Applied Biological Research 24(3): 393-398; (2022) **SHORT COMMUNICATION**
DOI: 10.5958/0974-4517.2022.00044.1

GENOTOXIC EFFICACY OF FRUIT EXTRACT OF *Cestrum diurnum* USING *Allium cepa* ASSAY

Sumanta Chakraborty

Department of Zoology, Sreegopal Banerjee College, Hooghly - 712 148, West Bengal (India)
e-mail: *sgzoostat@gmail.com*

(Received 24 March, 2022; accepted 31 July, 2022)

ABSTRACT

Onion *(Allium cepa)* root apical meristem cells were treated, for various exposure time, with various concentrations (0.1-0.4%) of fruit extract of *Cestrum diurnum* so as to assess its cyto-genotoxic potential. The treated root tips were fixed, stained and squashed; and mitotic anomalies observed under a light microscope. The micro-nuclei (MN) were observed at interphase and MN percentage calculated. The fluctuations in mitotic index (MI) and abnormality percentage were statistically co-related through ANOVA analyses with exposure periods and concentrations as random variables. Phytochemical analyses of fruit extract of *C. diurnum* were performed using standard methods. The MI was significantly reduced by all the treatments. Increase in concentration and exposure time significantly increased the anomaly percentage and frequency of MN induction as compared to the control. Phytochemical analysis revealed the presence of alkaloids, terpenoids, phenolic compounds, tannins, phytosterols, volatile oils, and saponins as secondary metabolites. *C. diurnum* fruit extract showed promising cyto-genotoxic effects on *A. cepa* root apical meristem cells. However, caution is essential for the safe administration of herbal preparations.

Keywords: *Allium cepa*, *Cestrum diurnum*, genotoxicity, micronucleus, mitotic index

INTRODUCTION

Medicinal plant usage has been a part of human culture from ancient times. The World Health Organization has estimated that 80% world population relies on habitual medicine (Smith-Hall *et al*., 2012). The practice based on habitual medication is generally crude extracts in water, alcohol, distillates, or essential oils, containing multiple secondary metabolites (SMs) with different structural groups, and their effectiveness is frequently dependent on the synergistic interactions of SMs present (Mulyaningsih *et al*., 2010; Eid *et al*., 2012). The apparent broad-spectrum activity of concoctions used in traditional medicine has been ascribed to the phenolic compounds and polysaccharides (Wink, 2015). At high concentrations, the secondary metabolites alter membrane fluidity and enhance permeability. As a result, many lipophilic secondary metabolites (particularly those found in essential oils) exhibit antimicrobial and cytotoxic properties (van Wyk and Wink, 2015). Standard plant assays can be used to explore the cyto-genotoxic effects of chemicals in live systems by assessing MI and phase, chromosomal aberrations, and the proportion of MN in somatic cells (Singh *et al*., 2008). Onion (*Allium cepa*) is the most frequently used plant to assess the potential cytotoxicity due to its easy availability, quick processing, and accurate results (Verma *et al*. 2016; de Souza *et al*. 2017). Chemicals that generate chromosomal aberration in plant cells also cause chromosomal aberration in cultured animal cells (Grant, 1978; Ma *et al*., 1994).

The genus *Cestrum* (family Solanaceae) has about 300 species and is widely dispersed across the world. *Cestrum diurnum* L. is a single- or multi-stemmed shrub commonly known as 'Day Jasmine'. The herb is used in folk medicine for a variety of purposes (Mello, 2003). Many herbal extracts have been explored for potential cytotoxicity and mutagenic potential (Feretti *et al*., 2007; Chaudhuri and Ray, 2015; Barman *et al*., 2020). However, no information is available on the genotoxic potential of *C. diurnum*. The present study was aimed to assess the potential cytotoxicity (based on mitotic index and certain nuclear abnormalities) and mutagenic potential (based on the detection of micronucleated cells) of phytoextract of *C. diurnum* in somatic cells of onion (*A. cepa*).

MATERIALS AND METHODS

Fruit collection and extraction

Healthy and green fruits of *C. diurnum* were collected from the outskirts of Bandel, West Bengal, India. The plant was recognized taxonomically, and a voucher specimen (GCSC05) herbarized in the Department of Zoology, Sreegopal Banerjee College, Mogra, West Bengal, India (latitude 22°59' North and longitude 88°22' East) for future reference. Freshly harvested fruits of *C. diurnum* were cleaned in running tap water, followed by distilled water, and then soaked in paper towels. Fresh extract was made by crushing the fruits (50 g) in a mortar and pestle. The contents were filtered through Whatman No. 1 filter paper and the filtrate maintained as stock solution (100% concentration) for future assays. After a preliminary toxicity study on *A. cepa* root tips with stock solution, four concentrations (0.1, 0.2, 0.3 and 0.4%) of extract were prepared by diluting the stock solution with sterilized distilled water for the *Allium cepa* assay.

