Effectivity of Plant Aqueous Extracts of *Curcuma longa* and *Nigella sativa* in Cancer Therapy through Ameliorating Colchicine Induced Polyploid Cells in *Allium* as Test System

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Summary Polyploidy is a typical trait in human cells ($2n=46$) in cancer diseases. In the present investigation, polyploid cells ($2n>16$) due to erroneous division of centromere at metaphase are induced in root tip meristem of *Allium cepa* following treatments (0.25%-72 h, 0.50%-48 h, and 1.00%-24 h) of colchicine. The objective of the work is to foresee whether or not the induced ploidy level can be ameliorated by administering the doses of aqueous extracts of *Curcuma longa* rhizome and *Nigella sativa* seeds. Cisplatin at its pharmaceutical dose (1 mg mL^{-1}) is used as a positive control in the study. Results show the formation of enhanced polyploid cell frequency in the root meristem of *A. cepa* in a dose-dependent manner and effectivity of the aqueous plant extracts in ameliorating the polyploid frequency is differential. Cisplatin demonstrates total inhibition of cell division and apoptosis of cells. From the results, it can be inferred that the plant resources having ethnomedicinal properties could be potential in cancer treatment if the selection of appropriate dose(s) could be made. Thus, the present investigation is significant in suggesting the further exploration of ethnomedicinal resources in the development of low cost, readily available, household treating agents in cancer therapy through proper scientific intervention followed by a clinical trial.

Keywords Polyploid cell, Mitotic index, Aqueous plant extract, Amelioration, Apoptosis.

Cancer, a group of diseases, can be developed in most of the tissue systems in the human body resulting in uncontrolled cell growth due to abnormal mitotic division followed by metastatic invasion to adjacent as well as distant organs of the patient (Obenauf and Massagué 2015). World Health Organization (2019; <https://www.who.int/health-topics/cancer>) reported cancer as the second major medical threat to human civilization accounting for approximately 9.6 million deaths globally in 2018. Among the vast pool of clinically reported cancer types, genomic instability serves to prove as one of the common characteristic hallmarks of cancer cells through the development of different types of ploidy in tumorigenic cells (Duensing and Duensing 2010). The catastrophic mitotic event promoted by centrosome malfunctioning coupled with spindle fiber disorganization is reported to be one of the major causes of ploidy level increment in the cancerous cell (Salisbury *et al.* 1999).

In light of characteristic spindle disarrangement and polyploid content of cancer cells, the present investigation is designed to develop colchicine induced polyploid cells in *A. cepa* root tip mimicking malignant cells at

first and then to study possible ameliorative potentialities of traditionally used two natural resources (*C. longa* rhizome and *N. sativa* seed extracts) having immense therapeutic properties (Datta *et al.* 2012, Sundar *et al.* 2018). *A. cepa* bioassay is reported as a convenient and reliable test system corroborating mammalian systems to study chromosomal damages and disturbances in the mitotic cycle (Leme and Marin-Morales 2009). The objective of the present study is to explore the polyploid frequency reduction potentiality, if any, by administering the *C. longa* rhizome and *N. sativa* seed extracts. This may open up a new dimension in this field of cancer research by the expansion of ethnomedicinal perspectives towards the development of an easily accessible, cost-effective natural treatment of neoplasia. For the study, cisplatin, a well-known anticancerous drug has been used as a positive control.

Materials and methods

Preparation of chemicals and plant extracts

Cisplatin or *cis*-diamminedichloroplatinum (II) (Cipla, India), well-known platinum-based anti-neoplastic drug, is used as a positive control in the present study. Working concentration of cisplatin is prepared using

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ready-to-inject stock (1 mg mL^{-1}) following dilution with ddH_2O .

A stock solution of colchicine (1 g in 100 mL ddH_2O ; 1.00%) was made by dissolving the colchicine powder in ddH_2O followed by storage in dark for future use. Working concentrations (0.25 , 0.50 , and 1.00%) were prepared following serial dilutions of stock solution using ddH_2O .

