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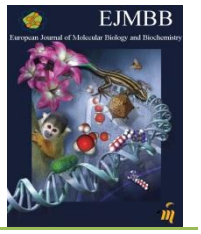

EVALUATION OF ANTHELMINTIC ACTIVITY OF ETHANOLIC EXTRACT OF *ANTIGONON LEPTOPUS*

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ABSTRACT

To evaluate the anthelmintic activity of ethanolic extract of leaves of *Antigonon leptopus*. The activity was carried out on adult Indian earthworm (*Pheretima posthuma)* and leech *(Hirudo medicinalis).* In this study, 20 mg/ml, 40 mg/ml, 60mg/ml, 80mg/ml, 100mg/ml, 120mg/ml and 140mg/ml concentrations of plant extract were tested which involved the time of paralysis and time of death. Piperazine citrate (10mg/ml) was taken as a reference standard drug whereas distilled water was referred as a vehicle. The extract exhibited significant anthelmintic activity which was more in higher concentration extract. So it was concluded from the present study that the plant of *Antigonon leptopus* exhibited significant anthelmintic activity.

INTRODUCTION

Alternative System of Medicine like Ayurveda, Siddha, Unani and Traditional Chines Medicine has gain its importance in the recent few years for its high potential in curing various disease with lesser side effects as compared with the synthetic drugs. These are due to the mainly presence of the secondary metabolites like proteins, flavonoids, alkaloids, steroids and phenolic substances which are in turn used to restore health and heal many diseases**.** .Natural products of plant and animal origin offer vast resource of newer medicinal agents with potential in clinical use [1].

Approximately 3 million people are infected with helminthes worldwide. Helminthes infections are commonly found in villages of developing countries and are being recognized as cause of much acute as well as chronic illness among the human beings as well as cattle's. Hence, the treatment for helmintic infection is of utmost need. The high cost of modern anthelmintics has limited the effective control of these parasites. However, increasing problems of development of resistance in helminthes against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity [2].

Antigonon leptopus family Polygonaceae, commonly known as Mexican Creeper is an important medicinal plant used in our Traditional System of Medicine to treat various health aliments. The plant is used as anti-thrombin [3], anti-diabetic [4], analgesic and anti-inflammatory [5], Hepatoprotective [6], anthelmintic [7], anti-microbial [8], lipid peroxidation inhibitory activity [9] and traditionally it is used for the treatment of asthma, liver and spleen disorder [10], cough and throat constriction [11]. Screening of methanol extract of the aerial part of the flower yielded n-hentriacontane, ferulic acid, 4- hydroxycinnamic acid, quercetin-3-rhamnoside, and kaempherol-3-glucoside along with ?- sitosterol, ?-sitosterol-glucoside and d-mannitol [12]. Therefore the present investigation was aimed at evaluating the pharmacognostical features and phytochemical analysis for authentication and identification of the plant [13].

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MATERIAL AND METHODS

Plant Material

The leaves of Antigonon leptopus were collected from Azamgarh city, Uttar Pradesh, India during 4 Oct 2013. There are to prepare the herbarium and botanical

identification was done by Dr S.L. Gupta, a Scientist 'E' and Head of Office, Botanical Survey of India, C.R.C., Allahabad- 211002.

Preparation of extract

There are the fresh plant material was washed thoroughly with distilled water to remove traces of contaminants. Then materials were dried well under shade and prepare coarse powder that pass through meshes no.40. The fine leaves powder was extracted by using soxhlet apparatus (a solvent system extraction by using ethanol and chloroform separately) at 40ºC to 60ºC. After 72 hr it was filter from Whatman filter paper. Then extracts were concentrated by using vacuum under pressure by using rotator flash evaporator. Finally the product was kept at room temperature in a vacuum desiccator until analysis.

Experimental animal, Drugs and Chemicals

Indian adult earthworms (*Pheretima posthuma)* and leech *(Hirudo medicinalis)* collected from Kandhrapur, Azamgarh, Uttar Pradesh, were used in experiment. Then select fresh, mature and disease free animal for experimental work to give better response. There are the following drugs and chemicals were used for the experiment: Piperazine citrate and ethanol (Sd Fine- Chem Limited Mumbai).

Phytochemical screening

Freshly prepared ethanolic extract was liable to phytochemical screening for observation of constituents by using a standard protocol [14].

Experimental protocol

The anthelmintic activity of ethanolic extracts of *Antigonon leptopus* was evaluated as per the method reported by Dash *et al.*, 2002 [15]. Nine groups with separately two earthworms and two Leech in each group were sorted and used to evaluate anthelmintic activity. Both earthworm and leech were separately released into 10 ml of desired formulation in distilled water, Group I_E (for Earthworm) and I_L (for Leech), earthworm and leech were released in 10 ml normal saline in a clean watch glass separately. Group II_E, III_E, IV_E, V_E, VI_E, VII_E, and $VIII_E$ earthworms were released in 20, 40, 60, 80, 100, 120 and 140 mg/10ml of ethanolic extract respectively. Similarly, group II_L, III_L, IV_L, V_L, VI_L, VII_L and $VIII_L$ Leech were released in 20, 40, 60, 80, 100, 120 and 140 mg/10ml of ethanolic extract respectively. Group IX_E and IX_L earthworm and leech were released in distilled water containing watch glass respectively.

