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Evaluation of Antimitotic and Genotoxic Effects of the Triterpenoid Enriched Extract from *Trichosanthes dioica* Root

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Abstract: *Trichosanthes dioica* Roxb. (Cucurbitaceae), called *Potol* in Bengali, *Palval* in Hindi and pointed gourd in English is a dioecious climber grown in Indian subcontinent and all parts of it have been traditionally used for various medicinal purposes. The present study aimed to evaluate the *in vitro* antimitotic and genotoxic effect of cucurbitacin enriched extract of *T. dioica* root using *Allium cepa* root meristems by keeping them in different concentrations of the test extract under specific experimental conditions, followed by determination of root growth inhibition (root length and number) and mitotic index. The extract significantly demonstrated concentration dependent inhibition of root length and number (EC_{50} value: 2.3 mg/ml) and reduction in mitotic index, indicating antimitotic activity demonstrating genotoxicity. The present study therefore, establishes remarkable *in vitro* antimitotic and genotoxic properties of *T. dioica* root against the test system and thereby suggesting the feasibility of its possible promise as natural antitumor agent.

Key words: *Allium cepa* · Antimitotic · Genotoxic · Cucurbitacins · Root

INTRODUCTION

Traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant therapeutic activity. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and low cost. Plants have a long history of use in the prevention and treatment of cancer. In recent years, there has been an increased interest in evaluating the anticarcinogenic properties of both dietary and medicinal plants all over the world.

Trichosanthes dioica Roxb. (Cucurbitaceae), called pointed gourd in English, *Potol* in Bengali, *Palval* in Hindi and *Patola* in Sanskrit is a dioecious climber found wild throughout the plains of North and North-East India from Punjab to Assam and Tripura states. It is also grown and commercially cultivated in India (Uttar Pradesh, Bihar, West Bengal and Assam states), Pakistan, Bangladesh and Sri Lanka for its consumable fruits, a common culinary vegetable in the Indian subcontinent. In India, all parts of this plant have been traditionally used for various medicinal purposes. According to Ayurveda, the traditional system of Indian medicine, its root is a strong purgative. The root has been traditionally used in India as hydrogouge cathartic, tonic, febrifuge, in treatment of jaundice, anasarca and ascites [1-4]. However, reports on the experimental studies on its root are comparatively scanty. In our previous studies, we reported anthelmintic effects of leaf and root, antibacterial and *in vitro* cytotoxic activities of the root of *T. dioica* [5-8]. In the present study, we have aimed to evaluate the triterpenoid enriched extract of *T. dioica* root for its *in vitro* anti-antimitotic and genotoxic efficacy using *Allium cepa* root meristems to evaluate the promise of *T. dioica* for possible antitumor efficacy.

MATERIALS AND METHODS

Plant Material: The mature tuberous roots of *T. dioica* were collected during December 2009 from Majdia, Nadia district of West Bengal, India. The species was

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identified by Dr. M. S. Mondal at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India and a voucher specimen (CNH/I-I/57/2009/Tech.II/493) was deposited at the Pharmacognosy Research Laboratory, Bengal School of Technology (A College of Pharmacy), West Bengal, India. Just after collection, the plant material was washed thoroughly with water and shade dried at room temperature (24-26°C) and ground mechanically into a coarse powder.

Chemicals: All the chemicals used were of analytical grade, obtained from Merck.

Preparation of Triterpenoid Enriched Extract (CETD): Just after collection, the fresh roots were washed thoroughly with water, cut into moderate pieces and immediately crushed thoroughly in tepid water (~50°C) using a mechanical grinder. After cooling to room temperature (23±2°C), the extract was separated from the remaining vegetable debris by pressing the material through muslin cloth. The resulting liquid is filtered and extracted once with *n*-hexane and the aqueous phase was further extracted successively with dichloromethane. The organic phases (dichloromethane extracts) were collected, pooled and evaporated to dryness *in vacuo* (at 35°C and 0.8 Mpa) in a Buchi evaporator, R-114. The dry extract i.e., triterpenoid enriched extract (CETD, yield: 6.55% w/w) was kept in a vacuum desiccator until use.

Standardization of CETD: Qualitative phytochemical analysis revealed the abundance of triterpenoids in CETD [9]. Presence of cucurbitacins in CETD was further ascertained by planar chromatography on silica gel pre-coated high performance thin layer chromatography (HPTLC) plates (Silica gel 60 F_{254} Merck, Germany) visually detected with vanillin-phosphoric acid reagent [10].

Test Samples: Test samples for *in vitro* cytotoxic bioassay were prepared freshly from the dry extract (CETD). Varying concentrations CETD, viz. 0.75, 1.50, 3, 6, 12 mg/ml were prepared by dissolving or suspending CETD in double-distilled water as per required concentrations and sonicated for 10 min immediately prior to use.

The *in vitro* Bioassay (Allium assay): This study was conducted as per the methods reported by previous workers with requisite modifications [11, 12].

***Allium cepa* Bulbs:** Approximately equal size bulbs (40±10 g) of the onions (*Allium cepa* L.) were obtained from the local vegetable market at Chendernagore, West Bengal, India. Any onions that were dry, moldy or have started shooting green leaves were discarded.

Growing *Allium cepa* Meristems: The outer scales were removed from the healthy onion bulbs leaving the root primordia intact. These bulbs were grown in dark for 48 h over 100 ml of tap water at ambient temperature until the roots have grown to approximately 3 cm. The water was changed daily during this period. The viable bulbs were then selected and used for subsequent studies.