Plant samples (rhizome of *C. longa* and mature seeds of *N. sativa* purchased from local market) of 1 g each were thoroughly surface cleaned with ddH_2O , soaked in blotting paper to remove excess surface water, and were separately homogenized in two sterilized mortar-pestles for 15 min . in a maintained laboratory environment ($22 \pm 1^\circ\text{C}$). In the homogenates, ddH_2O (100 mL for each) were added to make the plant extract solutions (1.00%). Before application, *C. longa* and *N. sativa* extract solutions were filtered using Whatman No. 1 papers and were marked as CL and NE respectively.

Treatments

Germinated *A. cepa* var. *aggregatum* bulbs (sprout-

ing made in 1:1 sand-saw dust trays) were dipped in test concentrations (three sets designed for each treatment) of colchicine (12 bulbs in each) for 24 h (1.00% colchicine), 48 h (0.50%) and 72 h (0.25%). Treated bulbs from each treatment were then transferred to ameliorating solutions of cisplatin, CE, NE, and to ddH_2O (three bulbs each) and were kept for 24 h . After completion of the duration, six roots (two roots from each bulb) from each ameliorating solution and ddH_2O were cut, fixed in acetic-ethanol ($1:1$) for 30 min and refrigerated (4°C) in 70% ethanol for further study. Control sets using ddH_2O were maintained ($21 \pm 1^\circ\text{C}$) simultaneously under uniform laboratory condition(s).

Cytological assessment

Root tip squash preparations were performed from treated and control roots following 2% aceto-orcein: 1 M HCl ($9:1$) staining and subsequent squashing in 45% acetic acid for cytological observations to assess variations between/among the different set of treatments. The prepared slides (triplicate for each treatment) were analyzed under a Leitz Laborlux S compound microscope

Fig. 1. Root tip mitosis of *A. cepa* showing cells with chromosomes from untreated control (a) and different colchicine-plant extract treatments (b–j) at metaphase (a–i) and anaphase (j) and cells in resting stage (k–l) and in apoptotic phases (m–p). a) Control showing $2n=16$. b–c) Cells showing the separation of chromatids at metaphase. d–i) Colchicine induced a varied degree of polyploid cells. h) Fragments (arrow) under col 1.00% - 24 h +CE. j) Bridge (col 1.00% - 24 h). k) Micro-nucleus (arrow; col 0.50% - 48 h +NE). l) Giant cell (col 0.25% - 72 h). m–p) Cells (cisplatin: m, o, p—sticky chromatin and chromatin mass degeneration, pseudo-compartmentalization, membrane shrinkage; col 1.00% - 24 h +NE: n—arrow marked, differential chromatin condensation with nuclear disorganization) in apoptotic phases. Scale bars= $10 \mu\text{m}$.

with a Leica E3 CCD camera.

Mitotic index [MI=(No. of dividing cells/No. of total cells scored)×100] was estimated from randomly taken 50 microscopic fields. Frequency (percent of total no. of cells scored) of polyploidy cells were scored based on the enhanced chromosome number in comparison to control (ddH₂O) scattered metaphase plate. Frequency of chromosomal aberrations (fragments and bridges; percent of total no. of dividing cells scored) and giant cells (percent of total no. of resting cells scored) were also studied. Suitable preparations were microphotographed.

Statistical analyses

Data scored from MI, frequency of polyploid cells, chromosomal aberrations, and giant cells in treatments with controls were statistically analyzed using one-way ANOVA (analysis of variance) followed by computation of CD (critical difference) at 0.05 probability level. Percentage (%) of amelioration of the treating drug (cisplatin) and CE and NE were estimated over the colchicine treatment data.

Results and discussion

Formation of polyploid cell

Mitotic cells in untreated control of test plant *A. cepa* root meristematic cells show $2n=16$ chromosomes (Fig. 1a) always. Induced treatments of colchicine in different concentrations with varying durations (1.00%-24 h, 0.50%-48 h and 0.25%-72 h) in the *A. cepa* root tip cells show polyploid cell frequency with enhanced chromosome numbers (>16; Fig. 1b–i). Polyploid cell is found to increase significantly ($p<0.05$) in a dose-dependent

manner (0.25%-72 h–3.28%, 0.50%-48 h–3.35%, 1.00%-24 h–7.91%) (Table 1). High colchicine concentration (1.00%) combining with shorter (24 h) treatment duration exhibits more pronounced induced autopolyploidization compared to the sets comprising lower concentration–longer treatment durations (0.25%-72 h and 0.50%-48 h). Sarathum *et al.* (2010) also reported enhancement in colchicine effectivity with increased working concentrations.

