INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

C-MITOTIC POTENTIAL OF AQUEOUS LEAF EXTRACT OF *MEMECYLON RANDERIANUM* SM & MR ALMEIDA.: A PROMISING NATURAL COLCHICINE ANALOG

P. R. Ramya Sree *[1] and J. E. Thoppil [2]
[1]Research scholar, Cell and Molecular Biology Division, Department of Botany, University of Calicut, Kerala, India
[2]Professor, Department of Botany, University of Calicut, Kerala. India
*Corresponding Author Email: ramyasreeprwynd@gmail.com

Article Received on: 08/09/18 Approved for publication: 29/10/18

DOI: 10.7897/2230-8407.0911269

ABSTRACT

Cytotoxic evaluation of *Memecylon randerianum* aqueous leaf extract on root tip cells of *Allium cepa* revealed its C-mitotic potential. Spindle formation was disturbed and abnormal mitotic stages were resulted during cytotoxic assay. Mitotic index decreased and abnormality percentage increased according to a dosage dependent way. The plant extracts induced major chromosomal aberrations that were apportioned to clastogenic and aneugenic viz., nuclear lesions, stickiness, multiple bridges, vagrance, chromosome laggards etc. Among the aneugenic aberrations, severe C-mitotic activity was observed at the prolonged exposure period (24 h) of highest concentration (0.1%), in which C-metaphase becomes the prominent one. Colchicine can induce the effects that interfere with spindle formation and keep the chromosomes in the metaphase plate at a seized condition. The highest percentage of mitotic division was found in the highest concentration at the prolonged exposure period, which strongly supports the presence of colchicine like compounds in the plant extracts. The C-mitotic activity of the plant extract of *M. randerianum* led to the exploration of a colchicine like compound, which can be exploited mainly in the field of plant breeding for inducing polyploidy. So this finding offers a natural colchicine analog that may be exploited in future.

Keywords: *M. randerianum,* Cytotoxicity, C-mitotic potential, colchicine, aneugenic aberration, C-metaphase.

INTRODUCTION

Colchicine is a natural alkaloid found in *Colchicum autumnale* that prevent spindle formation by muddling of the tubulin polymerization in to microtubules. It has also been obtained from *Gloriosa superba* of Lilliaceae and *Coleus forskohlii* of Lamiaceae[1, 2]. These can directly interfere with cell division and arrest mitotic spindle formation[3]. C-mitosis or colchicine mitosis is the interruption of spindle formation during mitosis. This mitotic spindle arrest causes various abnormalities in cell during mitosis viz., C-metaphase, C-anaphase, polyploidy, vagrance, cytostatic effect etc. In C-metaphase, a complete blockage of the chromosomes in the metaphase plate, and sometimes a partial blockage may occur. In C-anaphase, migrations of chromatids were prevented, and unequal separations of chromosome groups may occur as in vagrance formation[4]. A complete arrest of cell cycle will occur in the cytostatic condition. One of the useful effects of colchicine is, it can induce polyploidy, a reliable method in plant breeding for producing hybrid varieties[5, 6]. The therapeutic use of colchicine has been well documented in gout and Familial Mediterranean Fever (FMF). It has also been used in other diseases including Behcet's Disease (BD), pericarditis, coronary artery disease and other inflammatory and fibrotic conditions[7].

Plants showing the C-mitotic effect were reported earlier [8]. *Memecylon randerianum* SM & MR Almeida is a vigorous plant material in the Melastomataceae family having potential C-mitotic activity revealed during this study. So it can be used as a future perspective in the field of plant breeding and therapeutics. After colchicine treatments many hybrid varieties are now available in horticulture field[9, 10]. *M. randerianum* has many medicinal applications viz., flowers and twig decoction are used to treat skin diseases and chicken pox[11]. A paste of shoot tip is used to cure herpes and stomach disorders[12]. During the preliminary phytochemical analysis, the plant extract was found to contain several secondary metabolites like alkaloids, terpenoids, phenolics, coumarins etc. This may be the reason for the cytotoxic and C-mitotic effects of the plant extract.

