PRELIMINARY STUDIES ON ANTIMITOTIC AND ANTI CANCER ACTIVITY OF *CALOTROPIS GIGANTEA*

Pardesi Goldee S*., Gadgoli Chhaya., Vaidya Madhav D., Hasni Hamid Y., More Babita H and Bhuskat Pallavi P.

Department of Pharmacognosy,
Saraswathi Vidya Bhavan's College of Pharmacy, Shil road Dombivli (E) – 421203, Maharastra, India
**Corresponding Author: goldeepardesi@rediffmail.com*

Summary

Calotropis gigantea, commonly known as Ruvi belongs to family *Asclepiadaceae*. The dried aerial part of *C. gigantea* was extracted with water using decoction method. The Total aqueous extract (CAI) and Water soluble fraction of Latex of *C. gigantea* (CAII), were evaluated for Antimitotic activity by determining Mitotic index of growing root tip cells of *Allium cepa*, treated with CAI and CAII at concentrations 2, 5 and 10 mg/ml. Both CAI and CAII exhibited significant antimitotic activity. The results were found to be comparable with Methotrexate (0.1 mg/ml).

The extracts; CAI and CAII were also subjected to in- vitro anticancer screening in three commonly occurring cell lines viz. DWD (Human Oral Cancer), DU-145 (Human Prostrate Cancer) and COLO-205 (Human Colon Cancer) by performing SRB assay method. Both CAI and CAII possess anticancer activity which was observed comparable to Adriamycin (ADR).

Key Words: *Calotropis gigantea*, Antimitotic, Cell lines, *Allium cepa*.

Introduction

C. gigantea belonging to family *Asclepiadeae* mostly distributed in tropical and sub-tropical regions of Asia and Africa. In India the genus is represented by two species. Viz, *Calotropis procera* and *Calotropis gigantea*[1].

Cardenolides have been isolated and identified from both the latex and the leaves of the plant, triterpenoides and anthocyanins from the flower. The leaves and latex of *Calotropis* species were found to have cardiac glycosides and were identified as Calotropogenin, Calotropin, Uscharin, Calotoxin, and Calactin.[1] Three cardenolide glycosides Coroglaucigenin, Frugoside and 4'-O-beta-D-glucopyranosylfrugoside were reported as cytotoxic principles from the roots of *C. gigantea*. An active principle 'Mudarine' was isolated from the leaves of *C. gigantea*.

The plant has been reported to have various pharmacological activities like antifertility, cardiotonic, antimicrobial and many more [2].The literature also reveals that the plant *C. gigantea* possess anti oxidant activity[2], also alcoholic extracts of the root and the leaves were found to have anticancer activity against human epidermal carcinoma of the nasopharynx tissue culture[3]. Thus keeping the vision of cancer therapy and going through the literature, the research programmed was aimed to screen CAI and CAII for anticancer activity.

Most of anticancer compounds derived from plants are phase specific and possess antimitotic activity. Hence, it was thought worthwhile to evaluate antimitotic activity of CAI and CAII.

Methods

Procurement of Plant Material: The dried aerial parts of *Calotropis gigantea* were procured from the local market of Mumbai and the sample was authenticated at Agharkar Research Institute, Govt. of India, Pune. The latex oozing out from the plant was collected through giving 'V' shaped incision on the branches of the plant, planted in the medicinal garden of the institute which is an authenticated species.

Preparation of extract: The Total aqueous extract (CAI) of the aerial parts was prepared by decoction method using distilled water as solvent. The fresh latex was collected and dried in vaccum oven at 60°c for 48 hours. The dried latex was suspended in water and filtered to get the water soluble fraction (CAII).

Determination of Mitotic Index: [4 & 5] The red variety of *Allium cepa* (onions) were procured from the local market and stored for the entire study. The bulbs of *Allium cepa* were sprouted in tap water for 48 hours at room temperature. The roots thus developed were treated by dipping these in the aqueous solutions of *calotropis gigantea* with CAI and CAII of concentrations 2, 5 and 10mg/ml for 2 hours. Treatment of the roots with the distilled water and Methotrexate (GSK, India 2.5 mg) at concentration 0.1mg/ml served as negative and positive control respectively. The roots thus treated with the above solutions were then cut to separate root tips and the root tips were transferred to the fixing solution {acetic acid (45% v/v) ethanol (95% v/v) in ratio of 1:3 v/v} for 10-15 hrs. After 10-15 hours, these were treated with 1 N hydrochloric acid and warmed in an oven at 50°C for 15 min. These root tips were then washed with distilled water and were stained with 2-3 drops of Carmine stain (LR Central Drug House Pvt Ltd). The slide was then squashed and observed under microscope. The numbers of cells in each stage of cell division were counted in four fields for each group.

