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Research Article

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Anthelmintic activity of different extracts of *Calotropis procera* leaves

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ABSTRACT

Calotropis procera (Asclepiadaceae), a giant milk weed, is known for its pharmacological importance for centuries. The coarse shrub is a very prom ising source of anticancerous, ascaricidal, schizonticidal, anti-microbial, anthelmintic, insecticidal, anti-inflammatory, anti-diarrhoeal, larvicidal with many other beneficial properties. Plant is described as a golden gift for human kind containing calotropin, calotropagenin, calotoxin, calactin, uscharin, amyrin, amyrin esters, uscharidin, coroglaucigenin, frugoside, corotoxigenin, calotropagenin and voruscharine used in many therapeutic applications. Different compounds like norditerpenic esters, organic carbonates, the cysteine protease procerain, alkaloids, flavonoids, sterols and numerous cardenolides made this plant of scientific attraction for centuries. Different extracts of Calotropis procera leaves were found to possess in-vitro anthelmintic activity against Indian earthworms Pheritima posthuma, using piperazine citrate as reference standard. Dose dependent activity was observed in different extracts of plant leaves. But 70% hydroethanolic extract shown better activity as compared to n-butanol and chloroform extract of Calotropis procera leaves.

Keywords: *Calotropis procera*, *Pheritima posthuma*, piperazine citrate, Anthelmintic activity.

INTRODUCTION

Helminthiasis is a worldwide diseases of all ages and it is common. As per WHO more than two billion people harbor this infection. From the survey of WHO only few drugs are frequently used in the treatment of helminthes in human, due to the cost and the development of resistance against these drugs turned the attention of many researchers towards the evaluation of medicinal plants. After the successful research work of many scientists, the natural sources play a key role in the treatment of the anthelmintic. *Calotropis procera* (Ait.) R.Br. (giant milkweed) belong to the family Asclepiadaceae locally known as "*aak*" in India is being used as herbal medicine by people living the desert areas [1]. Arka (Calotropis procera) an important drug of Ayurveda is known in this country from the earliest time. It is mentioned by the earliest Hindu writers and the ancient name of the plant which occurs in the Vedic literature was Arka alluding to the form of leaves, which was used in the sacrificial rites. There are two common species of Calotropis, viz. Calotropis gigantean (Linn.) R.Br. and Calotropis procera (Ait.) R.Br described by the Sanskrit writers. Both the species are used as substitutes for one another and are said to have similar effects. In Dhanvantari Nigantu three varieties of Arka are mentioned viz. Rajarkah, Suklarkah and Sveta mandarah. It has been widely used in the Sudanese, Unani, Arabic and Indian traditional medicinal system for the treatment of various diseases namely leprosy, ulcers, piles and diseases of the spleen, liver and abdomen. In the traditional Indian Medicinal system, this plant has been used for a variety of disease conditions including asthma, cold, cough, piles, ulcers, diarrhea, heart diseases, leprosy, rheumatism and diseases of skin, spleen, liver and abdomen [2-4].

Different parts of the plant have been advocated for use in a variety of disease conditions. The leaf of *Calotropis procera* is sub-sessile, 6-15 cm by 4.5-8 cm, broadly ovate, ovate-oblong, elliptic or obovate acute, pubescent, when young and glabrous on both sides on maturity. The lamina which is dorsiventral with mesophyll differentiated into a palisade and spongy tissue.[5] Extracts from this plant have been found to possess various pharmacological activities.[6] In search of potential anthelmintic agent and to validate the anthelmintic claim of this plant, this study was designed. Morphologically the plant is erect, tall, large, much branched and perennial shrub or small tree that grows on a height of 5.4m, with milky late x throughout. Bark is soft and corky, branches stout, leaves sub sessile, opposite, decussate, broadly ovate, oblong, elliptic or obovate, acute, thick, g laceous, green coloured with fine cottony pubescent hair on young. Flowers in umbellate cymes and tomentose on young. Seeds broadly ovate, acute, flattened, minutely to mentose, brown coloured and silky.

Fig.1; Leaves of *Calotropis procera*

EXPERIMENTAL SECTION

Plant Material

Fresh leaves were collected from the *Calotropis procera* plant growing in medicinal garden of Rajiv Academy for Pharmacy, Mathura, U.P. India. The plant specimens were authenticated and voucher specimen is NISCAIR/RHMD/Consult/-2008-09/1144/176 for future reference. The leaves were cleaned by washing with running water and shade dried and then milled to coarse powder by mechanical grinder.

Preparation of Extracts

The dried powdered leaves were extracted by maceration for seven days with 70% ethanol and the solvent portion was evaporated under reduced pressure to yield 70% hydroethanolic fraction. This dried fraction was further extracted by maceration with *n*-butanol. The organic layer was evaporated under reduced pressure to yield *n*-butanol fraction. The residue was again macerated with chloroform and the organic layer was evaporated under reduced pressure to yield chloroform fraction. The prepared extracts were kept under refrigeration for screening of anthelmintic activity. [7]

