International Food Research Journal 23(6): 2449-2452 (December 2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Evaluation of cytogenotoxic and antimutagenic potency of water extract of *Centella asiatica* Linn. using the *Allium cepa* assay

[1, 2*]Akinboro, A., [2]Baharudeen, I., and [2]Mohamed, K.

[1]*Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, P.M.B. 4000, Nigeria*
[2]*School of Biological Sciences, Universiti Sains Malaysia, 11800, Pulau Pinang, Malaysia*

Article history

Received: 12 September 2015
Received in revised form: 21 February 2016
Accepted: 27 February 2016

Keywords

Antimutagenicity
Allium cepa
Chromosome aberrations
Centella asiatica

Abstract

In this study, toxicological safety (mutagenicity) and therapeutic potential (antimutagenicity) of water extract of *Centella asiatica* Linn., were evaluated using the *Allium cepa* assay. The mitotic index (MI) at 6.25% concentration of the extract decreased significantly from 3.13% to 2.05% after 24 h and 48 h, respectively, however, the MI increased significantly by 60% and 400% at 12.5% and 50.0% concentrations, respectively. There was total arrest of cell division at 100% concentration after 24 h and 48 h of onion's roots exposure to the water extract. The chromosomal aberrations (CA) induced by the extract were not significantly different from the negative control ($p \geq 0.05$) at the tested concentrations. The mutagenic activity of cyclophosphamide was significantly suppressed above 50% at the tested concentrations. These results suggest non-genotoxic effect, and antimutagenic potency of water extract of *C. asiatica* in *A. cepa* cells, being desirable characteristics of anticancer therapeutics.

Introduction

Herbs are usually consumed by humans because of their nutritive and medicinal values which are functions of phytochemicals in them. Recent scientific reports have shown that some plant extracts contain toxic phytochemicals which can interract with biomolecules in the cells to cause mutagenic, cytotoxic and genotoxic effects in in vitro and in vivo assays (Schimmer *et al.*, 1994; Akinboro and Bakare, 2007; Akinboro *et al.*, 2012).

Centella asiatica is an important medicinal herb recognized for its various healing activities such as the treatment of leprosy, ulcer, asthma, eczemza, anxiety and elephantiasis (Siddique *et al*., 2008; Seema and Meena, 2012), because it contains various biochemical compounds such as alkaloids, flavonoids, glycosides, triterpenoids and Saponins (Babu *et al*., 1995; Siddique *et al*., 2007; Siddique *et al.*, 2009; Hashim, 2011).

So far, the available results of scientific experiments on the genotoxic and antigenotoxic evaluations of the extract of *C. asiatica* were obtained from *in vitro* assays, suggesting the needs for validation of the reported results of the *in vitro* experiments in *in vivo* assays which better mimic the normal human system. Thus far, the present study aimed at evaluating water extract of *C. assiatica* for mutagenic and antimutagenic effects on cell division and chromosomes using the *in vivo Allium cepa* assay.

Materials and Methods

Centella asiatica herb was puchased at Tesco shopping mall, Sungai dua Pinang, it was identified and given voucher specimen number 11268 at the herbarium unit of the School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia. The leaves were dried at the room temperature and ground using an electric blender. The ground leave (50 g) was added with 1000 ml distilled water and placed in a water bath at 40°C for 10 h for aqueous extraction of phytochemicals to occur. The undiluted aqueous extract (stock decoction) was seived through a filter paper and kept at 4°C for cytological investigations. The stock extract (decoction) was diluted to 6.25% , 12.5% , 25% and 50% concentrations for purpose of inducing *A. cepa* root growth for mutagenic evaluation after 24 h and 48 h of exposure (Akinboro and Bakare, 2007; Akinboro *et al*., 2012).

The antimutagenic evalution of the extract was similar to the mutagenic evaluation except that 0.1% cyclophosphamide (CP) was added to the water extract. Distilled water and cyclophophamide served as the negative and postive controls, respectively. Root tips from the onions exposed to the extract were harvested after 24 h and 48 h root growth and fixed in ethanol acetic acid (3:1) fixative for 1 h. The roots were then prepared on microscope slides

*Corresponding author.
Email: *aakinboro@lautech.edu.ng*
Tel: +2348134727845

and scored as previously described (Akinboro *et al.*, 2011a; Akinboro *et al.*, 2011b). Mitotic index (MI), chromosomal aberration (CA) percentage and reduction percentage of CP-induced CA were calculated as follows:

$$MI\ (\%) = [A/B] \times 100 \quad \text{.....(1)}$$

Where MI = Mitotic index, A = Number of dividing cells, B = Total number of counted cells.

$$CA\ (\%) = [A/B] \times 100 \quad \text{.....(2)}$$

Where CA = Chromosomal aberration, A = Number of aberrant cells ,
B = Total number of counted cells

$$\text{Reduction of CP-induced CA (\%)} = [A - B] / [A - C] \times 100 \quad \text{.....(3)}$$

Where A = Proportion of CA in MI induced by the positive control (cyclophosphamide-CP) ,
B = Proportion of CA in MI induced by the mixture of CP and water extract of *C. asiatica,*
C = Proportion of CA in MI induced by the negative control (distilled water).

