheumatism [2]. The leaves and young shoots are used as flavouring agent, carminative, purgative, diuretic and stomach problems [3]. The main chemical constituents which are reported from the plant belong to various classes viz; anthraquinones, naphthalenes, flavonoids and phenolic compounds [4]. The plant has been screened for various pharmacological activities like anti-viral [5], anti-bacterial, antifungal [6], anti-diarrheal [7] and antioxidant [8]. As traditionally the plant is used in stomach problems, the present study was designed to evaluate the in -vitro anthelmintic potential of *Rumex hastatus*.

MATERIALS AND METHODS

Plant material

The collection of whole plant of *Rumex hastatus* was done in the month of September, 2010 from Darlaghat, Distt Solan, Himachal Pradesh (India). The plant specimen was identified and authenticated by Dr.H.B.Singh, Head, Raw Material, Herbarium and Museum Division, NISCAIR, New Delhi (Ref.NISCAIR/RHMD/Consult/-2010-11/486/84).

Preparation of extract

The air dried stem and root parts of the plant were ground to coarse powder individually. The dried powdered plant material was extracted with 90% ethanol in soxhlet apparatus for the preparation of ethanolic extract and for aqueous extract preparation, the plant material was extracted with distilled water by cold maceration method. All the extracts were further dried at low temperature under reduced pressure and used for the present study.

Phytochemical screening

The Phytochemical screening of ethanolic and aqueous extracts of stem and roots of *Rumex hastatus* was performed to detect the presence of various phytoconstituents in the plant [9].

Worm collection and authentication

Adult Indian earthworms (*Eisenia fetida*) were used for the evaluation of in vitro anthelmintic activity which were collected from Agronomy Department of Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar (Haryana) and authenticated by Dr. Thakral (Senior Scientist), Agronomy Department, CCSHAU, Hisar. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for whole experimental protocol.

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Drugs and chemicals

Albendazole (GlaxoSmithkline, Mumbai). All other chemicals or solvents used were of analytical grades.

Sample preparation

The preparation of the test samples for in-vitro study was done by dissolving and suspending 2.5 gm of ethanolic and aqueous extracts of stem and root in 1% acacia and the volume was adjusted to 25 ml with normal saline to obtain a stock solution of concentration of 100 mg/ml. The stock solution was further diluted to obtain concentration range of 25, 50 and 75mg/ml.

Evaluation of anthelmintic activity

The adult Indian earthworm *Eisenia fetida* was used in the experimental study as it has anatomical and physiological resemblance to the intestinal roundworm parasites of human beings. Moreover, they are easily available and are suitable model for screening of anthelmintic drugs [10-13]. The in-vitro studies were performed according to the method of Ghosh et al [14]. The worms were divided into eighteen groups containing six earthworms of approximately equal sizes, placed in petridishes for each concentration separately. 50 ml suspension of ethanolic as well as aqueous extracts (25, 50, 75 and 100 mg/ml) of stem and roots respectively were used as test samples, albendazole (20 mg/ml) as reference standard while 1% acacia in normal saline as control group were poured into the petridishes. The time taken to paralyse or cause death of the individual worm was observed. Paralysis was said to occur when the worms do not revive even in normal saline. Death was ascertained, when the worms lost their motility followed by fading away of their body color and was concluded by transferring it into a beaker containing hot water at 50°C, which stimulated and induced movements if the worms were alive [15]. The results were shown and expressed as mean ± SEM of six worms in each group.

RESULTS AND DISCUSSIONS

The preliminary phytochemical screening of ethanolic extracts of stem and roots of *Rumex hastatus* showed the presence of anthraquinone glycosides, tannins, carbohydrates, saponins and steroids.The ethanolic and aqueous extracts of stem and roots of the plant showed anthelmintic activity in dose dependent manner. The shortest time required for paralysis and death of earthworms was observed with 100 mg/ml of ethanolic extracts of root as 10.00 ± 0.3651 min and 20.50 ± 0.4282min respectively, followed by aqueous extracts of root which showed paralysis and death time as 30.67 ± 0.3333 min and 44.00 ± 0.3651min respectively at the same concentration. The same concentration of ethanolic extracts of stem showed paralysis and death time as 36.33 ± 0.3333 min and 73.67 ± 0.4944min respectively, while it was 41.33 ± 0.3333min and 79.83 ± 0.4773min with 100 mg/ml aqueous extract of the stem. The paralysis time with standard albendazole was 8.67 ± 0.3333 min and death time was

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16.67 ± 0.4216 min. In the present study, anthelmintic activity has been confirmed in the ethanolic and aqueous extracts of *Rumex hastatus* stem and roots at the concentrations of 25, 50, 75 and 100 mg/ml. [Table:1 and Figures1 to 4]. The ethanolic extract of root showed most significant anthelmintic activity, followed by the aqueous extract of roots. The ethanolic extract of the stem showed lesser activity than the ethanolic and aqueous root extract. The least activity was shown by aqueous extract of the stem. The lethal effect of albendazole was attributed to its inhibition of tubulin polymerization and blocking glucose uptake [16]. The presence of anthraquinone glycosides, tannins and saponins in the crude extracts of stem and root, as indicated by the preliminary phytochemical screening may be responsible for the anthelmintic activity. Further studies are required to isolate the active phytoconstituents which are responsible for the reported activity.

