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

Akinboro, A., Baharudeen, I., and Mohamed, K.

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

**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 ≥ 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 interact 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, eczema, 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 (Baba *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. asiatica* for mutagenic and antimutagenic effects on cell division and chromosomes using the *in vivo Allium cepa* assay.

**Materials and Methods**

*Centella asiatica* herb was purchased 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 sieved through a filter paper and kept at 4°C for cytological investigations.

The antimutagenic evaluation of the extract was similar to the mutagenic evaluation except that 0.1% cyclophosphamide (CP) was added to the water extract. Distilled water and cyclophosphamide served as the negative and positive 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.
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:

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

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

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

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

Reduction of CP-induced CA (%) = \left[ \frac{A - B}{A - C} \right] \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 1.40% which was significantly different ($p \leq 0.05$) from those obtained at the tested concentrations 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
inversely proportional to the concentrations except at 25.0% concentration which caused 89.29% reduction of CP-induced CA.

Discussion

The water extract of *C. asiatica* at the tested concentrations significantly reduced the number of dividing cells in the root tips of *A. cepa* compared to the effect of negative 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 disparity 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 continuous interference with the progression of cell cycle in the root tip of *A. cepa*.

However, the non dose dependent inhibition 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 division 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-radicals scavenging 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 effectiveness against cyclophosphamide-induced chromosomal aberrations in the *A. cepa* cells, suggesting its possible use as a promising anticancer chemotherapy.

Reference


