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Original Research Paper

CYTOTOXIC AND ANTHELMINTIC ACTIVITIES OF METHANOLIC EXTRACT OF *PARTHENIUM HYSTEROPHORUS* L.

M. Sessa Sai Durga, K. Satyavathi, P. Bhojaraju, P. Mercy Preeti, K. P. Lovaraju, K. Sony Priya, Y. Ratna Kumari, D. Venkatesh Prasad and **Lakshmi kanta Kanthal***

Koringa College of Pharmacy, Department of Pharmacology,
Korangi-533 461, Tallarevu (M), Andhra Pradesh, India

ABSTRACT

The present study has been designed to evaluate the cytotoxic and anthelmintic activities of crude methanolic extract of whole plant of *Parthenium hysterophorus* L. The *in-vitro* cytotoxic activity of methanolic extract was performed by MTT assay method against CHOK 1 (Chinese hamster ovary cell line) and A-549 (Human lung adenocarcinoma epithelial cell line). The crude methanolic extract (25, 50, 150 mg/ml concentration) of *Parthenium hysterophorus* whole plant was taken for anthelmintic activity against *Pheretima posthuma*. Effect of inhibition of cell growth showed significant cytotoxicity against A-549 with an IC_{50} of $183.00 \pm 5.8 \mu\text{g/ml}$ and against CHOK1 with an IC_{50} of $230 \pm 0.00 \mu\text{g/ml}$. The results obtained from the study indicate good anthelmintic activity against *Pheretima posthuma*. The present study concluded that the methanolic extract of *Parthenium hysterophorus* possess potent cytotoxic and anthelmintic activities. Further investigations are in progress to identify the mechanism of cytotoxic effect and anthelmintic activity.

Keywords: *Parthenium hysterophorus* L, Methanol extract, Cytotoxic activity, Anthelmintic activity.

INTRODUCTION

Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain parts of plants or other plant materials as active ingredients.¹ The anticancer property of nutrients derived from plants as well as non nutritive plant derived constituents has been proved in different *in vitro* and *in vivo* models.² Helminths infections are among the most common infections in man, affecting a large proportion of the world's population. *Parthenium hysterophorus* L. (congress grass, congress weed, carrot weed, wild feverfew, the "Scourge of India") is an exotic weed that was accidentally introduced in India in 1956 through imported food grains.³ It has become a common weed causing dermatitis of epidemic proportions.⁴ It

belongs to the family Asteraceae/Compositae (Daisy family). *Parthenium hysterophorus* is a native of the West Indies and North East Mexico.⁵ During the last hundred years, it has spread worldwide.^{6,7} It was used as a folk remedy against various afflictions such as ulcerated sores, certain skin diseases facial neuralgia, fever and anemia. Flowers of this plant were being used as tonic, blood purifier, abortive, vermifuge, ammenagogue, antimalarial and cytotoxic agent.⁸ To the best of our knowledge, there is no such previous study on the cytotoxic and anthelmintic activities of methanolic extract of *Parthenium hysterophorus* L. whole plant.

MATERIALS AND METHODS

Plant Material

The whole plant of *Parthenium hysterophorus* L. were collected on July 2013 from local areas of Korangi, Kakinada, Andhra Pradesh. The plant was identified and authenticated (Specimen No. BSI/DRC/2013-14/Tech/274) by Mr. P. Venu, Scientist-Additional Director, Botanical Survey of India, Deccan Regional Centre, Hyderabad-500048 where a voucher specimen has been deposited.

Extraction of Plant Material

325 gm fresh whole plant of *Parthenium hysterophorus* was washed with distilled water to remove dust particles. The shade dried whole plants were powdered. The ground fine powder (135 gm) of the whole plant was extracted with absolute methanol (1 liter) at room temperature (30⁰c) for three days. The extract was filtered through Whatman No: 1 filter paper and then concentrated at 45⁰C using a rotary vacuum evaporator. The extract (15 gm) was stored at -4⁰c till further analysis.

Preliminary Phytochemical Screening

The methanol extract was tested for alkaloids, anthraquinones, flavonoids, phenols, steroids, tannins, terpenoids,⁹ cardiac glycosides, saponins,¹⁰ phlobatannin,¹¹ reducing sugars,¹² volatile oils,¹³ carbohydrates and protein/amino acids.^{14,15}

Cytotoxic activity

Chemicals

3-(4,5-dimethylthiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT), Fetal Bovine Serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E. Merck Ltd., Mumbai, India.