Mitotic index was calculated using the formula,

$$\textbf{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

In vitro screening for Anti cancer Activity: In-vitro screening for Anti cancer Activity of CAI and CAII was carried out on the cell lines viz. DWD (Human Oral Cancer), DU-145 (Human Prostrate Cancer) and COLO-205 (Human Colon Cancer) at concentrations 10, 20, 40 and 80 µg /ml, by sulphorhodamine SRB assay method[6]. The experiment was conducted at the Advanced Center for Treatment Research and Education in Cancer (ACTREC) Navi Mumbai.

Results

.**Table 1: Mitotic index of *Alium cepa* root tips treated with different extracts of *Calotropis gigantea***

Sr. no	Groups	Average value ± S.D.
1	Distilled Water	87.00±2.00*
2	Methotrexate (0.1mg /ml Positive control)	12.00±2.00 *
3	CA1 (2mg/ml)	72.00±5.13*
4	CA1(5mg/ml)	60.00±4.50*
5	CA1(10mg/ml)	45.00±2.00*
6	CA2(2mg/ml)	60.00±3.21*
7	CA2(5mg/ml)	50.00±4.58*
8	CA2(10mg/ml)	32.00±2.00*

*Values are expressed as mean ± S.D. of three readings; *p<0.05, compared with control group; Least Significant Difference Procedure.*

Table 2: % Growth Inhibition of Cell Lines by incubation with various extracts of *Calotropis gigantea* and ADR using SRB assay

% Inhibition as compared to control												
	Colo205 cell line				**DWD cell line**				**DU145 cell line**			
µg/ml	**10**	**20**	**40**	**80**	**10**	**20**	**40**	**80**	**10**	**20**	**40**	**80**
CA1	24.7 ± 4.98	23.6 ± 1.62	29.9 ± 2.30	56.8 ± 1.27	3.0 ± 2.62	15.1 ± 10	44.1 ± 2.68	60.6 ± 1.47	7.6 ± 3.7	14.4 ± 3.41	30.8 ± 4.10	45.6 ± 1.75
CA2	69.4 ± 1.03	75.4 ± 0.65	74.6 ± 4.10	80.6 ± 5.06	82.5 ± 1.41	90.6 ± 1.40	94.2 ± 0.76	95.2 ± 0.43	54.2 ± 3.69	56.0 ± 1.87	56.4 ± 3.21	61.5 ± 3.26
ADR	70.9 ± 5.03	69.1 ± 1.78	70.5 ± 4.10	70.4 ± 2.67	95.5 ± 0.85	95.7 ± 2.12	96.6 ± 0.92	96.2 ± 0.57	60.7 ± 3.07	61.8 ± 6.19	62.1 ± 4.54	70.0 ± 1.86