Allium cepa assay

The present experiment was conducted in the Research Laboratory, Department of Zoology, Sreegopal Banerjee College, Hooghly, West Bengal (India). The *Allium cepa* test for genotoxicity was conducted according to the method of Rank and Nielsen (1994) with slight modifications. Small, healthy onion bulbs (2n = 16) of 1–2 cm dia. were purchased from a local market. Dried roots were removed from the base of stem disc with the help of a sharp blade; the outer scales of bulbs were removed without damaging the root primordia. The bulbs were planted in test tubes (10 mL) containing sterilized distilled water in such a way that only root primordia were immersed in the sterilized distilled water and allowed to sprout in darkness (for 48 h) in a culture room maintained at 25±1ºC. The germinated bulbs with healthy roots (1-2 cm) were transferred to test tubes (10 mL), between 9 a.m. and 10 a.m. (during peak mitotic phase), in such a way that only roots were immersed in the test concentration of *C. diurnum* extract (10 mL). To account for photosensitive nature of plant extracts and to reduce variations in cell division, the tests were conducted at 25±1ºC in dark. A control group, which did not receive any treatment, was kept in sterilized distilled water alongside the treatment groups. Each treatment was replicated five times in a completely randomized design, maintaining 5 roots per bulb and inspected at hourly interval up to 4 h. For MN assay, the roots were allowed to grow for 6, 12 and 24 h at the test doses (0.2, 0.3, and 0.5%) and inspected accordingly.

Preparation of mitotic phases

For mitotic squash preparation the improved technique of Sharma and Sharma (1980) was followed. Root tips (treated and untreated) were cut from the samples at 1, 2, 3, and 4 h interval, then rinsed in distilled water and promptly fixed in Carnoy's fixative for 24 h. The root tips are hydrolyzed for 10 min in 1 N HCl at 60ºC and stained with staining mixture (2% aceto-orcein) for 45 min. After staining, the meristematic regions were placed on clean slides in a drop of 45% acetic acid, squashed under a cover glass, and examined at 40X magnification using a bright field microscope (Make: Carl Zeiss, Model: Primo Star).

Phytochemical analysis

The phytochemical analyses of green fruit of *C. diurnum* were carried out using standard procedures (Harborne, 1984) for the presence of secondary metabolites like alkaloids, terpenoids, phenolic compounds, flavonoids, tannins, phytosterols, volatile oils, and saponins

Statistical analyses

ANOVA was performed for MI and abnormality percentage data, followed by Tukey's HSD test (Tukey *et al*., 1985; Benjamini and John, 2002). Results with $P < 0.05$ were considered statistically significant. Data analysis was performed by using the computer software "MS Excel 2003" and SPSS Statistical Software Package version 16.0 (SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, SPSS Inc.).

RESULTS AND DISCUSSION

Drugs of plant origin are extremely important in safe treatment of human illnesses. The therapeutic value of a plant is attributed to its presence of chemical components having medicinal characteristics (Prabhu *et al*., 2011). The phytochemicals like colchicine perform as spindle poisons and inhibit microtubule polymerization (Cocco *et al.*, 2010). The mitotic abnormalities due to the cytotoxicity and genotoxicity of aqueous extract of *C. diurnum* on onion root meristematic cells are given in Table 1. The MI showed decrease with increase in concentration and exposure time; while abnormality percentage showed increase with increase in concentration and exposure time. The two-way analysis of MI and abnormality percentage induced by *C. diurnum* fruit extract in response

Table 1: Mitotic index and abnormality percentage in *Allium cepa* cells exposed to concentrations of *Cestrum diurnum* fruit extract

