INTRODUCTION

Helminth infections are among the most widespread infections in humans. As per WHO, only a few drugs are frequently used in the treatment of helminth infestations in human beings. Anthelmintic resistance is a major problem for the control of many parasitic nematode species and has become a major constraint to livestock production in many parts of the world. Due to the prevalence of parasitic infections and the developed resistance of some anthelmintic drugs is now an enclosing area in the field of research [1].

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Calotropis procera (Ait) R. Br. is well known for its traditional medicinal uses belonging to the family Asclepiadaceae. It has numerous colorful names such as Sodom Apple, Akund Crown flower and Dead Sea Fruit, but the scientific name is C. procera (Ait.) R. Br. leaves to distinguish from substitutes identification and standardization of which is useful to supplement existing information with regard to the scientific name is C. procera, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [26-28] were used in this study.

Previously, our group reported the pharmacognostical studies including macroscopic and microscopic evaluation of C. procera leaves which is useful to supplement existing information with regard to the identification and standardization of C. procera leaves to distinguish it from substitutes [19]. The leaves were reported to contain calotropigenin, calotropin, calotoxin, calactin, α-amyrin, β-amyrin, stigmasterol, calotropenyl acetate, calotropenol, uscharin, wortchwarin, syringogenin, multiflorenol, 12β-hydroxyfrugoside, 4'-O-β-glucopyranosyl-12β-hydroxyfrugoside, 4'-O-β-celllobiosyl-12β-hydroxyfrugoside, 16α-hydroxy calotropagenin, uscharolic acid, and α-pyrocatechuic acid on the basis of the liquid chromatography-mass spectrometry (MS)/MS analysis of C. procera leaves [19]. Literature survey revealed that different parts of this plant have been used as anthelmintic [20-23]. In an attempt to confirm the claimed folk use, the present study was carried out to scientifically evaluate the anthelmintic activity of C. procera (Ait) R. Br. leaves.

METHODS

Collection and preparation of plant material

The leaves of C. procera (Ait) R. Br. were collected in the month of February from the local field of Mathura (Uttar Pradesh), India. The leaves were cleaned by washing with running water and shade dried, then powdered to pass through 100 mesh size. Powdered leaves were extracted with ethanol by maceration for 7 days at room temperature. The solvent was recovered under reduced pressure and vacuum dried. The n-butanol fraction was subjected to column chromatography. The column was run with different solvents and ethanolic extract, n-butanol fraction, water fraction and chromatographic elutes of n-butanol fraction (n-hexane, chloroform, chloroform: methanol: 9:1) were investigated for their anthelmintic activity.

Phytochemical screening

Qualitative assay, for the presence of plant phytoconstituents, such as carbohydrates, alkaloids, glycosides, flavonoids, tannins, and saponins, were carried out on the ethanolic extract, n-butanol fraction and water fraction of C. procera (Ait) R. Br leaves following standard procedure [24,25].

Animal

Healthy adult Indian earthworms, Pheretima postuma, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [26-28] were used in this study.
All earthworms were of approximately equal size of 5-6 cm length and 0.2-0.3 cm widths used for all the experimental protocol. They were collected from the local moist place, washed and kept in water.

**Drugs**

Piperazine citrate was purchased from GSK Pvt. Ltd. The solvents and other chemicals of analytical grade were used during experimental protocol.

**In-vitro anthelmintic activity**

Ethanolic extract, n-butanol fraction, water fractions and n-hexane, chloroform, chloroform: methanol (9:1) elutes of *C. procera* (Ait) R. Br leaves were investigated for their anthelmintic activity against *Pheretima posthuma* as a method of Ajayeeba et al. [29]. Six groups of equal size Indian earthworm consisting of six earthworms in each group were taken. Each group was treated with one of the following: vehicle (dimethyl sulfoxide [DMSO]), piperazine citrate (20 mg/mL), and ethanolic extract, different fractions and different elutes (10 mg/mL, 20 mg/mL) in DMSO (5.0 mL) and volume was made up to 40 mL by distilled water. Observations were made for the paralysis time and subsequently for death time of the worms. The mean paralysis and death time for each group were recorded (each reading was taken 6 times). The time taken by the worms to become motionless, was considered as paralysis time, was recorded, and the lethal time was also recorded by observing the time taken to become motionless on the application of external stimuli by pricking with pin. Piperazine citrate (20 mg/mL) was taken as reference drug.

**RESULTS AND DISCUSSION**

Preliminary phytochemical screening of ethanolic extract revealed the presence of carbohydrates, glycosides, alkaloids, saponins, tannins, flavanoids, and steroids. Water fraction showed the presence of carbohydrates, glycosides, alkaloids, and tannins while n-butanol fraction showed the presence of glycosides, alkaloids, saponins, and flavanoids.

The perusal of the data of Table 1 revealed that the ethanolic extract at the concentration of 20 mg/mL showed paralysis in 7.25 minutes and death in 14.10 minutes. Similarly n-butanol and water fraction, n-hexane, chloroform, and chloroform: methanol (9:1) elutes at 5.56, 7.3, 7.26, 7.26, and 5.4 min and death in 8.3, 13.5, 13.2, 13.26, and 10.5 min, respectively. This indicates that ethanolic extract, water fraction, n-hexane and chloroform elutes have paralysis time comparable with piperazine citrate but death time greater than piperazine citrate. The predominant effect of piperazine citrate on the worm is to cause a flaccid paralysis that result in the expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarization and reduced excitability that leads to muscle relaxation and flaccid paralysis [30].

Ethanolic extract, different fractions and chromatographic elutes caused paralysis followed by the death of the worms at all tested dose levels. The potency of the *C. procera* (Ait) R. Br. leaves was found inversely proportional to the time taken for paralysis or death of worms. The activity confirms the dose-dependent nature of extract (Fig. 1). It was observed that n-butanol fraction and chloroform: methanol (9:1) elutes showed better activity as compared to ethanolic extract, water fraction, n-hexane and chloroform elutes of *C. procera* (Ait) R. Br. leaves and refererence control piperazine citrate. These results indicate that activity of n-butanol fraction was due to the combined effect of n-hexane and chloroform elutes, and activity of the ethanolic extract was due to the combined effect of n-butanol and water fractions.

Phytochemical analysis of the ethanolic extract, water, and n-butanol fractions revealed the presence of flavonoids as one of the chemical constituent. Polyphenolic compounds show anthelmintic activity [31]. Some synthetic phenolic anthelmintics, e.g., niclosamide, oxyclozanide, and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation [32]. It is possible that phenolic content in the n-butanol fraction of *C. procera* (Ait) R. Br. leaves produced similar effects. It is also possible that tannins contained in the ethanolic extract of *C. procera* (Ait) R. Br. leaves produced similar effects because tannins can bind to free protein in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death [33].

**CONCLUSION**

This study indicated the potential usefulness of *C. procera* (Ait) R. Br. leaves against earthworm infections and provide a rationale for the traditional use of this plant as anthelmintic. Further studies need to establish the mechanisms of action are required.

**REFERENCES**

3. Patil SH, Adkar PP, Shalker TT, Oswal RJ, Borase SP. Antidiarrhoeal...