Cytotoxicity studies of *M. randerianum* leaf extract on *A. cepa* was a preliminary step into its bioactivity, which may prove to be useful as a prerequisite for the development of a natural drug. The species *A. cepa* has been used as an efficient standard organism to run genetic tests for cytotoxicity, especially cytogenetic and chromosome aberration tests[13, 14]. The present study is an attempt on the cytotoxicity and C-mitotic potential of *M. randerianum* leaf extract, which is a novel report.

MATERIALS AND METHODS

Plant material

Memecylon randerianum leaf was collected from Pathanamthitta district, Kerala, India (9.1553°N, 76.4713°E). Taxonomic authentification of the plant materials was done by Dr A. K Pradeep, Assistant professor, Angiosperm Taxonomy Division, Department of Botany, University of Calicut, Kerala, India and voucher specimen (CALI no: 123775) was deposited in the Calicut University herbarium. The plant material was shade dried, powered and stored in sealed containers.

The test material, onion bulbs (*Allium cepa* L., 2n = 16), procured from Tamil Nadu, Agriculture University, that is free from agricultural pesticides and growth inhibitors were used for the present study.

Determination of period of peak mitotic activity

Healthy uniform sized bulbs of *A. cepa* were kept in autoclaved moist sandy soil for 2 days and allowed to produce roots. In order to find out the time of peak mitotic activity, the germinated untreated root tips of *A. cepa* were fixed in modified Carnoy's fluid (1 acetic acid: 3 alcohol) mixture at different times from 8.30 to 11 am. After many trials, it was found that maximum dividing cells (peak mitotic activity) occurred between 9 am and 10 am under normal sunshine conditions.

Preparation of aqueous plant extracts

Fresh aqueous plant extract was prepared by grinding 1g of powdered sample using chilled mortar and pestle and stock solution was made in distilled water. Lower concentrations (0.1%, 0.05%, 0.025%, and 0.0125%) were prepared from this stock solution.

Cytotoxic evaluation - *Allium cepa* assay

Germinated bulbs of *A. cepa* with healthy roots (2-3 cm) were collected at the time of peak mitotic activity and washed thoroughly with distilled water. The onion bulbs were kept at the rim of the bottle in which different extracts was taken in such a manner, that only the roots remain completely immersed in the solution. Distilled water and hydrogen peroxide (CAS No: 7722-84-1, 0.01%), were taken as the negative control (NC) and the positive control (PC), respectively. A few root tips were cut from each sample after the treatment for different time duration viz., ½, 1, 2, and 24 hours. Root tips were washed thoroughly with distilled water and immediately fixed in modified Carnoy`s fluid for one hour. Mitotic squash experiments were conducted with the help of improved techniques [15]. Then root tips were subjected to hydrolysis with 1 N HCl for 5 –10 min and washed thoroughly in distilled water. Acetocarmine was used to stain the tissues for one hour and destained in 45% acetic acid. Slides were prepared and photomicrographs were taken using 40 X light microscope (Leica DM 2000 LED, Germany). From six different fields, numbers of mitotic cells, aberrant cells, and total cells were counted. Mitotic index (%) and abnormality percentage (%) were calculated using the following formulae, and values were expressed as mean ± SE.

$$\text{Mitotic Index (\%)} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

$$\text{Aberration percentage (\%)} = \frac{\text{Number of aberrant cells}}{\text{Total number of cells}} \times 100$$

Statistical analyses

Data obtained through the analyses were then subjected to Duncan's multiple range tests and one-way ANOVA to confirm the significance of the results using SPSS Version 20 (SPSS Inc., Chicago, IL, USA). All results were expressed as mean ± SE.

