Metaphase arrest and delay in cell cycle kinetics of root apical meristems and mouse bone marrow cells treated with leaf aqueous extract of *Clerodendrum viscosum* Vent

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Abstract

Objectives: To study cell cycle delay and metaphase arresting activity of leaf aqueous extract of *Clerodendrum viscosum* Vent. (LAECV) in root apical meristems and mouse bone marrow cells.

Materials and methods: Cell cycle delay and metaphase arresting activities of LAECV were analysed, in root apical meristems of onion and wheat, and in mouse bone marrow cells, by scoring mitotic index, metaphase frequency and transition of cells from metaphase to anaphase. Colchicine was used as the standard metaphase arresting drug. Phytochemicals present in LAECV were detected and their phytotoxic activity was evaluated by analysing green-gram (*Vigna radiata*) seedling's root growth retardation and branch root swelling phenomenon.

Results: LAECV treatment resulted in dose-dependent root growth retardation of green-gram seedling root length ($P < 0.01$) and half maximal growth inhibitory concentration (IC_{50}) of LAECV was 0.87 mg/ml at 144 h. In onion and wheat root meristem cells the mitotic index decreased, metaphase frequency increased and transition from metaphase to anaphase reduced. Experimentation with mouse bone marrow cells indicated that LAECV induced metaphase arrest (164.3% increase in arrested metaphases per 300 mg/kg body weight, over 2.5 h). Phytochemicals like carbohydrates, glycosides, saponins, terpenoids, triterpenoids, tannins and trace amounts of alkaloids were detected in LAECV.

Conclusion: It may be said that LAECV contains mitostatic and metaphase arresting components that are able to induce significant metaphase arrest in root apical meristems and also in mouse bone marrow cells.

Introduction

Ethno-medicinal herbs are an excellent source of ample varieties of secondary metabolites, with valuable bioactivities. Cancer is a worldwide health problem and the search for new chemotherapeutic agents from phytochemicals is of great interest. Efficient anticancer agents such as vinblastine and vincristine, isolated from *Catharanthus roseus* L., and paclitaxel (Taxol®) from *Taxus brevifolia*, offer reliable clues that natural products of plant origin are potential sources of cancer chemotherapeutic agents (1).

The genus *Clerodendrum* of the Lamiaceae family has been cited in many indigenous systems of health care for treatment of variety of disorders. *Clerodendrum viscosum* Vent. (common name Ghetu, Bhat) is a perennial shrub with several medicinal uses in traditional practices (2,3). It is commonly used for stomach pain, all types of worm infection, malarial fever and for many types of skin disease (4). *Clerodendrum viscosum* leaves are taken raw or are mixed with certain vegetables for treatment of diabetes, high blood pressure and asthma (5) and leaf juice is used for stomach troubles and headache. It has the reputation to have anti-tumour activity particularly with respect to benign tumours (4) and leaf juice has been prescribed for treatment of cancer in some tribal communities (6). Ethanolic extracts of *C. viscosum* leaves have been shown to have significant anti-microbial activity comparable to standard tetracycline-type drugs (7). The product's antioxidant and protective effects against CCl_4 induced oxidative stress in

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rats have been found to be significantly high (8) and apparently it has reduced duration of seizures and has provided protection, in a dose-dependent manner, against leptazol-induced convulsions (9). Methanolic extract of *C. viscosum* leaves has caused significant reduction in fatty degeneration and necrosis in damaged liver, indicating moderate hepatoprotective activity (10). Moreover, methanolic extract of the leaves has been demonstrated to reduce blood sugar levels in streptozotocin-induced diabetes in Wistar rats (11) and also to have anti-inflammatory, antinociceptive and neuropharmacological activities (3). Leaf aqueous extract of LAE-CV contains carbohydrates, glycosides, saponins, tannins, terpenoids, triterpenoids and a trace amount of alkaloids (12). Alcoholic extract from its roots yields sterol glycoside, a mixture of β -sitosterol and Δ^5 25-stigmastadien-3 β -ol. Aerial components of the plant yield several sterols, such as clerosterol and its 22E-dehydro derivative, 24 β -ethylcholesta-5,22E,25-trien-3 β -ol, as major sterols, in addition to campestral, cholesterol, sitosterol, 24a-stigmasterol, 24 β -stigmasterol (2,13), and a fixed oil consisting of linolenic acid, oleic acid, stearic acid and lignoceric acid (5).

