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RESEARCH ARTICLE

Rhizome extract of C*urcuma longa L.* exhibit mitodepressive effect, but no genotoxicity

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Abstract The genus *Curcuma* within the family Zingiberaceae has been used as a folk medicine from ancient times, and the species *C. longa* is one of the most important cultivated species that yields turmeric, and widely used in traditional and modern medicine. Growing concerns raised about the side effects of therapeutic principles have necessitated to ensure safe use even for phyto-chemicals, and the least being that such chemicals do not pose any genotoxic effect. Since, *Allium* test model is one of the most efficient bioassay to assess cytotoxic and genotoxic effects of plant extracts, it was therefore planned to evaluate the effect of *C. longa* rhizome extract on cell division. It is observed that the rhizome extract with curcumin as its main constituent imparts inhibitory effect on root growth mediated through mito-depression, but at the same time there is no genotoxic / chromosome aberration effect, suggesting its safe use. The correlation analysis between the different concentrations of rhizome extract and the mitotic index revealed that there was a high negative correlation of r = -0.971 at 24 hrs and r= -0.950 at 48 hrs of extract treatment. Further, the root length also showed a high negative correlation after 24 hrs (r = -0.986) and 48 hrs (r = -0.964). The result of this study indicates that the mitodepressive effect of *C. longa* rhizome extract is both time and concentration dependent.

Keywords Curcuma, Cytotoxic, Genotoxic, Mitodepressive

Introduction

Many plants used as food or in traditional medicine, as revealed by recent investigations, have mutagenic, cytotoxic and genotoxic effects in vitro and in vivo assays (Higashimoto et al. 1993, Askin and Aslanturk 2007). Thus, assessment of their cytotoxic and mutagenic potential is necessary to ensure a relatively safe use of medicinal plants.

The rhizome of the turmeric plant has been used for centuries in traditional medicine and is known to have cancer preventive or therapeutic capabilities (Anand *et al.* 2008). It has been shown to suppress multiple signalling pathways and inhibit cell proliferation, invasion, metastasis, and angiogenesis (Kunnumakkara *et al.* 2008, Aggarwal *et al.* 2003, Duvoix *et al.* 2005). Its safety combined with its low cost and multiple targeting potential makes turmeric an ideal agent to be explored for prevention and treatment of various cancers and fits very well as a candidate for chemoprevention by edible phytochemicals (Aggarwal *et al.* 2008). Anticancer activity of the rhizomes of turmeric has long been evaluated *in vitro* by Kuttan *et al*. (1985).

The bulbs of *Allium cepa* are good source of fast growing roots and mitotic division phases. Mitotic index and chromosomal aberration method of Allium cepa roots is validated by IPCS, WHO, UNEP, as a test efficient for the analysis and monitoring genotoxicity of environment *in situ* (Leme *et al.* 2009 and Dimuthu *et al.* 2019).The mitotic index and replication index are used as indicators of adequate cell proliferation (Gadano *et al*. 2002), which can be measured by the plant test system *Allium cepa.* Cytotoxicity tests, using plant test systems *in vivo*, such as *Allium cepa*, are validated by several researchers, who jointly performed animal testing *in vitro* and the results obtained are similar (Vicentini *et al.* 2001 and Teixeira *et al.* 2003), providing valuable

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information for human health. Fiskesjo (1985) efficiently applied the Allium test as a standard in environmental monitoring. Many recent studies (Sabeen *et al.* 2020, Ergin *et al.* 2020) also used the *Allium cepa* model to demonstrate genotoxicity.

Inhibitory and mitodepressive effect of aqueous extracts of five medicinal plants on *Allium cepa* root tips were reported by Akinboro and Bakare (2007) as the indicators of cyto- and genotoxicity. Evaluating the genotoxic effect of plant extract, Chauhan *et al* (1999) showed that the sensitivity of the *Allium cepa* test system is well correlated with the mammalian test system, thus validating the use of *Allium* chromosomal test as an alternative test for monitoring the potential genotoxicity. Keeping this in view an attempt has been made to illustrate the effect of rhizome extract of *C. longa* on root tip cells using *A. cepa* test model.

