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ANTI-HELMINTHIC ACTIVITY OF TABERNAEMONTANA DIVARICTA LEAVES

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ABSTRACT

The present investigation was aimed at investigating the Anti-helminthic activity of Tabernaemontana divaricata leaves. Leaves were collected from Husnabad village of Karimnagar district which were dried and made into the fine powder then subjected to soxhlation by chloroform and methanol as solvents. The preliminary phytochemical screening was performed for those extracts and results showed the presence of carbohydrates, alkaloids, glycosides, terpenoids, phenols, tannins, flavonoids, steroids, proteins, and amino acids. These extracts were screened for their antihelminthic activity against Indian earthworm Pheretima posthuma by using soxhlation extracts of chloroform and methanol. These extracts exhibited the dose dependent paralysis and death of earth worms. Among these methanol extracts was more effective in causing death of the worms as well as promoting paralysis.

KEY WORDS

Tabernaemontana divaricata, Anti-helminthic, Soxhlation, Maceration, Pheretima posthuma

INTRODUCTION

Helminthis are the most common infectious agents of humans in developing countries and produce a global burden of disease that exceeds better-known conditions, including malaria and tuberculosis. The eggs of intestinal helminths can be found in the mummified feces of humans dating back thousands of years.

The filarial worms that cause lymphatic filariasis (LF) and onchocerciasis, whereas the platyhelminths include the flukes, such as the schistosomes, and the tapeworms, such as the pork tapeworm that causes cysticercosis[2].

For reasons not well understood, compared with any other age group, school-aged children (including adolescents) and preschool children tend to harbor the greatest numbers of intestinal worms and schistosomes and thus experience growth stunting and diminished physical fitness as well as impaired memory and cognition.

Hookworm and schistosomiasis are also important diseases during pregnancy, causing neonatal prematurity, reduced neonatal birth weight, and increased maternal morbidity and mortality[1].

Among some adult populations living in impoverished areas of developing countries, onchocerciasis is a leading cause of blindness and skin disease, while LF is a major cause of limb and genital deformities.

Most helminth infections, if left untreated, result in multi-year, chronic inflammatory disorders that cause both concurrent and delayed-onset pathology to the afflicted human host[6]. In addition to the overt and dramatic effects of blindness and elephantiasis in individuals with onchocerciasis and LF, respectively, it is now appreciated that chronic helminth infections are also linked to more insidious persistent health conditions such as anemia, growth stunting, protein-calorie undernutrition, fatigue, and poor cognitive development[4]. These seemingly subtle and often overlooked morbidities are very important because of the high prevalence of helminthiases in the rural developing world, in which any health impairment is substantially magnified in terms of degradation of individual patient performance status[5].

Initially, in childhood, it is the presence of helminth infection and the intensity of infection that determine the risk for disease formation. It is also true that for

many of the tissue-invasive helminths, such as the schistosomes and filariae, tissue damage can continue into later adult life, with disease persisting and even increasing long after the infection is cleared. As such, measures of infection prevalence do not capture the prevalence of infection-associated disease, particularly in adult life. Conditions such as elephantiasis, which occurs in individuals with LF; visual impairment, which occurs in individuals with onchocerciasis; periportal fibrosis and hypertension, which occur in individuals with intestinal schistosomiasis; biliary obstruction, cholangitis, and cholangiocarcinoma, which occur in individuals with food-borne trematodiasis; and urinary obstruction and bladder cancer, which occur in individuals with urinary schistosomiasis, are potentially the most life-threatening consequences of helminth infections. Although most likely to contribute to hospitalization and to cause mortality, these advanced outcomes are rare when compared to the disease burden of the average patient, which is characterized by the subacute morbidities detailed earlier[8].

Tabernaemontana divaricata belongs to family Apocynaceae, commonly called pinwheel flower, crape jasmine, East India rosebay and Nero's crown. It is one among the Indian medicinal plants. The stem exudes a milky latex when broken, hence the name milk flower. The plant contains several alkaloids and, like many other Apocynaceae, is toxic and medicinal plant. Coronaria is a spreading, bushy, many-branched shurb with elliptic-oblong, wavy-margined, thin, glossy, mid- to dark green leaves, paler underneath. Produces cymes of 4 to 6 salver form waxy, pure white flowers. Scented at dusk and after dark. In general, this spreading, bushy shrub grows to a height of 6 to 10 feet tall and 5 to 8 feet wide. It bears white, waxy summer flowers and has oblong leaves with wavy margins that are dark green above and pale green beneath[7].