Concentrations (%)	Exposure time (h)	Total cells in mitotic stages (count)	Total cells with chromosomal aberrations (Mean ± SE)	Mitotic index (MI) (Mean ± SE)	Percent abnormality ± SE
0.4	1	1,384	143.8 ± 3.63	55.3 ± 1.53**	52.1 ± 1.83**
	2	1,374	147.4 ± 4.04	54.9 ± 1.59**	53.8 ± 2.44**
	3	1,358	149.8 ± 3.73	54.3 ± 1.67**	55.3 ± 2.10**
	4	1,189	157.0 ± 2.30	47.5 ± 0.71**	66.7 ± 3.25**
0.3	1	1,457	127.2 ± 3.72	58.2 ± 1.19**	43.7 ± 1.49**
	2	1,442	132.0 ± 5.57	57.6 ± 1.26*	45.8 ± 2.03**
	3	1,426	133.8 ± 3.32	57.0 ± 1.31*	47.1 ± 2.05**
	4	1,406	136.6 ± 3.09	56.2 ± 1.41**	48.6 ± 1.27**
0.2	1	1,527	106.4 ± 3.38	61.0 ± 1.24**	34.8 ± 1.19**
	2	1,515	110.6 ± 3.58	60.6 ± 1.23**	36.6 ± 1.68**
	3	1,499	112.2 ± 4.37	59.9 ± 1.27**	37.5 ± 2.04**
	4	1,478	118.6 ± 3.69	59.1 ± 1.17**	39.6 ± 2.00**
0.1	1	1,623	87.4 ± 2.01	64.9 ± 1.21**	26.9 ± 0.85**
	2	1,609	91.4 ± 3.96	64.3 ± 1.19**	28.3 ± 1.00**
	3	1,589	97.4 ± 3.23	63.4 ± 1.12**	30.7 ± 1.42**
	4	1,565	101.6 ± 2.94	62.6 ± 1.19**	32.5 ± 1.49**
Control	1	1,906	0.0 ± 0.00	76.2 ± 0.64	0.0 ± 0.00
	2	1,729	0.0 ± 0.00	69.1 ± 0.97	0.0 ± 0.00
	3	1,701	0.0 ± 0.00	68.0 ± 1.01	0.0 ± 0.00
	4	1,678	0.0 ± 0.00	67.1 ± 0.99	0.0 ± 0.00

*, Significant at p<0.01, **, Significant at p<0.001 when compared with their respective control as determined by Tukey's HSD test, SE= standard error; Total cells observed were 2500 in each case

Table 2: Two-way analysis of mitotic index induced by *Cestrum diurnum* fruit extract with concentration (C) and exposure time (E) as two random variables

Source of variation	df	Sum of squares	Mean sum of squares	*F* value†
C	4	3380.714	845.179	112.648**
E	3	278.024	92.675	12.352**
C × E	12	221.954	18.496	2.465*
Residual	80	600.224	7.503	
Total	99	4480.916		

†Level of significance is 0.05,*, P<0.05, **, P<0.001, df =degrees of freedom

Table 3: Two-way analysis of abnormality percentage induced by *Cestrum diurnum* fruit extract with concentration (C) and exposure time (E) as two random variables

Source of variation	df	Sum of squares	Mean sum of squares	*F* value†
(C)	4	37319.769	9329.942	678.336**
(E)	3	490.451	163.484	11.886**
(C)×(E)	12	385.492	32.124	2.336*
Residual	80	1100.333	13.754	
Total	99	39296.044		

†, Level of significance is 0.05, *, P<0.05, **, P<0.001, df =degrees of freedom

Table 4: Efficacy of fruit extract of *Cestrum diurnum* on micronucleus (MN) assay

Concentrations (%)	Exposure (h)	Cells with micronucleus (%±SE)
0.4	6	0.37±0.02*
	12	0.44±0.02*
	24	0.57±0.02*
0.3	6	0.33±0.02*
	12	0.36±0.03*
	24	0.40±0.01*
0.2	6	0.32±0.03*
	12	0.34±0.02*
	24	0.37±0.01*
Control	6	0.00±0.00
	12	0.00±0.00
	24	0.00±0.00

*, Significant at p<0.01, when compared with their respective control as determined by Tukey's HSD test
Total cells observed were 2500 in each case

to different concentrations and increasing concentrations and exposure time. exposure time as two independent variables are given in Table 2 and 3, respectively. The individual factors and all the possible factor combinations were found significantly related to the MI and abnormality percentage (at P<0.05, P<0.001) when compared with control treatment. MI is used as a biological signal of adequate cell increment (Gadano *et al*., 2002), which gives the ratio of cells in various mitotic phases and the frequency of cellular division (Fachinetto *et al*., 2007). The reduction in MI may be the consequence of cells not advancing from the synthesis phase of cell cycle or because of the blocked DNA synthesis (Karaismailoglu, 2015). Reduction in mitotic activity in any cell is often taken as an indicator of its exposure to genotoxic agents.