Some efficient anticancer plant extracts and anti-neoplastic agents exert their effects through cell cycle progression machinery. In our previous study, we reported antiproliferative and apoptosis inducing activity of LAECV (12). However, its delay in cell cycle and metaphase arresting activities remained unknown; the novel aspect of the study described here was to determine LAECV-induced delay in cell cycle kinetics and metaphase arresting activity.

Materials and methods

Chemicals

Colchicine, glacial acetic acid, methanol and orcein were obtained from BDH Chemicals Ltd, Poole Dorset, UK. Other chemicals used in this study were of analytical grade, from reputed manufacturers.

Plant product collection, storage and extract preparation

Fresh leaves of *C. viscosum* were collected from the Burdwan University campus, West Bengal, India in September 2010. The plant species was taxonomically identified by Dr. Ambarish Mukherjee (Taxonomist), Professor, Department of Botany, University of Burdwan, and the voucher specimen (No.BUTBSR011) is maintained in the Department of Zoology of the University of Burdwan, for future reference.

Collected leaves were washed in tap water, shade dried, directly crushed into small pieces and pulverized using an electric grinder (Philips Mixer Grinder HL1605, Kolkata, West Bengal, India); ground leaf powder was then stored in an air tight container for future use.

50 g dried, powdered, plant material was extracted in 500 ml distilled water for 6 h, under slow heating (50 °C), in a water bath; every 2 h it was filtered through Whatman filter paper #1 (Sigma-Aldrich, Inc., St. Louis, MO, USA). This procedure was repeated twice and after 6 h the filtrate was concentrated in a water bath at 60 °C for around 4 h to obtain final volume of one fifth original volume (14). The extract was sterilized by autoclaving then stored at -20 °C for further use. To determine extract value (14% w/w) and initial extract concentration, 10 ml of extract was evaporated to complete dryness in a hot air oven at 60 °C.

Plant models

Wheat (*Triticum aestivum* L.), onion (*Allium cepa* L.) bulbs and green-gram (*Vigna radiata*) were used as plant models. Wheat and onion root apical meristems were used for determining cell cycle delay and metaphase arresting activity. Green-gram roots were used for growth retardation and compared to colchicine induced root swelling.

Experimental animals

Male Swiss albino mice, aged 2–3 months and weighing 25–30 g, were maintained in the departmental animal house, in community cages at room temperature, with controlled lighting (12 h light:12 h dark). Standard mouse diet and water were available to them *ad libitum*. Rules of the Institutional Animal Care and Use Committee were strictly observed during the whole experiment and steps were taken to protect their welfare. Metaphase frequency was analysed for the study of cell cycle kinetics in mouse bone marrow cells.

Root growth retardation and root tip swelling

Culture and treatment of green-gram seedlings. Green-gram seeds were surface sterilized using 1% sodium hypochlorite solution, and allowed to germinate in the dark on wet filter paper (in glass Petri dishes containing seven different concentrations (0.125–8 mg/ml) of LAE-CV and six different concentrations (0.025–1 mg/ml) of colchicine), and root length was measured on the 3rd, 4th and 6th days. Three replicas of each with 10 seeds were prepared for each treatment. For branch root swelling of green-gram seedlings, 72 h aged similar-sized main roots where root branch sprouting just started, were treated with

LAECV (4 mg/ml) and colchicine (0.05 mg/ml) and the root swelling patterns were observed for 48 h. Tap water alone in a Petri dish was considered as untreated control.

