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Evaluation of *in vitro* anthelmintic activity of *Oenothera rosea* L'Hér. ex Aiton. stem and root

Sumitra Singh Dahiya*, Rupinder Kaur and Surendra Kr. Sharma

Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India

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ABSTRACT

Present study was undertaken to investigate the anthelmintic potential of ethanolic and aqueous extracts of stem and roots of Oenothera rosea L'Hér. ex Aiton on adult Indian earthworm Eisenia fetida. Various concentrations (25, 50, 75 and 100 mg/ml) of ethanolic and aqueous extracts were evaluated for anthelmintic activity by recording the time required for paralysis and death of worms. Albendazole (20 mg/ml) was used as reference standard and 1% acacia in normal saline water as a control group. The present study has shown that, the ethanolic and aqueous extracts of Oenothera rosea stem and roots at the concentrations of 25, 50, 75 and 100 mg/ml have been confirmed to have anthelmintic activity. But ethanolic extracts of stem showed most significant anthelmintic activity as compared to the ethanolic extracts of roots and also both the ethanolic extracts(stem and root) were more active than their aqueous extracts respectively. The preliminary phytochemical analysis indicated the presence of various phytoconstituents in all the tested extracts.

Keywords: Anthelmintic activity, *Oenothera rosea*, Albendazole , *Eisenia fetida*

INTRODUCTION

Oenothera rosea L'Hér. ex Aiton.(Onagraceae) also known as 'pink evening primrose' is a perennial herb, native to Central and South America. However, it is naturalized in India and grows wildly in the Himalayas. Traditionally, the aqueous infusion of the leaves of *Oenothera rosea* has been used in hepatic pains and kidney problems[1]. The plant has also been used in ill-defined stomach problems[2], cough, headache, inflammation[3], scabies, carbuncles and pimples[4]. Chemical constituents belonging to various classes *viz;* flavonoids, coumarins, tannins, glycosides, alkaloids have been reported from *Oenothera rosea* [5]. The plant has been screened for various pharmacological activities like anti-diarrheal[6], cardiovascular[7] and anti-inflammatory[5,8]. Purpose of the study is to evaluate the *in -vitro* anthelmintic potential of *Oenothera rosea* as traditionally the plant is used in ill-defined stomach problems.

MATERIALS AND METHODS

Plant material

The whole plant of *Oenothera rosea* was collected in the month of June, 2009 from Y.S.Parmar University of Horticulture, Nauni, 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/-2009-10/1323/126).

Preparation of extract

Fresh stem and roots of *Oenothera rosea* were collected and air dried in shade at room temperature. Dried stem and root parts of the plant were ground to coarse powder individually. For ethanolic extract, the dried powdered plant material was extracted with 90% ethanol in soxhlet apparatus 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 ethanolic and aqueous extracts of stem and roots of *Oenothera rosea* were screened for the presence of various phytoconstituents such as alkaloids, carbohydrates, glycosides, flavonoids, tannins, saponins, amino acids, steroids and triterpenoids[9].

Worm collection and authentication

Adult Indian earthworms (*Eisenia fetida*) were used for the evaluation of *in vitro* anthelmintic activity. They 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 collected worms were washed with normal saline to remove all the faecal matter and used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used in complete experimental protocol.

Drugs and chemicals

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

Sample preparation

Test samples for *in-vitro* study were prepared 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, from this stock solution further dilutions were prepared to obtain concentration range of 25, 50 and 75mg/ml.

Evaluation of anthelmintic activity

The anthelmintic activity was performed on adult Indian earthworm *Eisenia fetida* as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. These are easily available and are suitable model for screening of anthelmintic drugs[10-12]. The *in-vitro* studies were performed according to the method of Ghosh et al[13]. The worms were divided into eighteen groups containing six earthworms of approximately equal sizes, placed in petridishes for each concentrations 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 a reference standard while 1% acacia in normal saline as control group were poured into the petridishes. Observations were made for the time taken to paralyse or cause death of individual worms. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed by fading away of their body color and was ascertained by transferring it into a beaker containing hot water at 50°C, which stimulated and induced movements if the worms were alive[14]. The results were shown and expressed as mean ± SEM of six worms in each group.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of ethanolic extracts of stem and roots of *Oenothera rosea* revealed the presence of tannins, carbohydrates, saponins and steroids. The ethanolic and aqueous extracts of stem and roots has showed anthelmintic activity in dose dependent manner.The shortest time required for paralysis and death of earthworms was with 100 mg/ml of ethanolic extracts of stem as 8.00 ± 0.3651 min and 11.167 ± 0.3073 min respectively, followed by ethanolic extracts of root which showed paralysis and death time as 10.333± 0.4216 min and 17.333±0.4216 min respectively at the same concentration. The same concentration of aqueous extracts of stem showed paralysis and death time as 16.833± 0.4773min and 22.500 ± 0.6191 min respectively, while it was 26.833 ± 0.4773 min and 46.500± 0.4282 min with 100 mg/ml root aqueous extract respectively. The paralysis time with standard albendazole was 8.67 ± 0.3333 min and death time was 16.667 ± 0.4216 min. The present study has shown