Cell lines and culture medium

A-549 (Human lung adenocarcinoma epithelial cell line), and CHOK 1 (Chinese Hamster Ovary cell line), cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine

Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO₂ at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm² culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of Test solutions

For Cytotoxicity studies, the extract was separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cell viability by MTT Assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10⁵ cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed once with medium and 100 µl of different test concentrations of extracts were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37⁰ C for 3 days in 5% CO₂ atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the sample solutions in the wells were discarded and 50 l of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37⁰ C in 5% CO₂ atmosphere. The supernatant was removed and 100 l of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm.¹⁶ The percentage growth inhibition was calculated using the following formula and concentration of test sample needed to inhibit cell growth by 50% (CC₅₀) is generated from the dose-response curves for each cell line.¹⁷

% Growth Inhibition = $100 - \left\{ \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100 \right\}$

Anthelmintic Activity

The anthelmintic activity was performed on the adult Indian earthworm *Pheritima posthuma*.^{18,19} Albendazole, the standard drug, was diluted with normal saline to obtain 25, 50 and 100 mg/ml concentrations and was poured into Petri dishes. Methanol extract of the plant was diluted with normal saline to obtain 25, 50 and 100 mg/ml concentrations. Normal saline (0.9% NaCl) alone served as the negative control. All these dilutions were poured into the Petri dishes accordingly. Ten petri dishes of equal size were taken & numbered. Six earthworms (n=6) of similar sizes (about 8 cm) were placed in each petri dish at room temperature. Time for paralysis was noted down when no movement of any sort could be observed, except when the worms were shaken vigorously. Time of death for worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50 ° C). The paralysis time and lethal time were recorded in terms of minutes.

RESULTS AND DISCUSSION

The preliminary phytochemical study revealed that methanolic extract of *Parthenium hysterophorus* contains carbohydrates, alkaloids, glycosides, flavonoids, tannins, saponins, phenols. Through the MTT method, the median cytotoxic concentration (CC₅₀) on CHOK 1 cell line (Chinese Hamster ovary cell line) and A-549 cell line (Human Lung adenocarcinoma epithelial cell line) were established for methanolic extract of whole plant of *Parthenium hysterophorus*. There was gradual increase in the value of PGI (percentage of growth inhibition) as the concentration of extract was increased (25.19, 27.30, 55.55, 77.34, 80.26 % for the concentrations 62.5, 125, 250, 500, 1000 µg/ml, respectively) against the CHOK 1 cell line (figure 1). The median value of CC₅₀ observed for CHOK 1 cells was 230± 0.00 (significant). A similar observation was made on A-549 cell line

(Human Lung adenocarcinoma epithelial cell line). There was gradual increase in the value of PGI (percentage of growth inhibition) as the concentration of extract was increased (12.49, 33.24, 75.68, 78.95, 81.95 % for the concentrations 62.5, 125, 250, 500, 1000 µg/ml, respectively) against A-549 cell line (figure 2). The median value of CC₅₀ observed for A-549 cells was 183.00± 5.8 (significant). The result show that for the 25 mg/ml concentration, albendazole showed the best activity for death time 107±5.99 min and the methanolic extract of *Parthenium hysterophorus* showed a death time of 129.0±2.82 min. Also, for the 50mg/ml concentration, albendazole showed the highest activity against the worms 88.5±3.84 min and the methanolic extract of *Parthenium hysterophorus* showed a death time of 120.0±7.75 min. For the 100 mg/ml concentration, albendazole showed the least death time 66.83±2.16 min and the methanolic extract of *Parthenium hysterophorus* showed a death time of 79.63±3.26 min. The paralysis and death times of the plant along with the standard are given in (table 1). The study revealed that the methanolic extract of *Parthenium hysterophorus* had significant activity (moderate) at the higher concentration (100 mg/ml).

CONCLUSION

In conclusion, the present plant *Parthenium hysterophorus* can be considered as an important source of natural products that have anti-cancer potentials and potent anthelmintic activity, due to presence of various phytochemical components but it is too early to reach a final conclusion and further investigations are required to include further cell lines and worms, respectively.

