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Original Article

EVALUATION OF CYTOTOXIC ACTIVITY AND ANTHELMINTIC PROPERTY OF CHLOROFORM EXTRACT OF *CLITORIA TERNATEA* L.

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ABSTRACT

Objective: The objective of the present work was to evaluate the phytochemical study, cytotoxic activity and anthelmintic property of the whole plant chloroform extract of *Clitoria ternatea L*.

Methods: The chloroform extract was tested for protein, amino acids. alkaloids, glycosides, flavonoids, tannins, steroids, saponins. The *in vitro* cytotoxic activity of chloroform extract was performed by MTT assay method against HepG2 (Liver carcinoma), HeLa (Cervix carcinoma) cell lines. 25, 50 and 100 mg/ml concentration of chloroform extract of *Clitoria ternatea L*. whole plant were taken for performing anthelmintic activity against adult Indian earthworm *Pheritima posthuma*.

Results: The preliminary phytochemical tests revealed that chloroform extract of *Clitoria ternatea L*. contains amino acids, alkaloids, glycosides, flavonoids, tannins, saponins, steroids. Effect of inhibition of cell growth showed significant cytotoxicity against HepG2 with an IC50 of 110.00±1.9 µg/ml and against HeLa with an IC50 of 104.50±0.9µg/ml. The crude chloroform extract (25, 50, 150 mg/ml concentration) of *Clitoria ternatea L*. whole plant shows potent anthelmintic activity against *Pheritima posthuma*.

Conclusion: The present study concluded that *Clitoria ternatea L*. can be considered as an important source of natural products that have anti-cancer potentials and potent anthelmintic activity.

Keywords: *Clitoria ternatea L*., Chloroform extract, Cytotoxic activity, Anthelmintic activity

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INTRODUCTION

In the recent past, there has been a growing interest in Traditional medicine/Complementary and Alternative Medicine (TCAM) and their relevance to public health both in developed and developing countries. 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]. *Clitoria ternatea* L. is a very bioactive plant and used in various diseases as folklore medicine [3, 4]. A Recent study showed that it has anxiolytic, antidepressant, anticonvulsant and anti-stress activity [5]. The herb has the property to increase the strength of the body. *Clitoria ternatea L*. is often grown as an ornamental plant. They require little care for cultivation. The plants have the property to improve the soil quality [6].

This plant has a long use in traditional Ayurvedic medicine for several diseases, and the scientific study has reconfirmed those with modern relevance. This review is an effort to explore the chemical constituents, pharmacological and toxicity studies of CT, which have long been in clinical use in the Ayurvedic system of medicine along with a critical appraisal of its future ethnopharmacological potential in view of many recent findings of importance on this well-known plant species [7]. The roots, leaves, and stems are all frequently used in Ayurveda, but for slightly different purposes. The roots are most widely used and are bitter, refrigerant, laxative, intellect promoting, diuretic, anthelmintic and tonic and are useful in dementia, hemicrania, burning sensation, leprosy, inflammation, leucoderma, bronchitis, asthma, pulmonary tuberculosis, ascites and fever. The seeds are cathartic, while the leaves are used in otalgia and hepatopathy [8].

MATERIALS AND METHODS

Plant material

The whole plants of *Clitoria ternatea* L. were collected from local areas of Korangi, Kakinada, East Godavari Dist., Andhra Pradesh. The plant was identified and authenticated by Mr. P. Venu, Additional Director, BSI, Deccan regional centre, Hyderabad-500048 where a voucher specimen has been deposited.

Preparation of extract

The whole plant parts of *Clitoria ternatea* L. were shade dried at room temperature, powdered and passed through 60 mesh size sieves. 215 gms of powdered plant parts were weighed accurately and extracted with chloroform solvent (1200 ml) using cold maceration method. Thus obtained extract were filtered through Whatman No.1 filter paper and the filtrate was concentrated. The extract (2.3 g) was transferred to sterile screw cap bottles, labeled and stored in the refrigerator until use.

Preliminary phytochemical screening

The chloroform extract of *Clitoria ternatea* was tested for protein, amino acids. alkaloids, glycosides, flavonoids, tannins, steroids, saponins [9-15].

Cytotoxic activity

Chemicals

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyltetrazolium 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

HepG2 (Liver carcinoma), HeLa (Cervix carcinoma), 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% CO_2 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 cm^2 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, each weighed test drugs were 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 test different concentrations of extracts were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37o C for 3 d 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 37o 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% (IC_{50}) is generated from the dose-response curves for each cell line [17].