Culture and treatment of wheat seedlings. For the root swelling activity study, similar-sized seeds of *T. aestivum* were surface sterilized with 1% sodium hypochlorite solution and five replicas of each with 30 seeds were prepared for each treatment. Seeds were placed on filter paper kept in sterilized Petri dishes (90 mm), were covered then incubated at 25 °C in a dark culture room, for germination. Different concentrations of LAECV (1, 2, 3 and 4 mg/ml) were applied at the beginning of the culture period and seedlings were treated continuously for 48 h. In a further set of experiments, root swelling activity of LAECV (2.25 mg/ml) was compared to that of colchicine (0.2250 mg/ml) induced root swelling.

Cell cycle delay and metaphase arrest in apical root meristems

Treatment with LAECV and preparation of mitotic phases from wheat root meristem cells. 48 h-aged roots were treated with LAECV (2.25 mg/ml) for up to 24 h. Treated and untreated root tips were fixed in aceto-methanol (3 parts methanol: 1 part glacial acetic acid) for 24 h then hydrolysed for 10 min in 1 N HCl at 60 °C, stained with 2% aceto-orcein and squashed in 45% acetic acid for each treatment (15).

Culture of onion roots. Similar sized onion bulbs were used for root sprouting in test tubes containing distilled water at 25–27 °C. 48 h-aged onion roots were used for all experiments.

Treatment with LAECV and colchicine and preparation of mitotic phases in onion root meristem cells. Mitotic index depression and cell division phase frequency were analysed for evaluation of induced delay in cell cycle kinetics, and metaphase arresting effects of LAECV. The 48 h-aged onion root meristem cells were treated with three different concentrations, 0.25, 0.75 and 2.25 mg/ml LAECV for 3, 24, 48 and 72 h. Simultaneously, root tips were treated with colchicine (0.28 mg/ml) for 24 h. The control group, (no treatment), remained in distilled water. Slide preparation and staining were performed as described for wheat root tip cells.

Cell cycle delay and metaphase arrest in mouse bone marrow cells

Treatment and preparation of metaphases from mouse bone marrow cells. Filtered and autoclaved LAECV was

injected into intraperitoneal cavities of albino mice and an equal volume of double distilled water was injected to the control group. At each data point, a minimum of six mice and three different doses (100, 300 and 500 mg/kg body weight) were used. Metaphase arresting activity of LAECV was analysed in mouse bone marrow cells after 2.5 h treatment. Simultaneously, colchicine (10 mg/kg body weight) was given to a group of six mice as positive control (16). Treated and untreated animals were sacrificed by cervical dislocation. Femurs were dissected out and bone marrow cells were obtained by injecting 2 ml 0.075 M KCl (pre-warmed to 37 °C, hypotonic solution). Cells were treated for 20 min then were fixed in acetic acid and methanol (1:3). Slides were prepared by flame-drying method, stained in 2% Giemsa solution for 25 min and mounted in synthetic medium.

Scoring and statistical analysis. Green-gram seedling root growth was recorded and percentage growth retardation was calculated. Differences between control and treated group root length was evaluated using Student's *t*-test. With Probit analysis (17), IC₅₀ was determined for green-gram root growth retardation with statistical software, SPSS version 14.0 (SPSS Inc. Chicago, IL, USA). For onion and wheat root tip cells, slides were randomly coded and for each experimental set at least five slides were studied by bright field illumination light microscopy, $\times 40$ objective lens; a minimum of 1000 cells were scored. Mitotic index, prophase, metaphase and anaphase-telophase frequencies were analysed on the basis of nucleus and chromosomal characteristics. For mouse bone marrow cells, slides were randomly coded and patterns of cell cycle kinetics were determined by scoring mitotic index (MI), MI% = number of metaphases/total number of cells scored $\times 100$. Statistical significance of differences between control and treated groups for MI, analysed by 2×2 contingency χ^2 analysis testing.