Figure 1: % Growth Inhibition of Colo205 cell line using SRB Assay

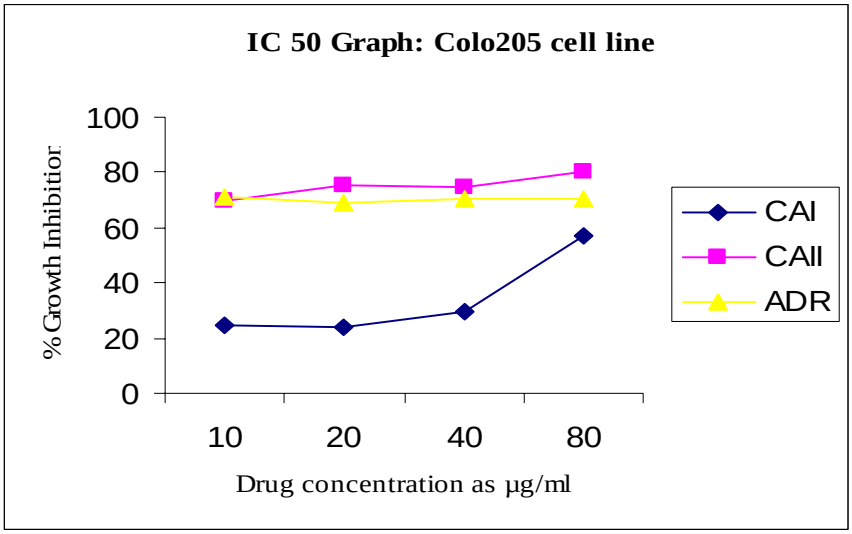


Figure 2: % Growth Inhibition of DWD cell line using SRB Assay

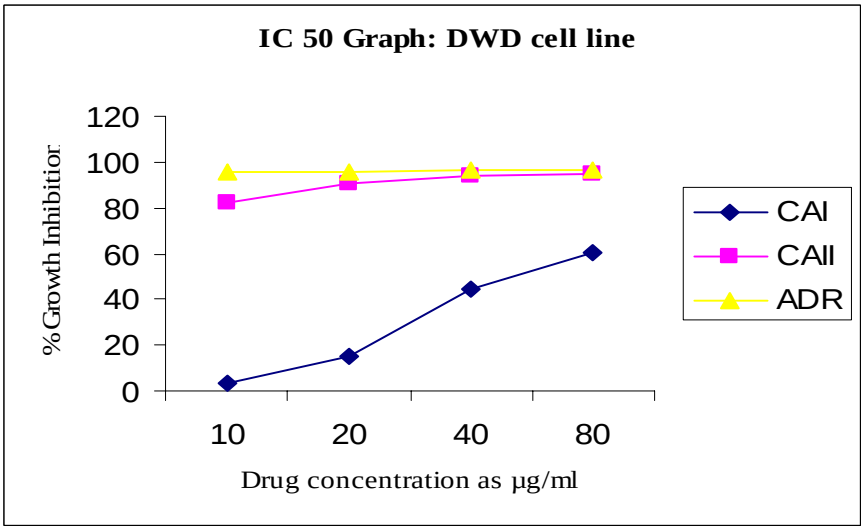
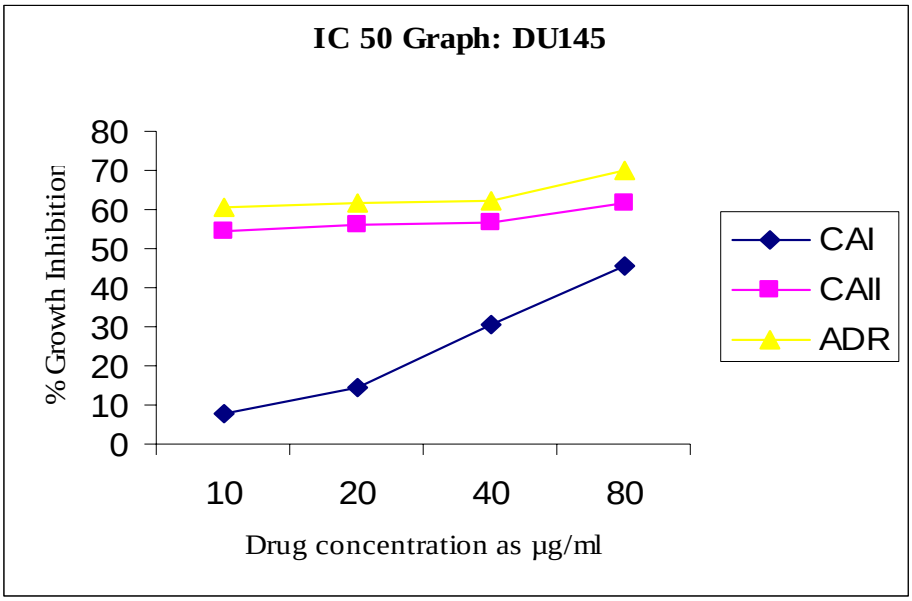


Figure 3: % Growth Inhibition of DU145 cell line using SRB Assay



CA1: Total aqueous extract of *Calotropis gigantea*.

CA2: Water soluble latex of *Calotropis gigantea*.

Colo205: Human Colon cell line.

DWD: Human Oral cell line.

DU145: Human Prostrate cell line.

ADR: Adriamycin as positive control.

The values are presented as average of three reading ± S.D.

Discussion

Mitotic index of root tips of *Allium cepa* treated with CAI and CAII at concentrations 2, 5, and 10 mg/ml was observed to be 72, 60, 45 and 60, 50, 32 respectively (Table 1). The mitotic index of groups treated with CAI and CAII was found to be significantly ($P< 0.05$) reduced when compared with distilled water. The maximum percentage of root tips cells were observed to be in prophase indicating inhibition of transition from prophase to metaphase and subsequent phases. The encouraging results were verified through in-vitro anticancer screening (Table 2).
In - vitro screening indicates that both CAI and CAII exhibited maximum inhibition in growth of cancerous cell lines viz. DWD (Human Oral Cancer), DU-145 (Human Prostrate Cancer) and COLO-205 (Human Colon Cancer) at concentration 80 µg /ml, by SRB assay method.

The percentage inhibitions in the growth of above mentioned cancerous cells when treated with (CAI) and (CAII) were observed to be comparable with standard drug Adriamycin.

From the results of the present study it can be concluded that *Calotropis gigantea* has potential to be explored as an Anticancer Agent.

Acknowledgement

The authors are grateful to ACTRECT for providing the services of In-vitro anticancer evaluation by SRB assay.

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