that, the ethanolic and aqueous extracts of *Oenothera rosea* stem and roots at the concentrations of 25, 50,75 and 100 mg/ml have been confirmed to have anthelmintic activity. But ethanolic extracts of stem showed most significant anthelmintic activity as compared to the ethanolic extracts of roots and also both the ethanolic extracts(stem and root) were more active than their aqueous extracts respectively.Tannins are polyphenolic compounds which were shown to produce anthelmintic activities[15-16]. Reported anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death[17-19]. It is possible that phenolic content reported in the ethanolic and aqueous extracts of stem and roots of *Oenothera rosea* produced similar effects and hence anthelmintic activity. The lethal effect of albendazole was attributed to its inhibition of tubulin polymerization and blocking glucose uptake[20]. All the results were comparable with the standard drug albendazole.

Anthelmintic activity of ethanolic and aqueous extracts of stem and roots of *Oenothera rosea*

Treatment	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
Control (1% acacia in normal saline).	--	--	--
Albendazole(Standard)	20	8.67 ± 0.3333	16.667 ± 0.4216
Ethanolic extract *(stem)*	25	74.333 ± 0.3333**	152.33 ± 0.4216**
	50	49.833 ± 0.4773**	100.00 ± 0.5774**
	75	13.667 ± 0.3333**	22.667 ± 0.4216**
	100	8.000 ± 0.3651**	11.167 ± 0.3073*
Aqueous extract *(stem)*	25	51.667 ± 0.4216**	78.167 ± 0.4773**
	50	33.833 ± 0.4773**	64.500 ± 0.5627**
	75	26.500 ± 0.4282**	51.167 ± 0.3073**
	100	16.833 ± 0.4773**	22.500 ± 0.6191**
Ethanolic extract *(root)*	25	72.333 ± 0.4216**	134.50 ±0.4282**
	50	49.833 ± 0.4773**	101.83 ± 0.4773**
	75	18.500 ± 0.4282**	51.333 ± 0.4216**
	100	10.333 ± 0.4216**	17.333 ± 0.4216
Aqueous extract *(root)*	25	176.17 ± 0.3073**	523.50 ± 0.7638**
	50	123.17 ± 0.7032**	371.50 ± 0.4282**
	75	34.167 ± 0.3073**	62.000 ± 0.5774**
	100	26.833 ± 0.4773**	46.500 ± 0.4282**

