Assessment of Cytotoxic Effects of Aqueous and Methanolic Leaf Extracts of *Clerodendrum inerme* (L.) Gaertn. and *C. viscosum* Vent. Using *Allium* Test

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Summary Aqueous and methanolic leaf extracts of two medicinal plants, *Clerodendrum inerme* (L.) Gaertn. and *C. viscosum* Vent. (Family: Lamiaceae) were evaluated on root tip mitotic cells of *Allium cepa* L. for assessment of cytotoxicity relevant to their potential applicability for therapeutic uses. Results revealed mitodepressive as well as cytotoxic effects in mitotic cells of *Allium* test system following treatments with the extracts; however, the manifestation of effects was differential in relation to extract types and concentrations. Aberration types encountered in dividing and resting cells of *A. cepa* highlight cytotoxic potentiality of the extracts. The present study is therefore significant for biomonitoring of the cytotoxic effects with studied leaf extracts.

Key words *Clerodendrum*, Leaf extract, *Allium* test, Cytotoxicity, Therapeutic use.

Modern medicines are intangible to destitute due to high cost, and therefore there is an upsurging demand for natural plant products in clinical uses. Use of plant extracts as source for traditional medicines in health care is an age-old practice (Farnsworth *et al.* 1985, Ajaiyeoba *et al.* 2003, Ettarh and Emeka 2004, Mishra *et al.* 2005, Chah *et al.* 2006, Akinboro and Bakare 2007, Grisi *et al.* 2012, Ping *et al.* 2012, Silva *et al.* 2013, Chukwujekwu and Van Staden 2014), but due to some phytoconstituents present in them they can be toxic and carcinogenic to human health causing hindrances to their potential exploration (Moody *et al.* 1999, Teixeira *et al.* 2003, Paes-Leme *et al.* 2005, Gadano *et al.* 2006).

C. inerme and *C. viscosum*, perennial shrubs of the family Lamiaceae, are therapeutically important plant species possessing immense traditional uses and pharmacological properties (Chethana *et al.* 2013, Nayeem and Mehta 2015, Nandi and Lyndem 2016, Wang *et al.* 2018). The present investigation describes the cytotoxic effect of aqueous and methanolic leaf extracts of *C. inerme* and *C. viscosum* using the *Allium* test. It is worth mentioning that the *Allium* test is widely used for monitoring toxicity due to its efficiency, operational simplicity and sensitivity corroborating analogous results in different cell lines including human lymphocytes (Fiskesjo 1985). The objective of the present work highlights the necessity of a safe dose(s) determination of the studied plant extracts in therapeutic uses.

Materials and methods

Plant species and leaf sample collection of plant species

Bulk collection of fresh leaves of *Clerodendrum inerme* (L.) Gaertn. and *C. viscosum* Vent. were made from fully grown leaves of matured plants during the months of January and July from University of Kalyani campus, Kalyani, West Bengal, India.

Extracts preparations

Both aqueous and methanolic leaf extracts of *C. inerme* and *C. viscosum* were prepared. Extract preparations were made in triplicate from the collected leaf samples in the studied period (January and July). After surface sterilization with 2% sodium hypochlorite solution for 2 min, leaf samples (5 g, fresh weight) were dipped in 50 mL double distilled water (ddH₂O) and 99.8% methanol separately in conical flasks for 24 h with intermittent shaking and subsequently filtered through a paper filter (Whatman No. 1) and centrifuged at 4000 rpm for 10 min. The supernatants of aqueous extracts were considered 1.0× and diluted to 0.5× and 0.25× with ddH₂O. The supernatants of methanolic extracts were dried under reduced pressure in a rotary vacuum evaporator (Büchi RII) and dissolved in 50 mL ddH₂O to form 1.0×, and serial dilutions were made to 0.5× and 0.25× with ddH₂O.

Treatments and determination of cytotoxicity

Bulbs of *A. cepa* L. (Sukhsagar, a local cultivar of West Bengal plains) were planted in sand-saw dust mixtures (1:1) in wooden trays and on sprouting the roots

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were exposed in 1.0×, 0.5×, and 0.25× aqueous and methanolic extracts for 5 days. The treatments were performed under controlled laboratory condition at 28°C. A control set was uniformly maintained using ddH₂O.

In each lot including control, five roots were collected between 12.30 p.m. to 1.00 p.m., fixed in acetic ethanol (1:3, v/v) overnight and preserved in 70% ethanol in a refrigeration at 4°C for further use. Roots were stained in 2% aceto-orcein-1M HCl mixture (9:1) for 2h following gentle warming over a spirit lamp for 10min and slides were prepared for each treatment including control. Root tips were squashed on glass slides in 45% acetic acid. The slides were observed under a Leitz Laborlux S microscope attached with a Leica EC 3 scientific camera. Mitotic cells with typical aberration were photographed.

Mitotic index [(the number of dividing cells/total cells scored)×100], and aberration types and frequencies were determined in each treatment including control. Data represented in Table 1 is pooled over all the preparations analyzed.

Statistical analysis

Critical difference (CD) at 0.05 probability level was determined for mitotic index and frequency of abnormal dividing cells using one-way analysis of variance for assessment of significant variations, if any, between/among treatments including control.