Subsequent treatments of the colchicine induced polyploid cells separately with aqueous plant extracts (1%) of *N. sativa* and *C. longa* for amelioration purposes document 3 to 4 folds upliftment in polyploidy cell frequencies as compared to the values noted in the cells induced by colchicine only particularly in the cells treated with 0.25%-72 h and 0.50%-48 h doses (Table 1). This additive effect of *N. sativa* and *C. longa* extracts in polyploidy cell formation may be attributed to the inability of the plant decoctions in ameliorating the chaotic cytoplasmic environment imposed due to longer durations (48 and 72 h) of colchicine treatments under arrested mitosis at the metaphase-anaphase junction. Polyploid cell formation due to *Curcuma* polyphenol (curcumin) treatments to several types of human cancerous cells (Wolanin *et al.* 2006, Banerjee *et al.* 2010, Zhang *et al.* 2017) are reported due to the downregulation of MAD2 (an essential component of mitotic spindle checkpoints and chromosome segregation—Prencipe *et al.* 2010) and reduced expression of CPC (chromosome passenger complex, essential for mitotic progression—Wolanin *et al.* 2006, Sharif *et al.* 2010) resulting in defective sister chromatid separation and disturbances in cytokinesis. Therefore, by enhancing polyploid frequency in the present in-

Table 1. Mitotic consequences of colchicine induced root apical meristems of *A. cepa* and its amelioration by plant aqueous extracts of *C. longa* and *N. sativa*.

Treatments	Total cells scored	Total no. of dividing cells	Pro-Meta	Ana-Telo	Mitotic index (%)	Frequency of polyploid cells (%)	Frequency (%)		Total no. of resting cells	Giant cells (%)
							Fragments	Bridges		
Control	1545	208	150	58	13.46	0.00	0.00	0.00	1337	0.00
Col 0.25%-72 h	1491	132	132	0	8.85	3.28	0.00	0.00	1359	2.21
Col 0.25%-72 h+NE	994	164	163	1	16.49	7.24	1.83	0.00	830	1.08
Col 0.25%-72 h+CE	746	107	107	0	14.34	12.60	0.93	1.87	639	3.13
						All cells are apoptotic				
CD at 5%					0.92	1.22				0.23
Col 0.50%-48 h	1164	132	132	0	11.34	3.35	0.00	0.00	1032	2.81
Col 0.50%-48 h+NE	979	181	181	0	18.49	10.11	2.21	2.21	798	1.75
Col 0.50%-48 h+CE	1081	139	139	0	12.86	10.91	1.44	0.00	942	1.48
						All cells are apoptotic				
CD at 5%					0.86	1.36				0.27
Col 1.00%-24 h	796	118	118	0	14.82	7.91	0.00	1.69	678	3.24
Col 1.00%-24 h+NE	1199	181	181	0	15.09	4.50	1.10	0.00	1018	0.20
Col 1.00%-24 h+CE	1581	190	188	2	12.02	3.23	1.58	0.00	1391	0.58
						All cells are apoptotic				
CD at 5%					0.88	0.72				0.13

col: Colchicine; NE: *N. sativa* seed extract; CE: *C. longa* rhizome extract; Cis: cisplatin.

vestigation, plant extracts possibly activate the ploidy-specific abortive cell cycle resulting in the triggering of cellular apoptosis. Different modes of cell mortality namely apoptosis, mitotic catastrophe, and autophagy (Wilson and Bedford 2010) in repairable cells induced by curcumin present in *C. longa* rhizome are observed by Aoki *et al.* (2007). Magalska *et al.* (2006) reported a subcellular decrease in survivin (a eukaryotic protein essential for cell division and inhibitor of cell death—Sally and Dario 2019) induced by *Curcuma* polyphenol is correlated with aberrant mitosis leading to increased DNA content, mitotic catastrophe, and cell death.