Results

Root growth retardation and root tip swelling

Data clearly indicate that both LAECV and colchicine induced dose-dependent growth retardation of green-gram roots (Fig. 1; Table s1; Fig. s1). Maximum root length was recorded from untreated groups while minimum root length was recorded from our highest concentration (1 mg/ml) of colchicine. Lowest concentration (0.125 mg/ml) of LAECV treatment showed a significant effect ($P < 0.01$) on root apical meristem growth of green-gram seedlings. Root growth inhibition was calculated as 29, 34, 38, 48, 61, 71 and 81% for LAECV concentrations respectively, of 0.125, 0.25, 0.50, 1, 2, 4 and 8 mg/ml, at

Figure 1. LAECV- and colchicine-induced root growth retardation in green-gram seedlings. Data are means $\pm$ SE of the mean.

144 h, and IC_{50} for root growth was determined to be 0.87 ± 0.08 mg/ml.

A further important observation was LAECV (4 mg/ml)-induced branch root tip swelling of green-gram seedlings was comparable to colchicine (0.05 mg/ml)-induced root swelling (Fig. 2d). Dose-dependent root growth

retardation and root swelling phenomenon were also seen in wheat root tips after treatment with LAECV (1–4 mg/ml) for 48 h (Fig. 3). Wheat root tips were found swollen after 48 h LAECV (2.25 mg/ml) and colchicine (0.225 mg/ml) treatment (Fig. 4). These results were analysed by comparing them to untreated controls.

Cell cycle delay and metaphase arrest in root apical meristems

Wheat root tip cells. Cell cycle data of wheat root tip cells (Table 1; Fig. 2a) indicated reduction in mitotic index of LAECV treated cells and also reduction in frequency of cell cycle phases, with the exception of metaphase and interphase. LAECV treatment also showed increased interphase percentage, reduced mitotic index, and cells which were in mitosis had arrested metaphases that resulted in subsequent reduction in anaphase and telophase frequencies. Significant effect ($P < 0.001$) in metaphase-arresting activity was observed after 2 h treatment with LAECV (2.25 mg/ml) in wheat root tip cells. Root tip cells which were treated continuously for

Figure 2. Comparable metaphase arresting and branch root swelling activity of LAECV and colchicine (0, 1 and 2 denote untreated, LAECV and colchicine, respectively; (a), (b), (c) and (d) denote wheat, onion, mouse bone marrow cells and green-gram respectively).

Figure 3. (a) Dose-dependent LAECV-induced wheat root and shoot growth retardation effects; (b) untreated; (c–e) effects of LAECV, treated from beginning of culture setting, for concentrations of 1, 2 and 4 mg/ml, respectively, at 48 h.

Figure 4. Comparable effects of colchicine and LAECV on wheat root tips. a, b and c, respectively, untreated, LAECV (2.250 mg/ml) and colchicine (0.225 mg/ml) treated for 48 h from beginning of culture setting, while ₀ and ₁ denote photographs and photomicrographs respectively.

24 h, showed slightly elevated anaphase-telophase frequency compared to untreated controls. In addition, notable observations were induction of chromosomal aberrations, metaphases with condensed and disorganized distribution of chromosomes.

Onion root tip cells. Data indicated tendency to mitodepression in LAECV-treated onion root meristem cells, compared to untreated controls; dose-dependent mitotic index reduction was observed in LAECV-treated samples at 48 h. Results were correlated to colchicine

(0.28 mg/ml)-induced reduction in prophase and anaphase-telophase percentage, and increased percentages in metaphase at 24 h treatment. Significant differences ($P < 0.01$) were seen in mitotic index and metaphase frequency between treated and untreated root apical meristem cells (Table 2).