Statistical Analysis

The data of anthelmintic evaluations were expressed as mean ± S.E.M of two animals in each group. The statistical analysis was carried out using one way ANOVA followed by Dunnet's Test. The difference in values at $P< 0.05$ was considered as statistically significant. The analysis of variance (ANOVA) was performed using Graph Pad Prism 6 Demo software to determine the mean and standard error of paralysis and death time of the earthworm and leech.

RESULT AND DISCUSSION

Phytochemical Screening

The preliminary phytochemical evaluation of *Antigonon leptopus* leaves showed the presence of different phytochemicals: alkaloids, steroids, carbohydrates, tannins, terpenoids, saponin glycosides and flavonoids glycosides.

Anthelmintic Test

As shown in table & figure 1 & 2 showed ethanolic extract of leaves of the plant of *Antigonon leptopus* exhibited anthelmintic activity using *Pheretima posthuma* (earthworm) and *Hirudo medicinalis* (leech) worms in dose dependent manner giving shortest time of paralysis and death with different concentration such as 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml, 100mg/ml, 120mg/ml and 140mg/ml. The standard drug Piperazine citrate(10mg/ml) also show a significant paralysis and death time when compared to control group.

Table 1. Anthelmintic effect on Earthworm (*Pheretima posthuma*) (mean ± SEM)

Group	Dose (in mg/kg)	Paralysis Time	Death Time
I	20	1.55± 0.05**	2.09± 0.02**
II	40	1.47± 0.04**	2.05± 0.01**
III	60	1.18± 0.06**	1.32± 0.04**
IV	80	1.08± 0.01**	1.27± 0.05**
V	100	0.50± 0.04**	1.08± 0.07**
VI	120	0.33± 0.01**	0.38± 0.03**
VII	140	0.17± 0.03**	0.25± 0.01**
Std.	2.5	0.54± 0.01	1.26± 0.02
Cont.	10 ml	_	_

All values are expressed in mean ± standard error mean (n=6).
All data were found to be significant at 5% level of significance where $^{**}p<0.05$.

Table 2. Anthelmintic Activity of ethanolic extract on Leech (*Hirudo medicinalis*) (mean ± SEM)

Group	Dose(in mg/kg)	Paralysis Time	Death Time
I	20	1.02 ± 0.01**	3.48 ± 0.01**
II	40	0.49 ± 0.01**	3.25 ± 0.0**
III	60	0.46 ± 0.01**	3.34 ± 0.01**
IV	80	0.36 ± 0.01**	3.08 ± 0.01**
V	100	0.30 ± 0.01**	1.53 ± 0.0**
VI	120	0.17 ± 0.03**	1.42 ± 0.01**
VII	140	0.24 ± 0.01**	1.51 ± 0.02**
Std.	2.5	1.07 ± 0.01	3.42 ± 0.02
Cont.	10 ml	_	_

All values are expressed in mean ± standard error mean (n=6).
All data were found to be significant at 5% level of significance where ** $p<0.05$.

Fig 1. Effect of ethanolic extract in the earthworm

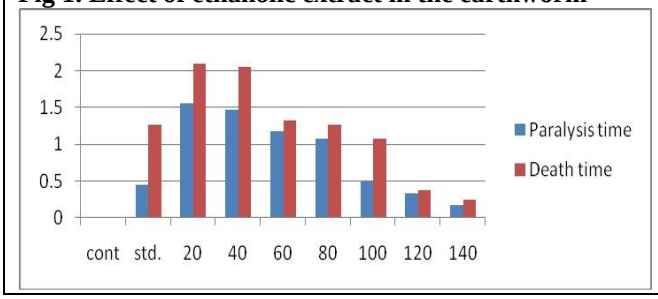
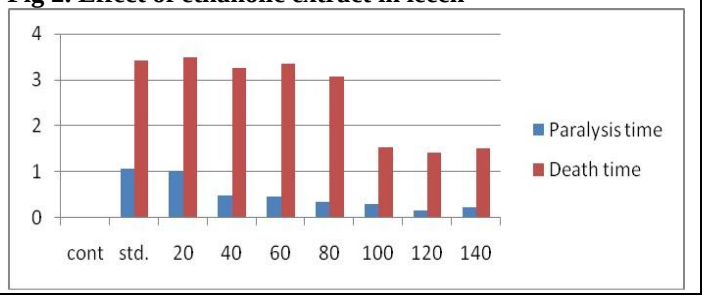


Fig 2. Effect of ethanolic extract in leech



From the observations made, higher concentration of extract produced paralytic effect much earlier and the time to death was shorter for all worms. These parts required the least time for causing paralysis and death of the earthworm and leech. The function of the anthelmintic drugs like Piperazine citrate is to cause paralysis of worms so that they are expelled in the feaces of man and animals. The extracts not only demonstrated this property, they also caused death of the worms, especially at 20 mg/ml as compared with the Piperazine citrate.

CONCLUSION

From the above results, it is concluded that *Antigonon leptopus* used to treat intestinal worm infections, showed significant anthelmintic activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as anthelmintic.

CONFLICT OF INTEREST

No conflict of interest

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