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

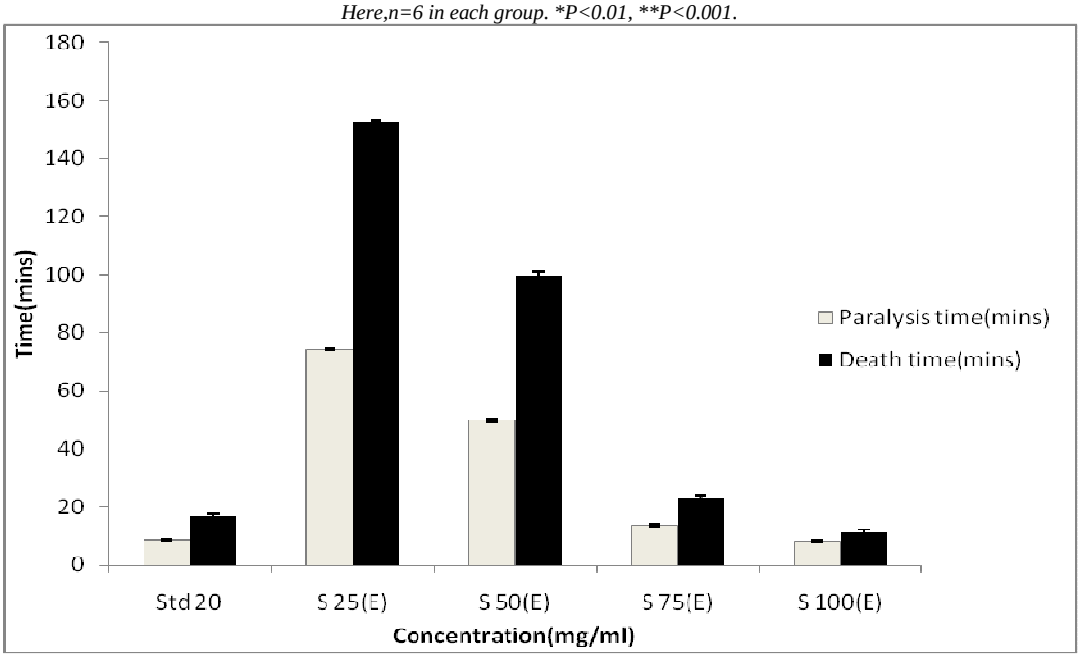

Fig. 1: Paralysis and death time of earthworms in stem ethanolic extract

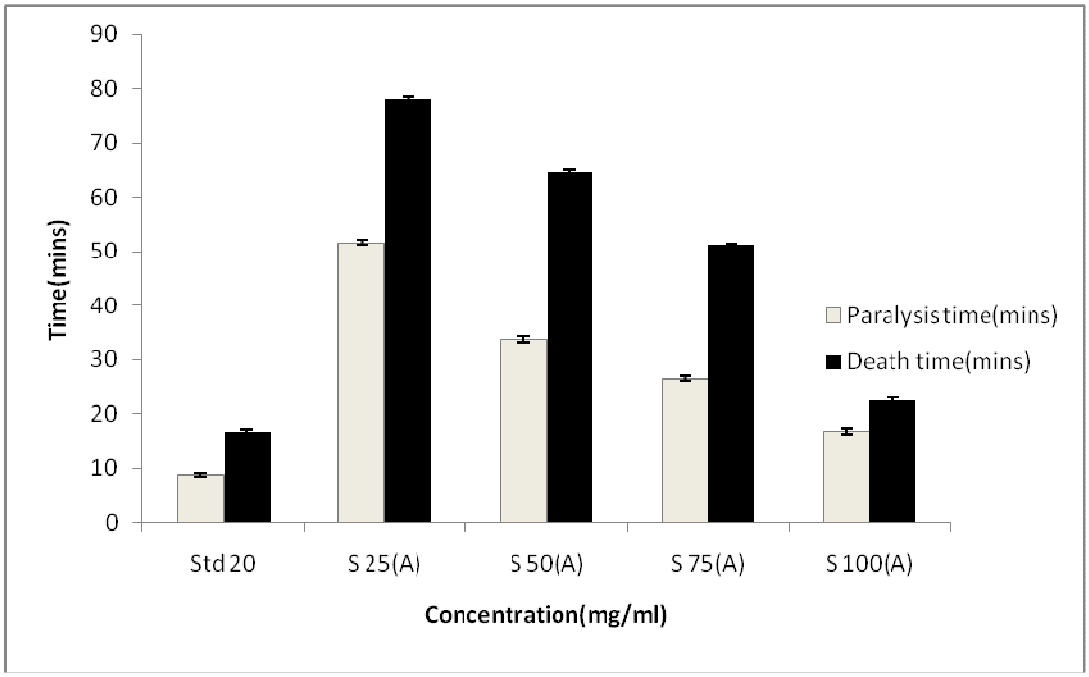

Fig. 2: Paralysis and death time of earthworms in stem aqueous extract

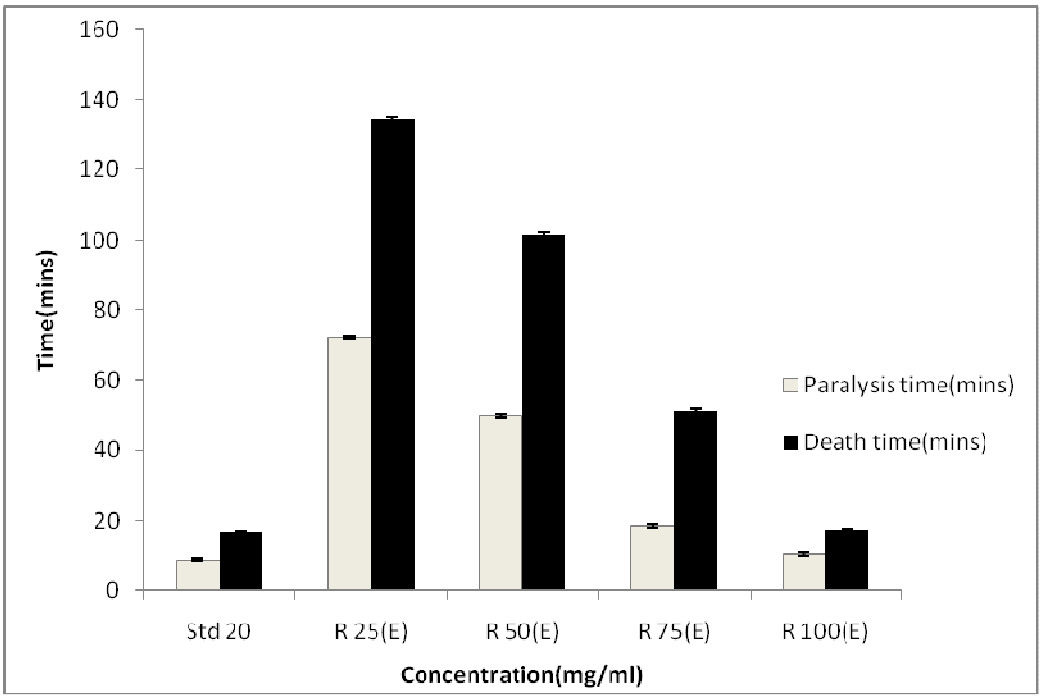