MATERIALS AND METHODS

Procurement of plant material:

For the present investigation, *Tabernaemontana divaricata* leaves were collected in the month of January 2016 from Husnabad village of the Karimnagar district. The plant was identified and authenticated by BSI/DRC/16-17/Tech.05. The leaves were dried in shade and stored at 25 °C. It was powdered, passed through sieve no.40 and stored in air tight bottles.

Drugs and chemicals

Albendazole (Piramal healthcare limited), Tween 80(Finar limited), Chloroform (Finar limted), Methanol (Merck life sciences [p] Ltd) was used during the experiment.

Selection of worms

An adult Indian earth worm *pheretima posthuma* was selected for antihelminthic evaluation as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings.

Instruments

Petridish

Preparation of extracts

Chloroform and methanolic extracts of *Taberneamonata divaricata* leaves were prepared by both soxhlation at suitable temperature. 50gms of the powder of leaves is dissolved in 200ml of solvent. Soxhlation process was carried out for about 6hrs for each solvent and maceration process was carried out for 5days. The extracts obtained were evaporated and dried in desiccator.

Antihelminthic evaluation

Experimental worms:

Indian adult earthworms (*pheretima posthuma*) were used to study anthelminthic activity. The earthworms were collected from moist soil and washed with distilled water to remove all faecal matter. Earthworms 3-5 cm in length and 0.1-0.2 cm in width were used for experiment.

Administration of albendazole:

Albendazole(10mg/ml) was prepared by using 1% v/v of Tween 80 as a suspending agent as administered as per method of extract.

Administration of extract:

The suspension of methanolic and chloroform extract of *Tabernaemontana divaricata* leaves of different concentrations (10, 20mg/ml) were prepared using 1% v/v of Tween 80 as a suspending agent. Total of 20ml for each concentration was prepared (200mg in 20ml for 10mg concentration, 400mg in 20ml for 20mg concentration). Albendazole was used as standard. Groups of approximately equal size worms consisting of two earthworms individually in each group were releases into each 20ml of desired concentration of drug and extract in the petridish.

EXPERIMENTAL DESIGN:

The antihelminthic activity was performed on adult Indian earth worm *pheretima posthuma* as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. *Pheretima posthuma* was placed in petridish containing two different concentrations (10 & 20 mg/ml) of methanolic & chloroform extract of leaves of *Tabernaemontana divaricata*. Each petridish was placed with 2 worms and observed for paralysis or death. Time for paralysis was noted when no movement of any sort could be observed, except when worm was shaken vigorously; the time of death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. The test results were compared with Reference compound Albendazole(10mg/ml) treated samples[3].

RESULTS

From the results, it is observed that *Tabernaemontana divaricta* shown potent anthelmintic activity while the *Pheretima posthuma* has taken long time for death (220 min-120 min) of worms. The earthworm selected for the anthelmintic activity was most sensitive to the different solvent extracts viz., chloroform and methanol leaves extract of *Tabernaemontana divaricta* as can be seen in Table 1. The anthelmintic activity result revealed dose-dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death at 10 and 20mg/ml concentrations, paralysis was observed respectively at 110min and 90 min and death at 160 and 140min in methanol extracts. The chloroform extracts of *Tabernaemontana divaricta* also exhibited dose dependent anthelmintic activities that caused paralysis at 90 and 105 min (at 10 and 20mg/ml) and death at 190 and 165 min (at 10 and 20mg/ml). The standard drug (Albendazole) shows paralysis within 125 min and time of death 180 min in the two solvents extracts. The observation of result show that the anthelmintic activity of methanol extract is more potent compare to the chloroform extract. The earthworms were more sensitive to the extracts of methanol at 20mg/ml concentrations as compared to the reference drug Albendazole (10 mg/ml). The results are furnished in the Table No:1.

Table 1 : Paralysis time and death time of the standard and solvent extracts.

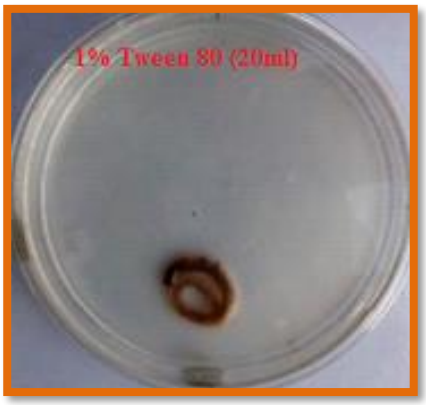
Group	Treatment	Concentration (w/v) mg/ml	*Pheretima posthuma*	
			Paralysis time (mins)	Death time (mins)
1	**1% Tween 80 (control) (ml)**	20	145±10	-
2	**Albendazole**	10	35	62.5
3	**MSLE**	10	108 ± 5	170±5
		20	**80±10**	**146±10**
4	**CSLE**	10	105±10	220±10
		20	95±10	165±10