% Growth Inhibition = 100–{(Mean OD of individual test group/Mean OD of control group) × 100}

Anthelmintic activity

The anthelmintic activity was performed according to the standard method [18] on the adult Indian earthworm *Pheritima posthuma*. Mebendazole, the standard drug, was diluted with normal saline to obtain 25, 50 and 100 mg/ml concentrations and was poured into Petri dishes. Chloroform 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. Seven petridishes of equal size were taken and numbered. Six earthworms (n=6) of similar sizes (about 8 cm) were placed in each petridish 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 o C). The paralysis time and lethal time were recorded in terms of minutes.

RESULTS AND DISCUSSION

The preliminary phytochemical study revealed that chloroform extract of *Clitoria ternatea L.* contains amino acids, alkaloids, glycosides, flavonoids, tannins, saponins, steroids.

In performing cytotoxic activity, There was gradual increase in the value of PGI (percentage of growth inhibition) as the concentration of extract was increased (39.68, 51.06, 60.92, 68.95, 78.00 % for the concentrations 62.5, 125, 250, 500, 1000µg/ml, respectively) against the HepG2 cell line (fig. 1) and (37.01, 55.76, 66.93, 87.31, 92.81 % for the concentrations 62.5, 125, 250, 500, 1000 µg/ml, respectively) against HeLa cell line (fig. 2). The median value of IC50 observed for HepG2 and HeLa cell lines were 110.00±1.9 and 104.50±0.9 respectively.

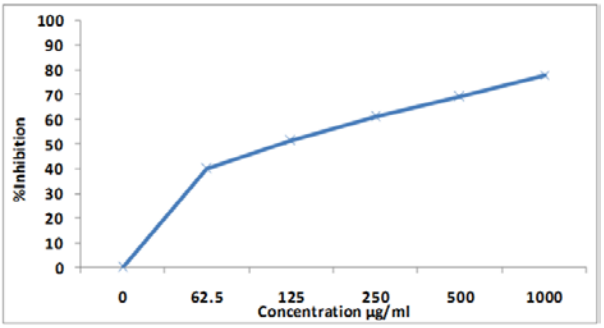

Fig. 1: Cytotoxic activity of *Clitoria ternatea L.* on HepG2 cell line

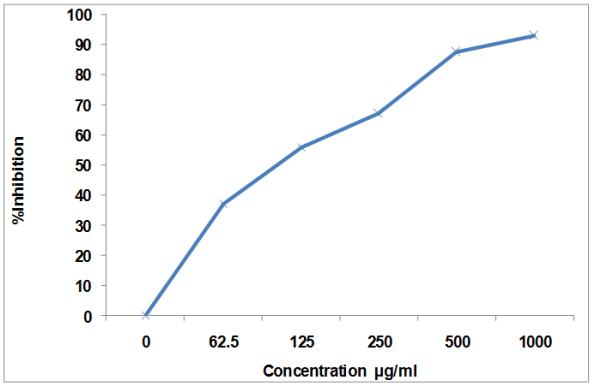

Fig. 2: Cytotoxic activity of *Clitoria ternatea L.* on HeLa cell line

The result of anthelmintic activity shows (table 1) that for the 25 mg/ml concentration, Mebendazole showed the best activity for death time (76.3±2.3 min) and the chloroform extract of *Clitoria ternatea* showed a death time of 97.0±2.0 min. Also, for the 50 mg/ml concentration, Mebendazole showed the highest activity against the worms (65.3±1.5 min) and the chloroform extract of *Clitoria ternatea* showed a death time of 75.0±2.0 min. For the 100 mg/ml concentration, Mebendazole showed the least death time 47.0±2.0 min and the chloroform extract of *Clitoria ternatea* showed a death time of 65.5±1.5 min.

Table 1: *In vitro* anthelmintic effect of *Clitoria ternatea L.* against *Pheritima Posthuma*

Treatment	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
Mebendazole	25	62.6±2.5	76.3±2.3
(standard)	50	48.0±2.0	65.3±1.5
	100	33.0±3.0	47.0±2.0
Chloroform extract of *Clitoria*	25	76.0±3.0	97.0±2.0
ternatea L	50	66.0±2.0	75.0±2.0
	100	48.0±2.6	65.5±1.5

±SD value, n=3.

CONCLUSION

In conclusion, the present plant *Clitoria ternatea* L. can be considered as an important source of natural products that have anti-cancer potentials and potent anthelmintic activity, due to the 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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CONFLICTS OF INTERESTS

Declared none

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