Mouse bone marrow cells. LAECV treatment was given for 2.5 h to analyse its metaphase arresting activity *in vivo* and this was compared to colchicine-induced metaphase arrest. Data indicated metaphase percentage

Table 1. Pooled data showing mitotic index and cell percentages at different phases in LAECV-treated and untreated wheat root tip cells

Dose (mg/ml)	Hours	Cells scored	Cells percentage at different phases (mean $\pm$ SEM)			
			Mitotic	Pro	Meta	Ana-Telo
0.00	1	1730	21.4 $\pm$ 2.5	68.19 $\pm$ 3.3	11.99 $\pm$ 3.9	19.91 $\pm$ 0.4
2.25		1687	20.7 $\pm$ 0.5	69.10 $\pm$ 1.8	14.13 $\pm$ 1.9	16.77 $\pm$ 0.9
0.00	2	1617	13.7 $\pm$ 0.5	63.98 $\pm$ 3.6	13.73 $\pm$ 2.3	22.29 $\pm$ 2.3
2.25		1630	08.8 $\pm$ 0.8***	58.58 $\pm$ 4.2	26.89 $\pm$ 4.2***	14.53 $\pm$ 3.0**
0.00	4	1706	11.4 $\pm$ 0.5	76.41 $\pm$ 4.6	16.50 $\pm$ 4.2	23.59 $\pm$ 3.6
2.25		1441	12.0 $\pm$ 1.9	46.57 $\pm$ 3.3	42.97 $\pm$ 5.0*	10.46 $\pm$ 2.6**
00	24	1940	17.3 $\pm$ 2.6	58.27 $\pm$ 3.6	23.3 $\pm$ 4.3	18.43 $\pm$ 3.2
2.25		1668	13.3 $\pm$ 1.4	53.93 $\pm$ 3.8	19.1 $\pm$ 2.2	26.97 $\pm$ 3.6

Significant at * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ 2×2 contingency χ^2 analysis compared to respective control.

Table 2. Effect of LAECV and colchicine on mitotic indices and frequency of different mitotic phases in onion root tip cells

Dose (mg/ml)	Hours	Cells scored	Cells percentage at different phases			
			Mitotic	Pro	Meta	Ana-Telo
0.00		1612	14.21	46.5	21.7	31.8
2.25	3	2814	07.72	37.8	54.1**	15.8**
0.00	24	1221	16.12	50.6	20.0	29.4
0.75		1791	09.31***	35.7	39.7**	25.0
0.28 ^s		1807	13.61	21.9***	76.9***	1.4***
0.00	48	1808	15.22	55.2	12.6	32.2
0.25		1201	10.31**	37.4	33.9***	28.7
0.75		1872	08.73***	20.8***	65.3***	13.6**
2.25		1658	07.22***	13.2***	78.9***	07.9***
0.00	72	1131	10.31	61.4	13.9	24.7
0.75		1186	08.62	27.8***	50.0***	22.3

Significant at ** $P < 0.01$ and *** $P < 0.001$ 2×2 contingency χ^2 analysis compared to respective control. ^ssymbol = point for colchicine treatment.

(increased%) 1.01 $\pm$ 0.04, 1.90 $\pm$ 0.34 (88.1), 2.67 $\pm$ 0.28 (164.3) and 1.56 $\pm$ 0.09 (54.5) after LAECV treatment with doses 0, 100, 300 and 500 mg/kg body weight, respectively (Table 3).

Discussion

Clerodendrum viscosum is a traditional medicinal plant with a number of uses, in India (2,4–6). Work presented here focuses on studying influences of LAECV on delay in cell cycle kinetics and metaphase-arresting activities, in root apical meristems and mouse bone marrow cells. Moreover, metaphase-arresting activity was analysed by comparison with the standard metaphase-arresting agent, colchicine. The LAECV-induced root growth retardation effect analysed on green-gram seedlings in the initial experiments, a wide range (0.125–8 mg/ml) of LAECV concentration was applied to determine IC₅₀, and then

Table 3. Pooled data showing influence of LAECV and colchicine on percentage of mitotic index in mouse bone marrow cells