The results of MN test in onion root tips exposed to the test concentrations of *C. diurnum* are given in Table 4. The frequency of MN induction was found time- and dose-dependent and was significantly distinct in all treatments in comparison to the control (p<0.05). MN assays have a key role in the evaluation of toxicity influences (Karaismailoglu, 2014a). MN may originate from a lagging chromosome at anaphase or from a chromosome fragment (Badr and Ibrahim, 1987), which may lead to the loss of genetic material and has been regarded as an indication of mutagenicity of their inducers (Ruan *et al*., 1992). These bridges are usually formed by joined the sister chromatids that stay together until late anaphase or telophase (Gömürgen, 2005).

The qualitative phytochemical screening of fruit extract of *C. diurnum* revealed the presence of various secondary metabolites *viz*., alkaloids, terpenoids, phenolic compounds, tannin, phytosterols, volatile oils, and saponin. The saponins have been reported to possess antiproliferative and anti-cancer activities (Kim *et al*., 2016). This indicates that a significant reduction in MI of onion root tips may be due to the individual or combined effects of secondary metabolites. The studies on toxicity and mutagenicity of botanicals help in the verification of their efficacy and safety before their use (Ferreira *et al*., 2009). Consequently, the cytotoxic menace of *C. diurnum* in *onion* emphasizes its judicious use in the form of drugs, while

the genotoxic and mito-depressive effects of extract can be targeted to destroy the cancer cells. It may be concluded that *C. diurnum* fruit extract induces cytotoxic effects at higher concentrations, and careful screening of this plant is required for its safe administration in the form of herbal preparations.

Acknowledgements: The author is grateful to the Principal of Sreegopal Banerjee College, Mogra, Hooghly, WB, India, for providing the laboratory space for the research and to Prof. A. Mukherjee for identifying the plant.

Conflict of interest statement: Authors declare that they do not have any competing interests.

REFERENCES

Badr, A. and Ibrahim, A.G. 1987. Effect of herbicide 'Glean' on mitosis, chromosomes and nucleic acids in *Allium cepa* and *Vicia faba* root tip meristems. *Cytologia*, **52**(2): 293-302.

Barman, M., Roy, S. and Ray, S. 2020. Colchicine like metaphase and cell cycle delay inducing effects of leaf aqueous extract of *Clerodendrum inerme* (L.) Gaertn. in *Allium cepa* root apical meristem cells. *Cytologia*, **85**: 197-201.

Benjamini, Y. and Braun, H. 2002. John W. Tukey's contributions to multiple comparisons. *The Annals of Statistics*, **30**(6): 1576-1594.

Chaudhuri, A. and Ray, S. 2015. Antiproliferative activity of phytochemicals present in aerial parts aqueous extract of *Ampelocissus latifolia* (Roxb.) Planch. on apical meristem cells. *International Journal of Pharma and BioSciences*, **6**: 99-108.

Cocco, G., Chu, D.C.C. and Pandolfi, S. 2010. Colchicine in clinical medicine. A guide for internists. *European Journal of Internal Medicine*, **21**(6): 503-508.

de Souza, R.B., de Souza, C.P., Bueno, O.C. and Fontanetti, C.S. 2017. Genotoxicity evaluation of two metallic-insecticides using *Allium cepa* and *Tradescantia pallida*: A new alternative against leaf-cutting ants. *Chemosphere*, **168**: 1093-1099.

Eid, S.Y., El-Readi, M.Z. and Wink, M. 2012. Synergism of three-drug combinations of sanguinarine and other plant secondary metabolites with digitonin and doxorubicin in multi-drug resistant cancer cells. *Phytomedicine*, **19**(14): 1288-1297.

Fachinetto, J.M., Bagatini, M.D, Silva, A.C.F. and Tedesco, S.B. 2007. Cytotoxic effects of infusions (tea) of Solidago microglossa DC. (Asteraceae) on the cell cycle of *Allium cepa*. *Brazilian Journal of Pharmacognosy*, **19**(2B): 632-636.

Feretti, D., Zerbini, I., Zani, C., Ceretti, E., Moretti, M. and Monarca, S. 2007. *Allium cepa* chromosome aberration and micronucleus tests applied to study genotoxicity of extracts from pesticide-treated vegetables and grapes. *Food Additives and Contaminants*, **24**(6): 561-72.

Ferreira, F.G., Regasini, L.O., Oliveira, A.M., Campos, J.A.D.B., Silva, D.H.S., Cavalheiro, A.J., Santos, R.A., Bassi, C.L., Bolzani, V.S. and Soares, C.P. 2009. Evaluation of mutagenicity and antimutagenicity of different fractions of *Pterogynenitens* (Leguminosae), using *Tradescantia pallida* micronuclei assay. *Revista Brasileria Farmacognosia*, **19**: 61-67.