Results and discussion

Mitotic consequences of aqueous and methanolic leaf extract of *C. inerme* and *C. viscosum* on root tip cells of *A. cepa* were documented in Table 1. Compared to control, the frequency of dividing cells reduces significantly ($p<0.05$) after treatments with aqueous extracts of both species; however, the reduction is not significant among their doses. In comparison to control, treatments with methanolic extracts also reduce mitotic index significantly ($p<0.05$) in 0.5× and 1.0× doses of *C. inerme* and in all the studied concentrations of *C. viscosum*; although among the doses the reduction is not significant excepting 1.0× dose of *C. viscosum*. Thus, both aqueous and methanolic leaf extracts of *C. inerme* and *C. viscosum* can bring about mitodepressive effect. Mitodepressive effects assessed from the *Allium* test following treatments with other plant extracts are also reported earlier (Kuraś *et al.* 2006, Akinboro and Bakare 2007, Aşkin Çelik and Aslantürk 2010, Ping *et al.* 2012, Silva *et al.* 2013, Chukwujekwu and Van Staden 2014).

Fernandes *et al.* (2007) opined that a decrease in the mitotic index by treatments is a contributing factor for determining the level of cytotoxicity induced. Both aqueous and methanolic leaf extracts induce cytotoxic effects in mitotic cells of *A. cepa* but only in specific doses of treatments (*C. inerme*: only in 1.0× aqueous extract, 2.47%; *C. viscosum*: aqueous extracts in all employed doses, 1.25 to 8.36%; methanolic extracts in 0.5×

Table 1. Mitotic index and aberration types and their frequency induced by plant extracts in root tip cells of *A. cepa*.

Extract type					Abnormal dividing cells (%)										
Plant species	Dose of treatment	Total number of cells scored	Number of dividing cells	Mitotic index (%)	Stickiness of chromosomes	Ring configuration of chromosomes	Fragments	Polyploid cells	Chromatin disorganization	Bridges	Laggards	Multipolarity	The frequency of abnormal dividing cells (%)	Cells with micronuclei (%)	Giant cells (%)
Aqueous															
CI	0	4645	733	15.78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.25×	1445	187	12.94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.5×	1905	233	12.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1.0×	2995	364	12.15	0.55	0.00	0.82	0.00	0.00	0.27	0.00	0.82	2.47	0.00	0.07
CV	0.25×	2529	240	9.48	0.00	0.00	0.00	0.00	0.00	0.42	0.83	0.00	1.25	0.00	0.00
	0.5×	2625	234	8.91	0.00	0.00	2.99	0.00	0.43	1.71	0.00	0.43	5.13	1.25	0.00
	1.0×	3225	287	8.89	2.43	0.35	1.74	1.74	0.69	1.04	0.00	1.39	8.36	1.32	0.07
Methanolic															
CI	0.25×	1610	244	15.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.5×	1295	136	10.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1.0×	1295	122	9.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CV	0.25×	1745	195	11.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.5×	1665	167	10.03	0.59	0.00	0.59	0.00	0.00	1.18	0.00	0.00	2.39	0.20	0.00
	1.0×	2025	140	6.91	0.71	1.42	2.14	2.14	0.71	0.71	0.71	0.71	7.14	0.69	0.26
CD at 0.05 probability level				1.546	1.792										

CI=*C. inerme*, CV=*C. viscosum*.

Fig. 1. Plant extracts of *C. inerme* and *C. viscosum* treated mitotic cells of *A. cepa* showing cytological normal (A, B) and abnormalities (C–I) at metaphase (A, C–E), anaphase to telophase (B, F, G) and resting (H–I) stages. A) Normal metaphase. B) Normal anaphase. C) Ring (arrow). D, E) Polyploid cells with fragments (arrow). F) Laggards. G) Bridge. H) Trinucleate cell with condensed micronuclei. I) Giant cell. Scale bars=10 μ m.

and 1.0 $\times$, 2.39 to 7.14%). Mitotic cells from control set (Fig. 1A, B) of *A. cepa* ($2n=16$) show no abnormalities whereas extract-treated cells document clumping and stickiness of chromosomes, ring configuration of chromosomes (Fig. 1C), chromatin disorganization, polyploid cells with (Fig. 1D, E) or without fragments, laggards (Fig. 1F), bridges (Fig. 1G) and multipolar groups in dividing cells and micronuclei (Fig. 1H), giant cells (Fig. 1I) and tripolar groups in resting cells (Fig. 1H). Both types of the extract of either of the species induce the identical type of abnormalities. However, the potentiality of aqueous extract is higher than methanolic extract and extracts of *C. viscosum* is more effective than *C. inerme* in inducing aberrations in root cells of *A. cepa*. Thus, plant extracts seem to bring about the blockage of cells into mitosis and induce chromosomal aberrations. Aberration types suggest that plant extracts as alike to mutagenic agents may cause change of chromosome organization resulting in stickiness of chromosomes and chromatin disorganization (Gaulden 1987) apart from inducing chromosomal breakages which form rings,

bridges, fragments and micronuclei, affecting spindle apparatus resulting to formation of polyploid cells, multipolarity and laggards and causing cellular metabolic disturbances (Ghosh *et al.* 2017) resulting in failure of cytokinesis and giant cell formation.

Cytological aberrations are not detected in any treatment of methanolic extracts for both the species of *Clerodendrum* excepting 0.5 $\times$ and 1.0 $\times$ extracts of *C. viscosum*. The aberrations encountered are alike to those that are studied in aqueous extracts of either of the species. Results, therefore, signify that methanolic extracts may be more useful for therapeutic studies due to lack of chromosomal aberrations in most of the administered doses.

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