Treatment	Dose (mg/kg bw)	Hours	TM/TC (No. of Mice)	MI	
				Range	Mean $\pm$ SEM (reduction%)
LAECV	000	2.5	153/15254 (7)	0.88–1.11	1.01 $\pm$ 0.04
	100		120/06332 (6)	1.53–2.21	1.90 $\pm$ 0.34*** (–88.1)
	300		171/06393 (6)	2.39–2.95	2.67 $\pm$ 0.28*** (–164.3)
	500		199/13207 (6)	1.24–1.90	1.56 $\pm$ 0.09*** (–54.5)
Colchicine	10		872/19213 (6)	4.03–5.74	4.64 $\pm$ 0.27***

Significant at *** $P < 0.001$ 2×2 contingency χ^2 analysis compared to respective control.

LAECV, leaf aqueous extracts of *Clerodendrum viscosum* Vent.; bw, body weight; TM, total metaphase; TC, total number of cells counted; MI, mitotic index.

the moderate concentration (4 mg/ml) was selected for branch root swelling activity analysis. Colchicine (0.025–1 mg/ml)-induced green-gram root growth retardation was compared to that of LAECV (Fig. 1), and for the root-swelling phenomenon 0.05 mg/ml of colchicine was selected. For wheat seedlings LAECV concentrations 1, 2 and 4 mg/ml were used for treating for root growth retardation and root swelling (Fig. 3). Moreover, colchicine (0.225 mg/ml) and LAECV (2.25 mg/ml)-induced root swelling patterns were compared (Fig. 4). For cell cycle kinetics and metaphase-arresting activity, analysis with wheat and onion root apical meristem cells, 2.25 mg/ml LAECV was considered to be the highest concentration.

In this study, all used concentrations of LAECV (0.125–8 mg/ml) induced dose-dependent reduction in

root length of green-gram seedlings. Growth retardation percentage increased from 28.98 ± 1.9 to 80.85 ± 1.7 for increased concentration from 0.125 to 8 mg/ml ($P < 0.001$); colchicine also induced growth retardation in green-gram seedlings in a dose-dependent manner. In our earlier study, growth retardation effect of LAECV was also observed in onion and wheat root apical meristems (12). Our results indicate that root apical meristems are sensitive to LAECV; these results are in agreement with previous study reports (18) and root growth retardation is a result of suppression of cell division and chromosomal aberration (19). A number of earlier studies have suggested that levels of root growth inhibition increase with increasing extract concentration (20). Aqueous extract from leaves of *Toona sinensis* R. has also been shown to have an anti-proliferative effect on human lung cancer cells (21,22); secondary metabolites acting as allelochemicals include alkaloids, phenols and terpenoids. Phenols are the most abundant substances that affect seedling growth and cell division (23). For wheat root tip cells, LAECV treatment (2.25 mg/ml for 1, 2 and 4 h) resulted in increased percentages of interphase and metaphase cells, while overall mitotic index and anaphase-telophase frequency decreased, indicating anti-proliferative and metaphase arresting activities of LAECV (Table 1). Like wheat, onion root tip cells treated with LAECV also had lower mitotic indices ($P < 0.001$) and increased interphase cell frequency; percentage of metaphase cells increased in dividing cell populations and metaphase to anaphase-telophase transition frequency decreased. It is well-known that cells arrest in metaphase when the mitotic spindle is disassembled and inactivation of MPF (that signals metaphase to anaphase transition) is blocked (24).