Exposure to Text Samples: The bulbs with root tips grown up to 2-3 cm were removed from the water and placed on a layer of tissue paper to remove excess of water. The bulbs were divided into six groups (n =12). The first group served as control. Immediately, the bulbs were placed in test tubes filled with test samples (one bulb in each) and control (tap water) and incubated at 22±2°C for 96 h away from direct sunlight. The test samples were changed daily with fresh ones. The length of roots grown during incubation (newly appearing roots not included), root number and the mitotic index were recorded after 96 h. After this period the root appearance was also noted. The effective concentration for 50% root length inhibition (EC_{50} value) was determined by plotting the treatment concentrations against mean root lengths as percentage of tap water control group.

Microscopic Studies and Determination of Mitotic Index: After 96 h, the root tips (2-3 mm) were collected and prepared for microscopic studies by standard aceto-orcin squash preparation technique [13]. For each root tip, the numbers of mitotic cells and total meristematic cells were counted manually in 5-8 fields of view using high resolution (100x) bright field light microscopy. Mitotic index was represented in terms of dividing cells/total cells and expressed as percentage.

Statistical Analysis: The data are presented as the mean±Standard Error of Mean (SEM). The data were analyzed for statistical significance by Student's 't' test. P values less than 0.01 (p = 0.01) were considered as statistically significant.

Table 1. Influence of CETD on root length, root number and mitotic index of *Allium cepa* roots.

Concentration (mg/ml)	Mean root length (mm)	Mean root number	Mitotic index (%)
Control (tap water)	31.7±0.13	31.1±0.23	64.6±0.41
0.75	24.3±0.31¶	29.5±0.61^{ns}	53.8±0.22¶
1.50	18.6±0.40*	23.3±0.42¶	40.1±0.31*
3	13.9±0.57*	17.0±0.55*	27.9±0.33*
6	8.5±0.32*	12.7±0.30*	15.3±0.49*
12	5.3±0.45*	10.2±0.27*	5.4±0.23*

Data are expressed as mean±SEM (n = 12); ¶$p < 0.01$ and *$p < 0.001$ compared to tap water control. ns: not significant.

RESULTS AND DISCUSSION

The results for antimitotic and genotoxic effects of the triterpenoid enriched extract from *T. dioica* root (CETD) are summarized in Table 1. The CETD exhibited significant ($p < 0.001$) inhibitory effects on root growth (root length and number) and reduction in mitotic index (antimitotic effect) in a concentration dependent manner when compared with the control group. The EC_{50} value of CETD was found to be 2.3 mg/ml. The test samples did not cause any change in color of roots, however, at 3 and 6 mg/ml concentrations of CETD the color of roots turned pale brownish and became blackish brown at 12 mg/ml of CETD concentration. The morphology of root tips was not found affected at any concentrations of the test extract.

In the present investigation, the *in vitro* antimitotic and genotoxic effects cucurbitacin enriched extract from *T. dioica* were evaluated by using *Allium cepa* root meristem model, commonly known as Allium assay, where root growth inhibition and antimitotic effects provided the indication of genotoxicty. Allium assay is a rapid, highly sensitive and reproducible bioassay for detecting cytotoxicity and genotoxicity; and shows good agreement with results obtained from other test systems [12, 14]. Here, cytotoxicity at all concentrations test extract were evidenced by evaluating macroscopic parameters, i.e., reduction in root number and the most importantly, root length, both of which were indicative of root growth inhibition. The genotoxic effect was further confirmed by microscopic studies involving determination of mitotic indices reduction of which allowed assessment of impaired cell division (antimitotic effect) thereby providing definitive information regarding the extent of genotoxic action. Both growth inhibition and antimitotic effects were found to be in concert, as growth inhibition had been the inevitable consequence of diminished cell division.

In the current study, there were irreversible changes in root tip cells resulting in their growth retardation and cessation. The change in color of CETD treated roots to brownish was due to cytotoxic effects causing cell death [14].

Being triterpenoid enriched extract, the abundance of triterpenoids especially cucurbitacins were affirmed in CETD by qualitative phytochemical tests and planar chromatography (HPTLC). Cucurbitacins are known to possess several biological activities especially cytotoxic and antitumor activity [15]. The presence of putative cucurbitacin aglycones could provide the chemical basis of its anti-mitotic and genotoxic actions [16].

Previously the authors reported variable antimitotic effects of different solvent extracts from *T. dioica* root [8], but in the present study, the effect of triterpenoid enriched extract form *T. dioica* root was found to be the maximum, thereby indicating enrichment of the extract with triterpenoids (including cucurbitacins) resulted in better antimitotic and genotoxic activities.

With respect to present findings it has been apparent that *T. dioica* root contains cytotoxic constituents, plausibly cucurbitacin type triterpenoids, that can inhibit or cease the cell division. These constituents plausibly affected the cytoskeleton or inhibited the activity of one or more components of the cell cycle.

CONCLUSION

The present preliminary toxicological investigation confirms the *in vitro* antimitotic and genotoxic effects of triterpenoid enriched extract from *T. dioica* root against Allium assay. However, more positive results are definitely necessary in other test systems involving *in vitro* and *in vivo* animal and human cancer cell lines for qualifying prospective anticancer activity. The present preliminary investigation provides comprehensive *in vitro* evidence that *T. dioica* root demonstrates remarkable cytotoxic and genotoxic properties thus suggesting the feasibility of its possible promise as natural antitumor agent. Further antitumor studies on *T. dioica* root are presently underway.

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