Surprisingly in the present study, an opposite trend regarding polyploid frequency is recorded in 1.00%-24 h colchicine (7.91%) demonstrating significant ($p < 0.05$) reduction in polyploidy cell formation in both *Nigella* (4.50%) and *Curcuma* (3.23%) extracts after colchicine exposures. The reductive effect of both the plant extracts in polyploidy cell formation suggests amelioration of cells with increased DNA content by NE and CE rather than extermination of damaged cells by apoptotic pathways. Shorter duration (24 h—one cell cycle event) exposure of colchicine to root tip cells possibly leads to ploidy increment and disturbed mitotic events but, falls within the limit of restoration potentiality and protective ability of the plant extracts. Sikora-Polaczek *et al.* (2011) reported that curcumin prohibits p53 deficient cells from entering the tetraploid state following upregulation of the p53 transcription factor. Talib *et al.* (2018) documented the upregulation of p53 and p21 promoted by curcumin is associated with G₂-M blockage which possibly inhibits the cells from entering aberrant mitosis caused by colchicine induced microtubular deformation and dysfunction.

Results of the present study related to the polyploid cell frequency data suggest preferential activation of bimodal amelioration mechanism of the plant extracts against colchicine induced mitotic disturbances—a) in prolonged duration of colchicine exposure (48 and 72 h—multiple cell cycle event), enhancement in polyploid cell formation possibly leads to the apoptotic exclusion of damaged cells possessing beyond recoverable chaotic mitotic event; b) in a shorter duration (24 h—single cell cycle event) of colchicine (1.00%) exposure, restoration of the cellular environment from enhanced ploidy contentment resulting in a significant reduction in polyploid cell frequency. Comparative assessment between two plant extracts, CE is found to be more efficient in both ploidy-specific apoptotic termination and ameliorative reduction-restoration of polyploid cells than NE.

Mitotic index

MI recorded in untreated control and test materials is presented in Table 1. In relation to control (13.46%), significant ($p < 0.05$) mitodepressive effect is

documented in 0.25%-72 h (8.85%) and 0.50%-48 h (11.34%) colchicine (col.) treatments, whereas MI value has been increased significantly in 1.00%-24 h colchicine (14.82%) suggesting opposite trends of cell cycle progression prevailing between higher concentration (1.00%)—shorter duration (24 h) and lower concentrations (0.25 and 0.50%)—prolonged durations (48 and 72 h) of colchicine treatments. In comparison to colchicine treatment, significant ($p < 0.05$) increments in dividing cell frequency are observed in both the plant extracts (NE—16.49%, CE—14.34% followed by col. 0.25%-72 h—8.85%; NE—18.49%, CE—12.86% followed by col. 0.50%-48 h—11.34%; and NE—15.09% followed by col. 1.00%-24 h—14.82%), excepting CE (12.02%) after colchicine 1.00%-24 h treatment. Enhancement in mitotic index value by the plant extracts possibly suggests the inductive effect of NE and CE on the faster progression of interphase cells towards divisional events to overcome blockage of growth by arrested cells for maintenance of meristematic extension in the root tip region. Upliftment in the mitotic index value by *Curcuma* polyphenol is reported due to the upregulation of cyclin B1 and prolonged dephosphorylation of cyclin B1/Cdk 1 complex (Wolanin *et al.* 2006).

In the present study, cell count data of different mitotic substages in treatments exhibit cell frequency increment in pro-metaphase event accounting for enhancement in the mitotic index followed by complete inhibition of ana-telophase event resulting in blockage of the cell cycle at the metaphase point of cell division. Mitotic disruption at the metaphase–anaphase junction is possibly attributed to curcumin mediated inhibition in cyclin B1 and downregulation and dysfunction of the anaphase-promoting complex (Mosieniak *et al.* 2012). Comparative analysis of NE and CE treatments along the line of MI documents a more pronounced effect on divisional frequency enhancement by NE than CE.

Cytotoxicity

Cytotoxicity induced by the test materials is depicted in the form of chromosomal fragments (Fig. 1h) and bridges (Fig. 1j) in divisional stages and formation of occasional micronucleus (Fig. 1k) and giant cells (Fig. 1l) in the resting stage (Table 1).