RESULT

Cytotoxic assay

Analysis of the cytotoxicity of *M. randerianum* leaf extract on A. cepa root tip cells, revealed a significant dosage dependent effect. Mitotic index is a measure on the proliferation status of a cell population. Here *M. randerianum* leaf extract treated roots revealed reduction in the number of dividing cells along with the concentration gradients. Maximum cell division (62.80 ± 2.05^a) and abnormality percentage (82.32 ± 2.38^a) occur in the highest concentration (0.1 %) during 24 hour exposure (Table 1). In all time durations the highest concentration of the extract induces maximum mitotic inhibition which ranges from 38.73± 2.86 (1/2 hour-0.1%) to 35.57 ± 4.00^a (2 hour-0.1 %) except in the prolonged exposure period. When compared to the negative control (Distilled water), plant extract induces a severe decline in the mitotic index and inversely the abnormality percentage was found to be higher than that of positive control (H_2O_2) (Table 1). Lowering of mitotic index is generally considered as a result of cytotoxicity, but during this study prolonged exposure at the higher concentration results in the maximum mitotic index. Researchers explained the increase in the mitotic index after colchicine treatment as a result of accumulation of abnormal mitoses. Sbrana et al. [16] also reported the increase in the mitotic indices and C-mitoses of lymphocytes, after treatment with spindle poisons. These results confirm that *M. randerianum* extract has potent cytotoxic and C-mitotic activity.

The cytotoxic effect of plant extract leads to several chromosomal aberrations, which are mainly clastogenic (Fig. 1) and aneugenic (Fig. 2). In the former abnormal effects are induced on the genetic material and later interfere with mitotic spindle formation [17, 18]. In clastogenic aberrations, stickiness, pulverization, chromosomal clumping, nuclear lesions, erosions etc. are observed. Hypoploid condition, stellate chromosomes, polyploidy and induction of vagrants may occur due to aneugenic aberrations. Among the aneugenic aberrations, C-mitosis was the prominently observed one. The spindle poisoning may occur due to the presence of colchicine like compound in the leaf extract of *M. randerianum*. Colchicine will disturb the mitotic spindle by inhibiting the α, β tubulin of microtubule systems and there by interrupt chromosomal movements. This microtubule drugs are often effective only in certain taxonomic groups, while other organisms remain resistant [19]. The connection with chemical constitution and C-mitotic activity of many compounds like naphthalene and acenaphthalene derivatives were studied earlier [20]. Thus the study of C-mitotic potential of *M. randerianum* strongly revealed the presence of colchicine like compounds in it. The major abnormalities associated with C-mitotic effects may include C-anaphase, vagrant formation, polyploidy, cytostatic effect etc. The plant extract shows C-mitotic activity in a time and dosage independent manner. As a result of prolonged exposure (24 h) of root tips in the plant extract, the C-mitotic efficiency increases, and C-metaphase becomes prominently resulted in 0.1 concentration (Table. 2, Fig. 3 a, b, c, d). C-metaphase is one of the main consequences of inactivation of spindle apparatus connected with the delay in division of centromere [21]. Vagrant formation by the abnormal spindle function leads to the unequal separation of chromosome groups, which was observed in all the time period of all concentrations (Fig. 2 f, g). Polyploidy occurred by the poisoning effect in the mitotic spindles found in all concentrations except in 0.0125% of plant extracts at all time durations (Table. 2, Fig. 2 d). Vagrant formation is another frequently observed aberration (Fig. 3 e). The aberrations shown by the plant extract is significantly ($p \leq 0.05$) emphasizes the presence of colchicine like compound *M. randerianum*. Chromatids in C-anaphase that cannot separate into opposite poles were found only in the highest time period of treatments **(**Fig. 3 l**)**. Shift in microtubule organizing centres are resulted by the effect of C-mitosis **(**Fig. 2 j**)**. Partial inactivation of spindle fibers leading to partial C-mitosis [22] was also observed during the study (Fig. 3 i, j, k**)**.

CONCLUSION

The present study is pointing to the C- mitotic effects of the plant extract. During the cytotoxic evaluation using *A.cepa* assay, increased mitotic index and C-mitotic activity of the plant extract in the 24 hour treatment is very significant, so that the proposed concentration and time period will be effective for the future formulation of a colchicine analog.