Gadano, A., Gurni, A., López, P., Ferraro, G. and Carballo, M. 2002. *In vitro* genotoxic evaluation of the medicinal plant *Chenopodium ambrosioides* L. *Journal of Ethonopharmacology*, **81**: 11-16.

Gömürgen, A.N. 2005. Cytological effect of potassium metabisulphite and potassium nitrate food preservative on root tips of *Allium cepa* L. *Cytologia*. **70**: 119-128.

Grant, W.F. 1978. Chromosome aberration in plants as monitoring system. *Environmental Health Perspective*, **27**: 37-43.

Harborne, J.B. 1984. *Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis*. Chapman and Hall, London, UK.

Karaismailoglu, M.C. 2014. Investigation of the cytotoxic and genotoxic effects of *Artemisia annua* methanol extract with the *Allium* test. *Ekoloji*, **23**(91): 64-74.

Karaismailoglu, M.C. 2015. Investigation of the potential toxic effects of prometryne herbicide on *Allium cepa* root tip cells with mitotic activity, chromosome aberration, micronucleus frequency, nuclear DNA amount and comet assay. *Caryologia*, **68**(4): 323-329.

Kim, K.M., Im A-R, Kim, S.H., Kim, S.H., Hyun, J.W. and Chae, S. 2016. Timosaponin AIII inhibits melanoma cell migration by suppressing COX-2 and *in vivo* tumor metastasis. *Cancer Science*, **107**: 181-188.

Ma, T.H., Cabrera, G.L., Cebulska-Wasilewska, A., Chen, R., Loarca, F., Vandererg, A.L. and Salamone, M.F. 1994. Tradescantia-stamen-hair-mutation bioassay. *Mutation Research*, **310**(2): 211-220.

Mello, J.R.B. 2003. Calcinosis - Calcinogenic plants. *Toxicon*, **41**(1): 1-12.

Mulyaningsih, S., Sporer, F., Zimmermann, S., Reichling, J. and Wink, M. 2010. Synergistic properties of the terpenoids aromadendrene and 1, 8 cineole from the essential oil of *Eucalyptus globulus* against antibiotic-susceptible and antibiotic-resistant pathogens. *Phytomedicine,* **17**(13):1061-1066.

Prabhu, K., Karar, P.K., Hemalatha, S. and Ponnudurai, K. 2011. Comparative micromorphological and phytochemical studies on the roots of three *Viburnum* (Caprifoliaceae) species. *Turkish Journal of Botany*, **35**: 663-670.

Rank, J. and Nielsen, M.H. 1994. Evaluation of the *Allium* anaphase-telophase test in relation to genotoxicity screening of industrial waste water. *Mutation Research,* **312**(1): 17-24.

Ruan, C., Lian, Y. and Linn, J. 1992. Application of micronucleus test in *Vicia faba* root tips in the rapid detection of mutagenic environmental pollutants. *Chinese Journal of Environmental Science*, 4: 56-58.

Sharma, A.K. and Sharma, A. 1980. *Chromosome Techniques - Theory and Practice*. Butterworths, London, UK.

Singh, P., Srivastava, A.K. and Singh, A.K. 2008. Cell cycle stage specific application of cypermethrin and carbendazim to assess the genotoxicity in somatic cells of *Hordeum vulgare* L. *Bulletin of Environmental Contamination and Toxicology,* **81**(3): 258-261.

Smith-Hall, C., Larsen, H.O. and Pouliot, M. 2012. People, plants and health: A conceptual framework for assessing changes in medicinal plant consumption. *Journal of Ethnobiology and Ethnomedicine*, **13**: 8-43.

Tukey, J.W., Ciminera, J.L., and Heyse, J.F. 1985. Testing the statistical certainty of a response to increasing doses of a drug, *Biometrics*, **41**: 295-301.

van Wyk, B.E. and Wink, M. 2015. *Phytomedicines, Herbal Drugs and Poisons*. Cambridge University Press, UK.

Verma, S., Arora, K. and Srivastava, A. 2016. Monitoring of genotoxic risks of nitrogen fertilizers by *Allium cepa* L. mitosis bioassay. *Caryologia*, **69**: 343-348.

Wink, M. 2015. Modes of action of herbal medicines and plant secondary metabolites. *Medicines*, **2**(3): 251-286.