Statistical analysis

Data were analyzed for the level of significance set at $p \leq 0.05$ using Duncan's multiple range comparison in One-way ANOVA of the SPSS version 18.0.

Results

The effects of water extract (decoction) of *Centella asiatica* on cell division in the root tips of the exposed onions for 24 h and 48 h are shown in Figure 1. There was a significant reduction ($p \leq 0.05$) in the number of dividing cells (MI) when compared to the negative control at the tested concentrations, except after 48 h at 12.5% concentration which induced the highest MI value of 3.69%. Complete arrest (0%) of cell division was observed at 100% concentration after both durations of onion root growth in this study. The MI obtained at 6.25% and 25% concentrations after 24 h were 3.13%, 2.52%, respectively, higher than those obtained after 48 h. The MI obtained at 50.0% concentration after 48 h was 1.62% whereas, it was 0.35% recorded after 24 h. The MI produced by positive control were 2.30% and 1.93% after 24 h and 48 h, respectively.

The induced CA by 0.1% CP was the highest at

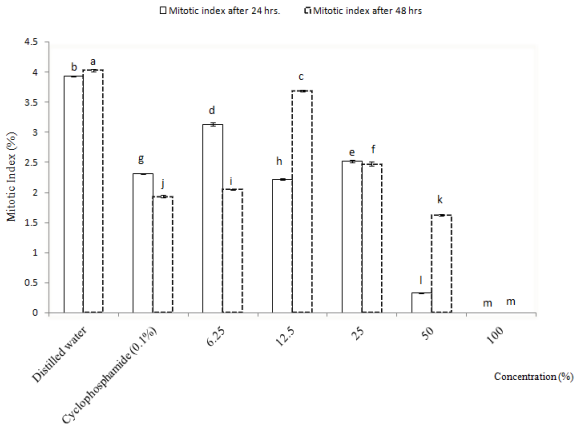

Figure 1. Mitotic indices caused by water extract of *Centella asiatica* in *Allium cepa* cells after 24 h and 48 h of root growth

MI values with different alphabelts are significantly different from the negative control at $p \leq 0.05$.

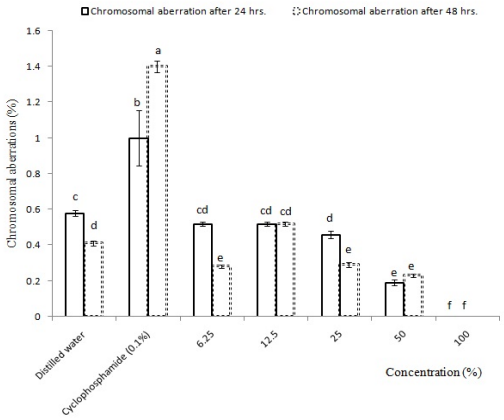

Figure 2. Chromosomal aberrations induced by water extract of *Centella asiatica* in Allium cepa after 24 h and 48 h of root growth

CA percentages with different alphabelt are significantly different at $p \leq 0.05$.

1.40% which was significantly different ($p \leq 0.05$) from those obtained at the tested concetrations of water extract of *C. asiatica* and negative control after 48 h. No CA was observed at 100% concentration after 24 h and 48 h of root growth, however, at 6.25%, 12.5%, 25.0% and 50.0% concentrations, the percentage of induced CA was not significantly different from the negative control ($p \geq 0.05$) (Figure 2.).

Table 1 shows the reduction of CP-induced chromosomal aberrations (CA) by the water extract of *C. asiatica.* The highest reduction percentage of 92.86% was recorded at 6.25% concentration, while the least percentage reduction of CP- induced CA was 53.57% recorded at 50.0% concentration. The percentage reduction of CA at 100.0% could not be calculated because there was no dividing cells at this concentration. The manner of reduction of CP-induced CA by the water extract of *C. asiatica* was

Table 1. Antimutagenic activity of water extract of *Centella asiatica* against CP-induced chromosomal aberrations in *A. cepa* cells

Concentration (%)	Mitotic index (%)	% Chromosomal aberrations	CA/ MI	% reduction of CP-induced CA
Distilled water	3.93	0.58	0.15	-
Cyclophosphamide	2.31	1.00	0.43	-
6.25 + CP	3.13	0.52	0.17	92.86
12.5 + CP	2.22	0.52	0.23	71.43
25.0 + CP	2.52	0.46	0.18	89.29
50.0 + CP	0.33	0.19	0.58	53.57
100.0 + CP	0.0	0.0	0.0	TA

TA: Total arrest of cell division; CA: Chromosomal aberration; MI: Mitotic index; CP: Cyclophosphamide.

inversely proportional to the concentrations except at 25.0% concetration which caused 89.29% reduction of CP-induced CA.