Table 1: Mitotic index and abnormality percentage of *M. randerianum* leaf extract treated *A. cepa* root meristem at various time periods and concentrations

Duration	Concentration of plant extract (%)	Dividing cells	Mitotic index % ± SE	Abnormality percentage ± SE
1/2 hour	0.1	387	38.73 ± 2.86^{a}	81.27 ± 1.68^{a}
	0.05	404	40.40 ± 4.65^{a}	58.39 ± 2.53^{b}
	0.025	453	45.33 ± 3.7^{a}	49.18 ± 7.97^{b,c}
	0.0125	632	58.22 ± 8.73^{b}	35.66 ± 5.58^{c}
	NC	906	90.68 ± 1.53^{a}	21.71 ± 3.43^{a,b}
	PC	408	40.83 ± 12.71^{c}	80.53 ± 3.11^{c}
1 hour	0.1	414	41.47 ± 7.41^{a}	79.30 ± 3.27^{a}
	0.05	436	43.67 ± 2.58^{a,b}	73.35 ± 5.45^{a}
	0.025	516	51.60 ± 6.97^{a,b}	64.97 ± 7.80^{a,b}
	0.0125	619	57.95 ± 6.75^{b}	52.03 ± 8.74^{b}
	NC	904	90.41 ± 1.75^{d}	22.05 ± 3.69^{c}
	PC	411	41.16 ± 7.71^{a}	80.10 ± 2.49^{b}
2 hours	0.1	355	35.57 ± 4.00^{a}	82.29 ± 2.74^{a}
	0.05	439	43.97 ± 3.57^{a,b}	79.48 ± 3.00^{a}
	0.025	544	54.48 ± 7.02^{b,c}	76.73 ± 4.37^{a}
	0.0125	616	56.67 ± 6.22^{c}	51.36 ± 8.24^{b}
	NC	891	89.10 ± 2.16^{d}	19.76 ± 2.56^{d}
	PC	411	41.13 ± 3.74^{a,b}	81.63 ± 2.62^{a}
24 hours	0.1	728	62.80 ± 2.05^{a}	82.32 ± 2.38^{a}
	0.05	666	62.69 ± 5.61^{a}	78.56 ± 2.22^{a}
	0.025	645	64.55 ± 5.69^{a,}	67.76 ± 7.61^{a,b}
	0.0125	693	69.36 ± 8.94^{b}	54.51 ± 9.04^{b}
	NC	913	91.33 ± 2.10^{c}	19.76 ± 2.49^{d}
	PC	434	43.48 ± 7.67^{a}	81.83 ± 5.76^{a}

NC: Negative control (distilled water), PC: Positive control (H_2O_2), SE - Standard error. Means within a column followed by the same letters are not significantly different at $P < 0.05$ as determined by Duncan's multiple range test.

Table 2: C-Mitotic aberrations in *M. randerianum* leaf extract treated *A. cepa* root meristem at various time periods and concentrations.