Fig No: 1

Fig No: 2

Fig No: 3

Fig No: 4



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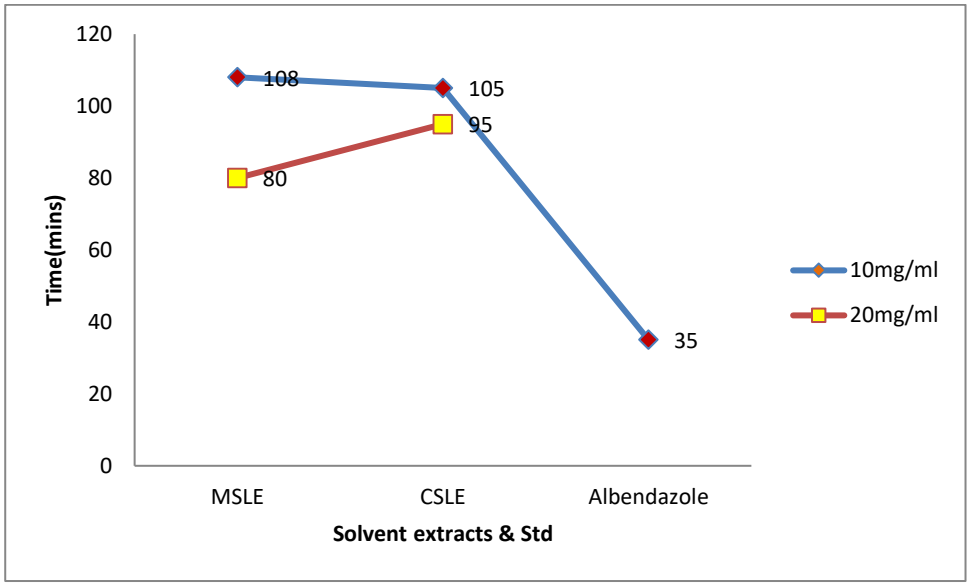
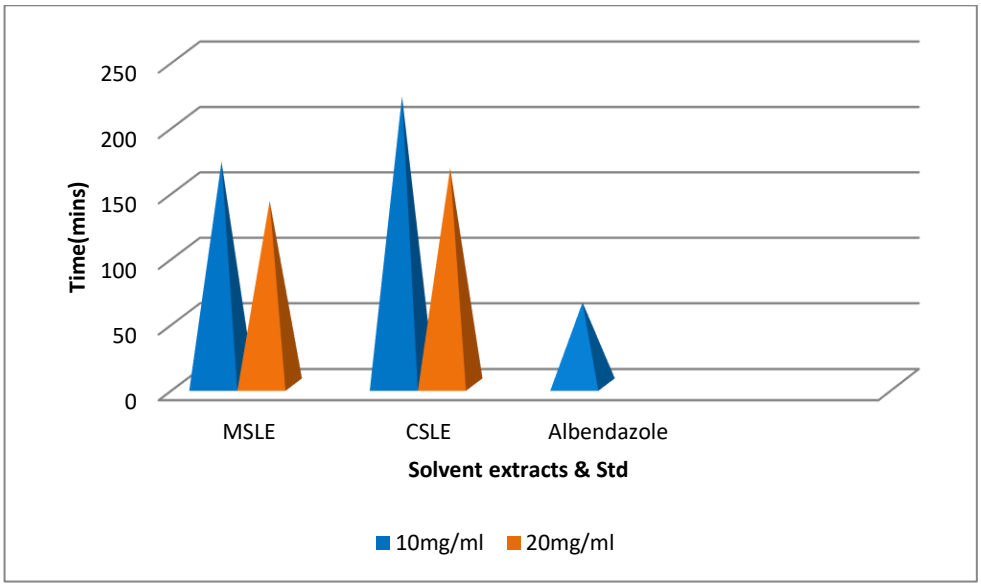


Graph 1: Comparative data of paralysis time at different concentrations against pheretima posthuma



Graph No 2: Comparative data of death time at different concentrations against pheretima posthuma

CONCLUSION

From the above results, it was concluded that chloroform and methanol soxhlation extracts of Tabernaemontana divaricata leaves exhibited the dose dependent antihelminthic activity. Among these methanol (20mg/ml extract causes paralysis in 80mins death in 146mins) extracts was more effective in causing death of the worms as well as promoting paralysis compared to standard.

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