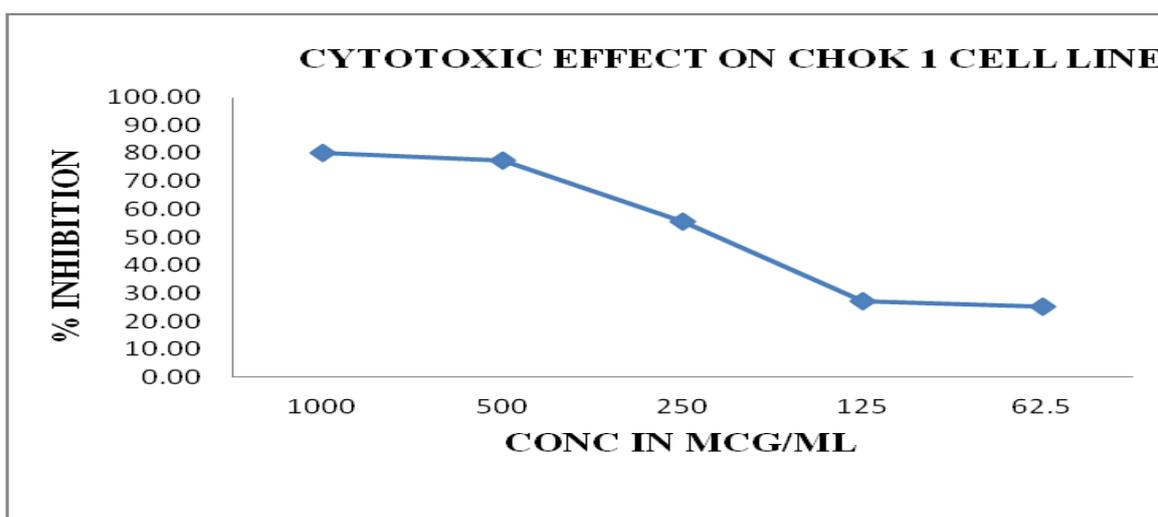
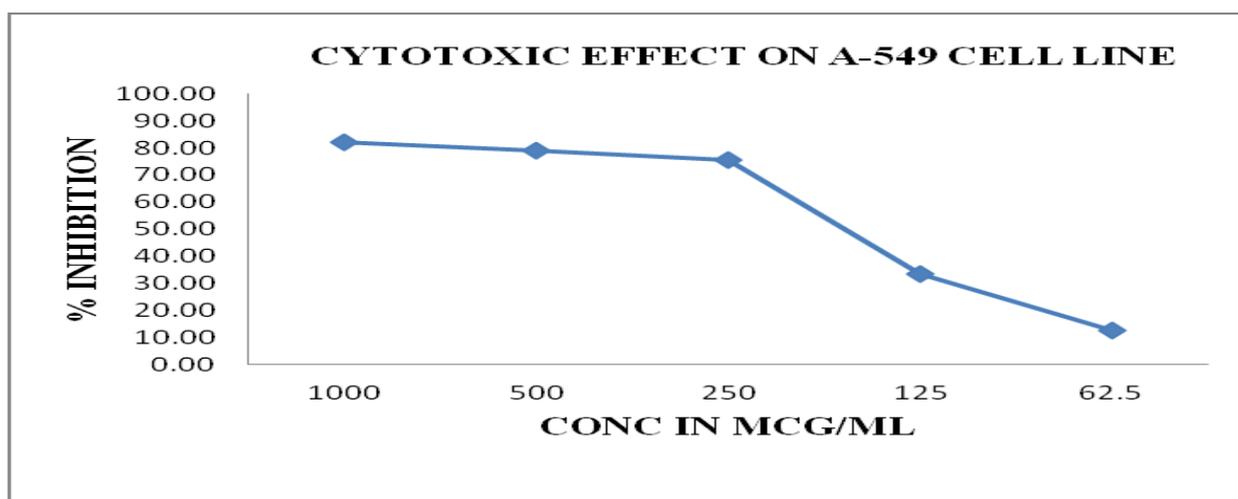
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Table 1: Anthelmintic effect of *Parthenium hysterophorus* L. against *pheritima posthuma*

Treatment	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
Albendazole (standard)	25	55.66±1.59	107±5.99
	50	45.33±2.32	88.5±3.84
	100	32.66±0.88	66.83±2.16
<i>Parthenium hysterophorus</i>	25	71.16±2.65	129.0±2.82
	50	54.33±4.13	120.0±7.75
	100	41.0±4.38	79.63±3.26
Control	-	-	-

±SD value, n=6, P <0.01

**Figure 1: Cytotoxic activity of *Parthenium hysterophorus* L. on CHOK 1 cells****Figure 2: Cytotoxic activity of *Parthenium hysterophorus* L. on A-549 cells****REFERENCES**

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Correspondence Author:

Lakshmi kanta Kanthal

Koringa College of Pharmacy, Department of Pharmacology, Korangi-533 461, Tallarevu (M), Andhra Pradesh, India

Email: lkkhaldia@gmail.com

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