Duration	Concentration of plant extract (%)	C-metaphase ± SE	C-anaphase ± SE	Polyploidy ± SE	Shift in MTOC ± SE	Vagrant chromosomes ± SE	Colchicine like activity (%)
PC	0.1	1 ± 0.25^{a}	000^{a}	2 ± 0.22^{a}	2 ± 0.21^{a}	2 ± 0.33^{a}	7 ± 0.58^{a}
	0.05	2 ± 0.21^{a}	1 ± 0.31^{a}	3 ± 0.35^{a}	1 ± 0.54^{b}	1 ± 0.36^{a}	8 ± 1.50^{b}
	0.025	2 ± 0.21^{a}	000^{a}	5 ± 0.41^{a}	3 ± 0.21^{a}	2 ± 0.25^{a}	12 ± 0.90^{b}
	0.0125	4 ± 0.33^{a}	000^{a}	4 ± 0.33^{a}	000^{a}	1 ± 0.31^{a}	9 ± 0.76^{b}
1/2 hour	0.1	6 ± 0.33^{a}	000^{a}	0.33 ± 0.21^{a}	000^{a}	1 ± 0.00^{b}	7.99 ± 0.54^{b}
	0.05	1.00 ± 0.25^{a}	000^{a}	3 ± 0.21^{a}	6 ± 0.16^{a}	1 ± 0.34^{a,b}	11 ± 0.80^{b}
	0.025	5 ± 0.34^{a}	000^{a}	000^{a}	000^{a}	2 ± 0.16^{a,b}	7 ± 0.50^{b}
	0.0125	2.16 ± 0.16^{a}	000^{a}	000^{a}	000^{a}	4 ± 0.33^{a}	6.16 ± 0.49^{a}
1 hour	0.1	12 ± 0.42^{a}	000^{a}	0.16 ± 0.16^{a}	000^{a}	2.00 ± 0.36^{b}	14.16 ± 0.78^{b,c}
	0.05	7 ± 0.40^{b}	000^{a}	000^{a}	1 ± 0.40^{b}	0.66 ± 0.21^{a}	8 ± 1.01^{b}
	0.025	5 ± 0.34^{a}	000^{a}	000^{a}	2 ± 0.33^{a,b}	1.33 ± 0.21^{a,b}	8.33 ± 0.88^{b}
	0.0125	1.50 ± 0.61^{a}	000^{a}	1.00 ± 0.44^{b}	0.16 ± 0.16^{a}	1.16 ± 0.41^{a,b}	3.82 ± 1.62^{a}
2 hours	0.1	5 ± 0.22^{a,b}	000^{a}	2 ± 0.22^{a}	000^{a}	3 ± 0.16^{a}	10 ± 0.60^{b}
	0.05	000^{a}	000^{a}	3 ± 0.34^{a}	000^{a}	4 ± 0.21^{a}	7 ± 0.55^{a}
	0.025	1.16 ± 0.47^{b}	0.83 ± 0.30^{b}	0.50 ± 0.34^{a}	000^{a}	0.83 ± 0.16^{a}	3.32 ± 1.27^{a}
	0.0125	4.00 ± 0.36^{b}	1.66 ± 0.42^{c}	0.50 ± 0.22^{a}	000^{a}	0.33 ± 0.33^{a}	6.49 ± 1.33^{a}
24 hours	0.1	15.33 ± 1.33^{b}	1.16 ± 0.16^{a}	0.66 ± 0.33^{a,b}	0.50 ± 0.34^{a}	1.00 ± 0.25^{a}	18. 65 ± 2.41^{c,d}
	0.05	8.50 ± 0.34^{a}	0.16 ± 0.16^{a}	2.50 ± 0.34^{b}	0.66 ± 0.33^{a}	10.50 ± 0.76^{b}	15.32 ± 1.93^{c,d}
	0.025	4 ± 0.51^{a}	7.50 ± 0.34^{a}	0.50 ± 0.34^{a}	0.83 ± 0.30^{a}	0.66 ± 0.33^{a}	14.49 ± 1.82^{d}
	0.0125	2.66 ± 0.33^{a}	0.66 ± 0.33^{a}	000^{a}	000^{a}	7.33 ± 0.21^{a}	11.65 ± 0.87^{b}

Means within a column followed by the same letters are not significantly different at $P < 0.05$ as determined by Duncan's multiple range tests. PC: Positive control (H_2O_2).

ACKNOWLEDGEMENTS

The first author gratefully acknowledges Council of Scientific and Industrial Research (CSIR), Government of India (09/043(0182)/2017-EMR-I), for the award of fellowship.

REFERENCES

1. Ade R, Rai MK. Review-colchicine current advances and future prospects. Nusantara Bioscience 2010; 2: 90-96.
2. Finnie JF, Van Staden J. *Gloriosa superba* L. (Flame Lily): Micropropagation and in vitro production of colchicine. Medicinal and Aromatic Plants 1991; 4: 146-166.
3. Taylor EW. 1965 The mechanism of colchicine inhibition of Mitosis. Journal of Cell Biology 2013; 25(1): 145-160.
4. Hervas JP, fernandez Gomez ME, Gimenez Martin G. Colchicine effect on mitotic spindle: Estimate of multipolar anaphase production. Caryologia 1974; 27(3): 359-368.
5. Bayliss MW. Variation of cell cycle duration within suspension cultures of *Daucus carota* and its consequence for the induction of ploidy changes with colchicine. Protoplasma 1976; 88(2-4): 279-285.