LAECV-induced metaphase arrest seemed to be comparable to colchicine-induced metaphase arrest in onion root apical meristem cells (Table 2) although concentrations of LAECV required were considerably higher than of colchicine, which may be due to LAECV being in a crude form. Levan first introduced the *A. cepa* root tip assay and later it was proposed as a standard method to study genotoxicity (19,25–28); and there are previous reports of mitotic index depression (29–31). Metaphase arrest has formerly been reported after use of vinblastine, vincristine and colchicine alkaloids (24,32). Each molecule of colchicine binds to one of tubulin by replacement of a methyl group thus preventing its polymerization (33). By our observation, cells treated with LAECV may also have experienced disturbance in the microtubule polymerization process as cells were arrested at metaphase and chromosomes were found to be relatively condensed and haphazardly distributed. In this study, LAECV-induced green-gram and

wheat root tip swelling patterns were very similar to colchicine-induced root swelling. LAECV also influenced cell cycle kinetics of mouse bone marrow cells. Here there was a tendency towards increased percentages of metaphases after LAECV treatment for 2.5 h, compared to untreated controls (Table 3) at 300 mg/kg body weight dose. These observations were perhaps, accomplished due to presence of bio-active compounds in LAECV similar to those that interact with the mitotic spindle, as with colchicine, as shown by the similar patterns of root swelling, chromosome condensation, haphazardly arranged metaphase chromosomes, reduced frequency of metaphase to anaphase transition and increased frequency of metaphases, in both plant and animal systems. Microtubule disrupting agents are thought to arrest cells in mitosis by triggering activation of a mitotic checkpoint, a series of biochemical reactions that ensure accurate attachment of chromosomes to the mitotic spindle, before cells enter anaphase (34–37). As a result of drug treatment, when microtubules fail to attach to one or more kinetochores, components of the checkpoint continue to generate signals that inhibit metaphase to anaphase transition. Disruption of the spindle with the drug treatment would be expected to produce a strong signal that greatly prolongs metaphase (33).

Unlike the dose-dependent root growth retardation phenomenon of root apical meristems, *in vivo* study using mouse bone marrow cells, metaphase-arresting activity of LAECV was not dose-dependent and this may be due to its overall antiproliferative activity. Our previous study with mouse bone marrow cells indicated that colchicine-induced metaphase frequency reduction with increasing dose of LAECV treatment (12). 500 mg/kg body weight LAECV-induced cell cycle delay in interphase may be the cause of reduced number of cells at mitotic phases, hence, less cells being available to be arrested at metaphase by LAECV.

The cell cycle kinetic study revealed that LAECV treatment arrested cells at metaphase ($P < 0.001$). These higher frequencies of metaphase and subsequent reduction in metaphase to anaphase transition frequency, indicate distinct metaphase-arresting activity of LAECV. Moreover, dose-dependent increased interphase frequency and reduced prophase frequency, indicate LAECV-induced cell cycle delay in interphase, and thus it may correlate with antiproliferative activity (12). In this study, colchicine, a microtubule assembly inhibitor, was used as positive control for metaphase arrest (16,25). Our findings also support the notion that antiproliferative tests with the *A. cepa* correlated with *in vivo* testing on mouse bone marrow cells, and this also was validated using animal cells (38,39). The medicinal properties of plants have

been claimed to lie in their phytochemical ingredients, which can produce specific results for human physiology (40–45). Efficient anticancer and anti-neoplastic agents exert their effects through the cell cycle progression machinery (46). The result described here indicates that LAECV may contain bio-active compound(s) that interact with the mitotic apparatus. Other and our previous studies have indicated presence of bioactive compounds such as specific carbohydrates, glycosides, saponins, terpenoids, triterpenoids, tannins and trace amounts of alkaloids in LAECV (12,47).

In conclusion, LAECV contains active component(s) for arresting cells at metaphase as shown by somewhat comparable results with colchicine treatment, in both plant and animal systems, although it is very early to be able to say that there might be colchicine-like microtubule disruptive activity. Further studies are in progress to isolate active LAECV metabolite(s) and to discover whether they have interactions with microtubules of the mitotic spindle.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Pooled data showing dose-dependent root growth retardation of *Vigna radiata* treated with LAECV and colchicine.

Fig. S1 Photographs showing influence of LAECV and colchicine on root growth of green-gram seedlings.