Discussion

The water extract of *C. asiatica* at the tested concentrations signficantly reduced the number of dividing cells in the root tips of *A. cepa* compared to the effect of nagetive control distilled water after 24 h and 48 h of the treatment. This effect of the water extract was mitostatic in *A. cepa* cells, and its total arrest of cell division at 100% concentration (stock extract) could mean cytotoxic possibly via the induction of cell death. The result of similar investigation using cultured human peripheral blood lymphocytes in *in vitro* assay is in contrary to our results of *in vivo Allium cepa* assay (Seema and Meena, 2012). This disperity could be due to the series of metabolic enzymatic reactions on the water extract of *C. asiatica* to produce cytotoxic metabolites capable of causing non continous interferrence with the progression of cell cycle in the root tip of *A. cepa.*

However, the non dose dependent inhibiton of mitosis after 24 h and 48 h of root growth at the tested concentrations except at 100% suggests mitostatic effect, possibly to be observed with the consumption of water extract of *C. asiatica* as food or medicinal herb by humans.

The frequencies of aberrant cells observed in non-concentration dependent manner, and which were not significantly different from the negative control suggests lack of mutagenic activities in the *in vivo A. cepa* assay. This is in accordance with the previous results of *in vitro* genotoxic investigations of the extract of *C. asiatica* on cultured human lymphocytes and human peripheral blood lymphocytes (Siddique *et al*., 2008; Siddique *et al*., 2009; Seema and Meena, 2012).

The antimutagenic activity of the extract against cyclophosphamide-induced mutagenicity was similar to the earlier reports. The suppression of mutagenicity of cyclophosphamide above 50% at the tested concentrations, except 100%, (where total arrest of cell dividion was observed) could be considered a strong antimutagenic effect of this extract. It is possible that metabolic activation of cyclophosphamide to free radicals mutagenic metabolites was perfectly inhibited by the water extract of *C. asiatica* through its free-radcals scanvenging phenolic compounds with antioxidant properties (Siddique *et al*., 2008; Siddique *et al.*, 2009; Hashim, 2011).

Conclusion

This study revealed that the effect of water extract of *C. asiatica* on cell division in *A. cepa* was mitostatic and not cytotoxic except at 100% concentration (stock extract solution used in this study). Its activity on the chromosomes of the treated cells of *A. cepa* was not mutagenic. However, it showed strong antimutagenic effectivness against cyclophosphamide-induced chromosomal aberrations in the *A. cepa* cells, suggesting its possible use as a promising anticancer chemotherapy.

Reference

Akinboro, A. and Bakare A. A. 2007. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. Journal of Ethnopharmacology 112(3): 470-475.

Akinboro, A., Mohamed, K. B., Asmawi, M. Z. and Sofiman, O. A. 2011a. Mutagenic and antimutagenic potentials of fruit juices of five medicinal plants in *Allium cepa* L.: Possible influence of DPPH free radical scavengers. African Journal of Biotechnology 10(51): 10520-10529.

Akinboro, A., Kamaruzaman, B.M., Zaini, M.A., Shaida, F.S. and Sofiman, O.A. 2011b. Antioxidants in aqueous extract of *Myristica fragrans* (Houtt.) suppress mitosis and cyclophosphamide-induced chromosomal

aberrations in *Allium cepa* L. Cells. Journal of Zhejiang University-Science B (Biomedicine and Biotechnology) 12(11): 915-922.

Akinboro, A., Kamaruzaman, M.B., Mohd Zaini, A., Ahmad, S.O., Tang, H.Y. and Siti Marina, M. 2012. Mutagenic and antimutagenic assessment of methanol leaf extract of *Myristica fragrans* (Houtt.) using in vitro and in vivo genetic assays. Drug and Chemical Toxicology 35(4): 412-422.

Babu, T.D., Kuttar, G. and Padikkla, J. 1995. Cytotoxic and antitumour properties of certain taxa of umbelliferae with special reference to *Centella asiatica* (L.) Urban. Journal of Ethnopharmacology 48: 53-57.

Hashim, P. 2011. Centella asiatica in food and beverage applications and its potential antioxidant and neuroprotective effect. International Food Research Journal 18(4): 1215-1222.

Schimmer, O., Kruger, A. and Paulini, H. 1994. An evaluation of the 55 commercial plant extracts in the Ames mutagenicity test. Pharmazie 49(6): 448-451.

Seema, C.C. and Meena, V. 2012. Cytotoxic and genotoxic effects of *Centella asiatica* extract in the cultured human peripheral blood lymphocytes. Research in Pharmaceutical 2(4): 31-40.

Siddique, Y.H., Ara, G., Beg, T., Faisal, M., Ahmad, M. and Afzal, M. 2007. Protective role of *Centella asiatica* L. extract against methyl methanesulphonate and cyclophosphamide induced genotoxic damage in cultured human lymphocytes. Recent Progress in Medicinal Plants 19: 369–381.

Siddique, Y.H., Beg, T. and Afzal, M. 2008. Antigenotoxic effect of ascorbic acid against cypropterone acetate induced genotoxicity in cultured mammalian cell". In: Recent Trends in Toxicology, YH. Siddique (Eds), Transworld Research Network, Trivandpuram, Kerala, India. 85-94.

Siddique, Y.H., Beg, T. and Afzal, M. 2009. Effect of *Centella asiatica* L. Extract against ethinylestradiol induced genotoxic damage on cultured human lymphocytes. Biomedical Research 20(2): 141-147.