6. Glendon Ascough D, Johannes van Staden. Effectiveness of colchicine and oryzalin at inducing polyploidy in *Watsonia lepida* N.E. Brown. HortScience 2008; 43(7): 2248-2251.
7. Leung YY, Laura Li Yao Hui, Virginia Kraus B. Colchicine - update on mechanisms of action and therapeutic uses. Seminars in Arthritis and Rheumatism 2015; 45(3): 341–350.
8. Neelamkavil, Thoppil JE. C-mitotic effect of aqueous extracts of *Isodon coetsa* (Bunch.-Ham. ex D. Don) Kudo (Lamiaceae). Nucleus 2013; 56(2): 117-122.
9. Nilanthi Dahanayake, Xiao-Lu Chen, Fu-Cheng Zhao, Yue-Sheng Yang, Hong Wu. Induction of tetraploids from petiole explants through colchicine treatments in *Echinacea purpurea* L. Journal of Biomedicine and Biotechnology 2009; https://doi.org/10.1155/2009/343485
10. Yasar sajjad, Muhammad jafar jaskani, Asim mehmood, Iftikhar ahmad, Haider abbas. Effect of colchicine on in vitro polyploidy induction in african marigold (*Tagetes erecta*). Pakistan Journal of Botany 2013; 45(3): 1255-1258.
11. Krishnan MKS. The Wealth of India-A Dictionary of Indian Raw Materials CSIR, New Delhi.1963; 6: 336-337.
12. Prakasha HM, Krishnappa M, Krishnamoorthy YL, Poornima SV. Folklore of NR Pura taluk in Chickmangalore district of Karnataka. Indian Journal of Traditional Knowledge 2010; 9(1): 55-60.
13. Feretti D, Zerbini I, Zani C, Ceretti E, Moretti M, Monarca S. *Allium cepa* chromosome aberration and micronucleus tests applied to study genotoxicity of extracts from pesticide-treated vegetables and grapes. Food Additives & Contaminants 2014; 24(6): 561-572.
14. Leme DM, Marin-Morales MA. *Allium cepa* test in environmental monitoring: a review on its application. Mutation Research 2009; 682: 71–81.
15. Sharma AK, Sharma A. Chromosome techniques, theory and practice 3rd edition Aditya Books; New Delhi 1990.
16. Sbrana Isabella, Alessandra Di Sibio, Alessandra Lomi, Vittoria Scarcelli. C-Mitosis and numerical chromosome aberration analyses in human lymphocytes: 10 known or suspected spindle poisons. Mutation Research 1993; 287(1): 57-70.
17. Bhagyanathan NK, Thoppil JE. Genotoxic potential of *Cynanchum sarcomedium* Meve & Liede coupled with its modulatory action on oxidative-stress–mediated genotoxicity by hydrogen peroxide. Turkish Journal of Biology 2016; 40: 120 -129.
18. Parry EM, Parry JM, Corso C, Doherty A, Haddad F, Hermine TF, *et al*. Detection and characterization of mechanisms of action of aneugenic chemicals. Mutagenesis 2009; 17(6): 509-521.
19. Dostal V, Libusova L. Microtubule drugs: action, selectivity, and resistance across the kingdoms of life. Protoplasma. 2014; 251:991-1005.
20. Levan A, Ostergren G. The mechanism of C-mitotic action observations on the naphthalene series. 1939; 29(3-4): 381-432. https://doi.org/10.1111/j.1601-5223.1943.tb03297.x.
21. Somashekar RK, Gowda MTG. Effect of a fungicide vitavax on *Allium cepa.* Cytologia 1984; 49(1): 177-181.
22. Levan A. The effect of colchicine on root mitoses in *Allium*. Hereditas 1938; 24: 471- 4

Cite this article as:

P. R. Ramya Sree and J. E. Thoppil. C-mitotic potential of aqueous leaf extract of *Memecylon randerianum* SM & MR almeida.: A promising natural colchicine analog. Int. Res. J. Pharm. 2018;9(11):115-118 http://dx.doi.org/10.7897/2230-8407.0911269

Source of support: Council of Scientific and Industrial Research (CSIR), Government of India, Conflict of interest: None Declared