Table 1: Anthelmintic activity of ethanolic and aqueous extracts of stem and roots of *Rumex hastatus*

Treatment	Concentration	Paralysis time(mins)	Death time(mins)
Control (1% acacia in normal saline).	--	--	--
Albendazole(Standard)	20mg/ml	8.67 ± 0.3333	16.67 ± 0.4216
Ethanolic extract of *Rumex hastatus* (stem)	25mg/ml	125.50 ± 9916**	285.33 ± 1.667**
	50mg/ml	57.50 ± 0.4282**	102.50 ± 0.5627**
	75mg/ml	42.17 ± 0.3073**	83.50 ± 0.7638**
	100mg/ml	36.33 ± 0.3333**	73.67 ± 0.4944**
Aqueous extract of *Rumex hastatus* (stem)	25mg/ml	136.50 ± 0.8062**	326.83 ± 0.7923**
	50mg/ml	62.00 ± 0.3651**	119.83 ± 0.4773**
	75mg/ml	50.83 ± 0.3073**	91.50 ± 0.4282**
	100mg/ml	41.33 ± 0.3333**	79.83 ± 0.4773**
Ethanolic extract of *Rumex hastatus* (root)	25mg/ml	67.833 ± 0.7923**	87.33 ± 0.6146**
	50mg/ml	50.33 ± 0.4216**	68.83 ± 0.4944**
	75mg/ml	20.33 ± 0.4216**	33.33 ± 0.4944**
	100mg/ml	10.00 ± 0.3651**	20.50 ± 0.4282**
Aqueous extract of *Rumex hastatus* (root)	25mg/ml	71.33 ± 0.4944**	100.33 ± 0.6667**
	50mg/ml	58.50 ± 0.4282**	88.33 ± 0.4216**
	75mg/ml	47.50 ± 0.4282**	61.83 ± 0.4773**
	100mg/ml	30.67 ± 0.3333**	44.00 ± 0.3651**

Values are expressed as MEAN±SEM, One way ANOVA followed by Dunnett's test. Here, n=6 in each group.
*P<0.01, **P<0.001.

Statistical analysis

The values were expressed as mean ± standard error of mean (S.E.M.) and statistical analysis was carried out by using one-way analysis of variance (ANOVA) method followed by Dunnett's test. P<0.05 was considered statistically significant when compared with standard references.

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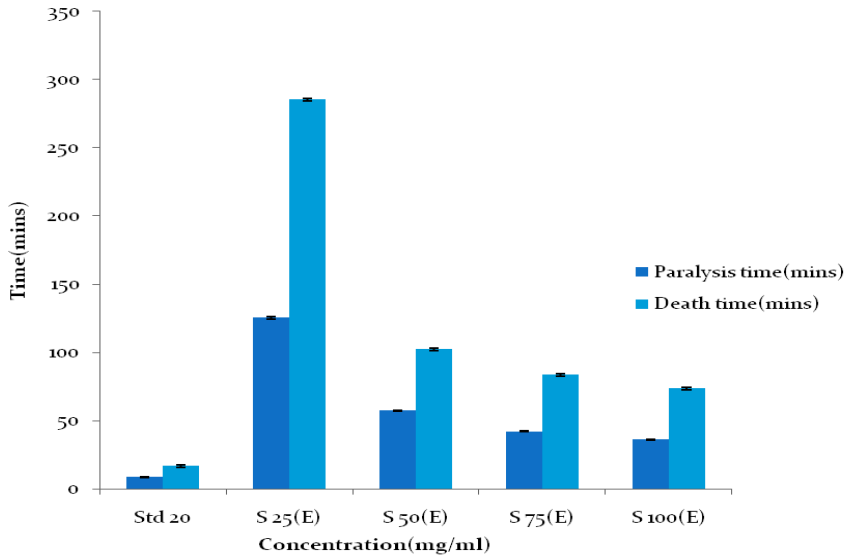

Fig1: Paralysis and death time of earthworms in stem ethanolic extract (R. hastatus)