In-vitro Anthelmintic activity

The anthelmintic activity was evaluated on adult Indian earthworm *Phaeritima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. [8-11] The earthworms are collected and washed with normal saline with removal of fecal matter. The earthworms are 5 to 6 cm length and 0.2-0.3 cm widths were used for experiment protocol. [12,13] Different extracts that were prepared from *Calotropis procera* leaves were examined systematically for their *in-vitro* anthelmintic activity against *Phaeritima posthuma.* The *in-vitro* anthelmintic assay procedures were carried out as per method of Mathew et al. [14] and Dash et al.[15] with slight modifications.[16-19] Five groups of equal size Indian earthworm consisting of six earthworms in each groups were released into 50ml of desired formulation. Each group was treated with one of the following: Vehicle

(0.9%w/v NaCl), piperazine citrate (15mg/mL), and different extracts of (50 mg/mL, 25mg/mL, and 12.5mg/mL) in normal saline. Observations were made for the paralysis time and subsequently for death time of the worms. The mean paralysis and/or death time for each group was recorded (each reading taken for 6 times). The time taken by the worms to become motionless, consider as paralysis was recorded and the lethal time was recorded by observing the time taken to become motionless on application of external stimuli by pricking with pin.Piperazine citrate (15mg/mL) was taken as reference drug. (Table-1,2& 3)

Table-1; Anthelmintic activity of 70% hydroethanolic extract of *Calotropis procera* leaves

Treatment	Concentration(mg/ml)	Paralysis Time (min.)	Death Time (min.)
Vehicle	-----	-----	------
Piperazine- citrate	20.0	14.32±0.360	30.10±0.230
70% Hydroethanolic extract	60.0	5.62±0.143	13.25±0.232
70% Hydroethanolic extract	30.0	12.53±0.704	26.50±0.207
70% Hydroethanolic extract	15.0	17.58±0.64	30.05±0.628

Table-2 Anthelmintic activity of *n*-butanol extract of *Calotropis procera* leaves

Treatment	Concentration(mg/ml)	Paralysis Time (min.)	Death Time (min.)
Vehicle	-----	-----	-----
Piperazine-citrate	20.0	14.32±0.360	30.10±0.230
***n*-Butanol extract**	60.0	6.62±0.26	14.25±0.12
***n*-Butanol extract**	30.0	17.03±0.20	30.52±0.61
***n*-Butanol extract**	15.0	21.03±0.22	49.26±0.16

Table-3; Anthelmintic activity of chloroform extract of *Calotropis procera* leaves

Treatment	Concentration (mg/ml)	Paralysis Time (min.)	Death Time (min.)
Vehicle	-----	------	------
Piperazine-citrate	20.0	13.32±0.360	30.10±0.230
Chloroform extract	60.0	7.31±0.497	12.0±0.369
Chloroform extract	30.0	16.00±0.290	29.20±1.90
Chloroform extract	15.0	28.53±0.350	58.25±0.25

❖ *Results expressed as ±SEM of six worms in each group.*

RESULTS AND DISCUSSION

The antihelmintic activity using adult earthworms from the crude latex of C. procera was evaluated. Both fresh as well as aqueous extracts of dried latex exhibited a dose-dependent inhibition of spontaneous motility (paralysis) and evoked responses to pin-prick. With higher doses (100mg/ml of aqueous extract of dry latex and 100% fresh latex),the effects were comparable with 3% piperazine. However, there was no final recovery in the case of worms treated with latex in contrast to piperazine with which the paralysis was reversible and the worms recovered completely within six hours. The results showed that latex possesses wormicidal activity and thus, may be useful as an anthelmintic.The anthelmintic activity of C.procera latex in sheep was investigated that had been infected with single oral doses of 12000 infective Haemonchus contortus larvae. Inappetence, dullness, erosive abomasitis, decreased haemoglobin concentration and increased eosinophils were the main features of haemonchosis in the sheep. In the sheep treated with single oral doses of 0.01 ml or 0.02 ml/kg body weight of C. procera latex, egg production was significantly reduced, but not completely suppressed, and fewer adult Haemonchus worms were found in the abomasum. Although the appetite improved, the haemoglobin concentration and serum copper, iron and zinc levels were still reduced after therapy with Calotropis latex. Calotropis latex showed a concentration-dependent larvicidal activity in vitro within 20 min of application. The perusal of the data reveals that the 70 % hydroethanolic extract at the concentration of 12.5 mg/mL showed both paralysis and death in 18.58 and 29.05 min. Similarly *n*-butanol and chloroform extract at the concentration of 12.5 mg/mL showed both paralysis and death in 21.03 and 48.26 min. & 26.53 and 51.25 min. respectively. The effect increased with concentration. It was observed that 70% hydroethanolic extract shown better activity as compared to *n*-butanol and chloroform extract of *Calotropis procera* leaves and reference control piperazine citrate. Different extracts caused paralysis followed by death of the worms at all tested dose levels. The potency of the extract was found inversely proportional to the time taken for paralysis or death of worms. The activity confirms the dose dependent nature of extract.

CONCLUSION

C. procera (giant milk weed) has been used as traditional folk medicine by many cultures, and it has been the subject of extensive phytochemical and bioactive investigations. It had shown significant pharmacological importance representing asa strong contender in the medical arena. It is a useful b otanical monitor of pollution because of the variation found in the concentrations of Br, Mn, Se, Cr and Zn between urban and suburban samples.The plant has proved to be a good indicatorfor the determination of above elements when it is exposed to them from any source. In conclusion the 70% hydroethanolic extract of *Calotropis procera* leaves had significant anthelmintic activity. It deserves further studies to identify its active components which responsible for anthelmintic activity and investigate their mechanism.

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