Fig. 3: Paralysis and death time of earthworms in roots ethanolic extract.

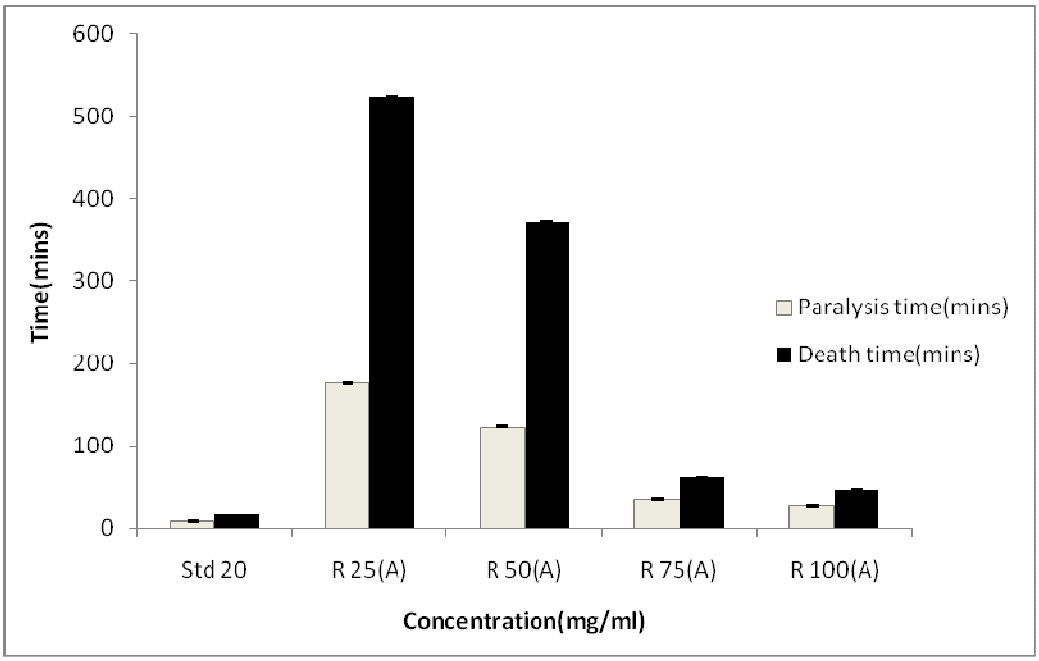

Fig. 4: Paralysis and death time of earthworms in root aqueous extract

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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