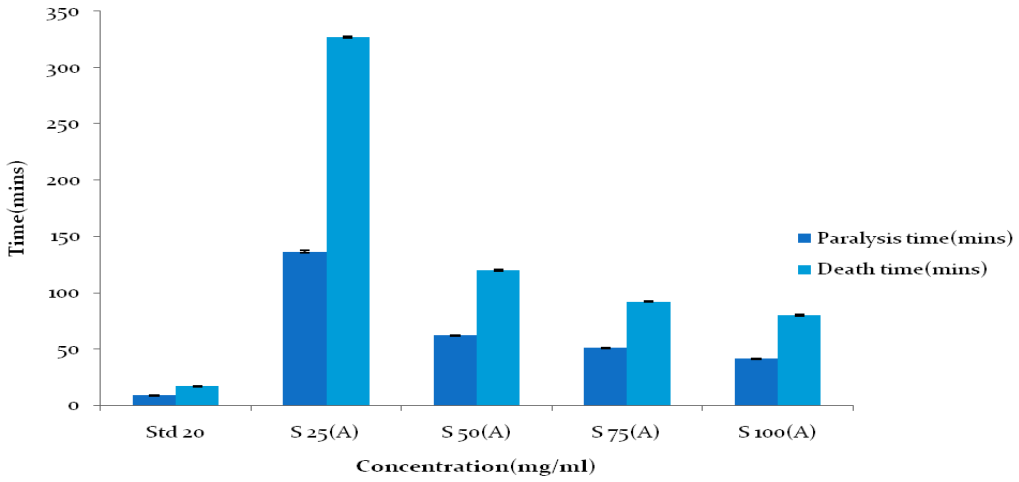

Fig 2: Paralysis and death time of earthworms in stem aqueous extract (R. hastatus)

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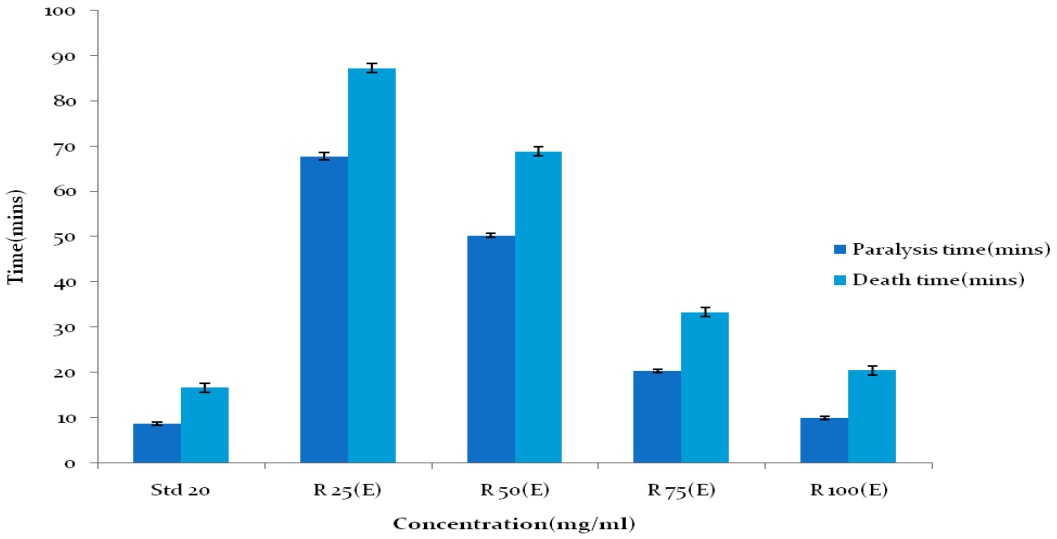

Fig 3: Paralysis and death time of earthworms in root ethanolic extract (R. hastatus)

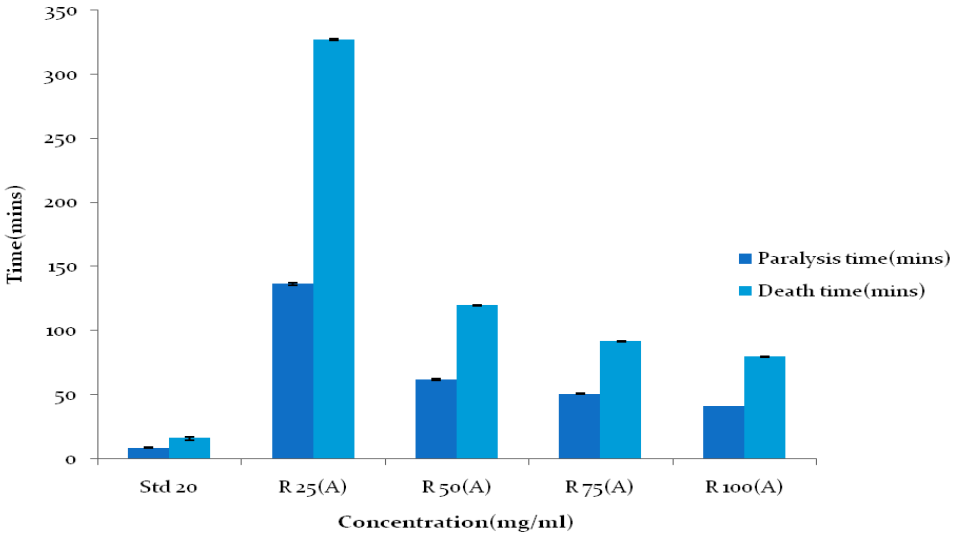

Fig 4: Paralysis and death time of earthworms in root aqueous extract (R. hastatus)

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