No cellular abnormality is encountered in the untreated control. Chromosomal fragments and bridges are absent in all the colchicine treatment excepting 1.00%-24 h (bridge—1.69%). Fragments (Table 1) are recorded only in plant extracts treatments with a maximum recorded frequency 2.21% (in NE after col 0.50%-48 h) and minimum—0.93% (in CE after col 0.25%-72 h). The appearance of bridges studied sporadically in CE (1.87%, after col 0.25%-72 h) and NE (2.21%, after col 0.50%-48 h). A comparative study between the colchicine and phytoextracts suggests the formation of chromosomal fragments as the outcome of plant extract treatments to root tip

mitosis. The appearance of chromosomal fragments is significant in the context of tissue reviving mechanism following the exclusion of damaged cells (Stevens *et al.* 2007). In the present study, fragment generation by plant extract treatments can act as predisposing factor signaling at the onset of cellular apoptosis. Aqueous extracts of *N. sativa* seeds and *C. longa* rhizome are reported to possess reactive oxygen species (ROS) generation potentiality (Thayyullathil *et al.* 2008) in both dividing and resting cells resulting induction of oxidative damage to the cells with enhanced chromosomes content and subsequent generation of chromosomal fragments which can help in apoptotic switching of polyploid cells.

Giant cells (cells with larger dimensions in comparison to untreated control cells in mitotic squash preparations) in the resting stage are the possible outcome of physiological defects accumulated due to disturbed mitosis (Hayashi *et al.* 2013) under stress environment imposed by the treating agents. Such cells are recorded dose-dependently in colchicine treatments (2.21% in col 0.25%-72 h to 3.24% in 1.00%-24 h) and the reduced frequencies are observed in plant extract treatments (excepting CE after col 0.25%-72 h) with maximum amelioration estimated in NE (16.20 fold decrement) followed by CE (5.58 fold) after col 1.00%-24 h. Decrement in the appearance of giant cells significantly in plant extract treatments possibly indicates the restitution of the sham-bolic subcellular environment after colchicine treatments. In this context, it is worth mentioning that plant extract treatments following col 1.00%-24 h also show a significant reduction in polyploidy cell frequency in line with amelioration of giant cell formation suggesting activation of protective effects of phytoextracts.

Cisplatin (a chemotherapeutic drug widely used in the treatment of malignant tumors particularly in testicular and ovarian metastasis—Jordana and Carmo—Fonseca 2000) treatments in root tip cells pre-exposed to colchicine (all three treating concentrations-durations) exhibit utterly all cells in the apoptotic phase (Fig. 1m–p) as evinced from the disintegration of the nucleus indicating rapid and broad-spectrum (irrespective of polyploid, non-polyploid state) antineoplastic mechanism of action. Shen *et al.* (2012) reported that cellular apoptosis triggered by cisplatin is attributed to non-selective inhibition of DNA synthesis and G₂-cell cycle arrests both in cancerous and healthy cells.

By combining all the results explored in the present study, it can be inferred that

- A. polyploid cells induced by colchicine treatments in a dose-dependent manner in the *Allium* test system;
- B. doses of aqueous plant extracts (*C. longa* rhizome and *N. sativa* seed) are found to be effective as ameliorating agents though variably by reducing the frequency of induced polyploid cells;
- C. reduction ability of polyploid frequency follows the restoration of chromosomal content in the root

tip cells especially treated with shorter duration (24 h) of colchicine (1.00%);

- D. significant mitoinpressive property resulting in a significant increment in pro-metaphase cell frequency;
- E. chromosomal fragments generation ability indicating genotoxicity mediated apoptotic switching of damaged cells;
- F. formation of giant cells suggesting the restoration of the cellular environment from a chaotic physiological state;
- G. ameliorative (in the form of apoptosis) progression in plant extract treatments takes place substantially at a slower rate as compared to anticancerous drug cisplatin (apoptotic cell frequency-100%).
- H. the actions of ameliorating potentiality by plant aqueous extracts exhibit selective (mostly on dividing polyploid cells) rather than the broad-spectrum mass killing of cells as followed by chemotherapeutic drug cisplatin.

Therefore, the results of the present study indicate that the potentiality to use the ethnomedicinal properties of the plant resources in the development of low cost, readily available, household treating agent in cancer therapy through proper scientific intervention followed by a clinical trial.

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