Materials and methods

Plant material

Fresh rhizomes of *Curcuma longa* L. were collected in the month of November, from Kannur District of Kerala. The taxonomic identity of the sample was determined by comparison with the authentic herbarium specimens deposited at the herbarium of JNTBGRI Thiruvananthapuram, Kerala. The voucher numbers is C. longa (36252, Pathanamthitta, 9-10-1999).

Preparation of the rhizome extract

100 g of fresh rhizome of *C. longa* were cut into small pieces and soaked in100 ml of acetone for 24h and then evaporated the solvent. 2.5 g of the dry extract (the amount of curcumin was quantitatively estimated as 16.56 mg/g in various dry samples of *C. longa*; Seema and Lavania, 2015) was suspended in 25ml of distilled water as stock solution. The test solution was prepared by taking 1, 2, 3, 4 and 5ml of the stock solution followed by diluting it in 10ml of distilled water.

Allium cepa assay

Allium cepa bulbs of 2.5 to 3 cm in diameter were rinsed in water. The outer scales of the bulbs, old root remnants were removed and the bottom plates cut off to leave root primordial intact. Bulbs were sprouted in containers of distilled water with narrow neck of diameter such that the lower third base of the bulb is submerged. Bulbs were kept for germination for two days for the root length to reach 1 cm to 2 cm. The bulbs with proliferating roots were placed over the vials containing different concentrations of rhizome extract with growing root meristem zone immersed in the treatment solution. Each extract was tested by three bulbs, as a clone. The control series were kept in distilled water. Distilled water and the extracts were changed daily at the same time to avoid contamination. The experiment was conducted at room temperature (20°C) and protected from direct sunlight. The lengths of the growing roots were monitored after 24, 48, 72 and 120 hrs. The root tips were harvested after 24 hrs and 48 hrs at the same time between 1:00 pm to 2:00 pm since the mitotic cycle was found at the peak during this period and hence this time has been standardized and fixed for uniformity in the study. Root tips were immediately transferred to 3:1(v/v) ethanol-acetic acid (Carnoy's fluid) for 24 hrs, subsequently placed in 70% aqueous ethanol under refrigeration at 4°C.

Slide preparation

Excised roots were used for study of mitotic cell division. The roots were first transferred to 45% acetic acid for 5 minutes, and then transferred of 2% aceto- Orcein N. HCl (9:1) for two hours for staining, and then squashed over glass slide in 45% acetic acid to obtain cell spreads. Such cell spreads were observed for frequency and spectrum of division phases under the light microscope at 400× and 600× magnification. A Nikon, HC00L light microscope with digital camera was used in order to get the clear images of cell division. Photomicrographs were made and minimum of 100 cells per slide were analysed. The mitotic index (M.I.) was determined by the examination and counting of minimum 100 cells per slide, using following formula;

$$\text{M. I.} = \frac{\text{No. of dividing cells}}{\text{Total no. of cells}} \times 100$$

Results

The length of the *Allium cepa* roots was found decreasing with increasing concentration of the rhizome extract. At 24 hrs and 48 hrs the root growth rate decreased in parallel to the increasing concentrations of the rhizome extract when compared with the control.The mitotic index of *Allium cepa* root tips treated with increasing concentrations of rhizome extracts was also decreasing when compared with the control (Table 1).

The inhibition of both root growth and the mitotic index was both concentration and time dependent. The result showed that when the concentration doubled from 1% to 2%, the rate of mitotic index decreased from 8.62% to 5.47%, with a 33% decrease in 24 hrs (Figure 1) and from 8.26% to 4.41%, with 42% decrease in 48hrs (Figure 2 and Figure 3.

The reduction of the mitotic index can be explained by the arrest of the division of the interphase nucleus, as well as by death of interphase nucleus, hindering the onset of the prophase and, thus, the division of the cells. No chromosomal aberrations or abnormalities were observed in the dividing root tip cells. However, the extracts exhibited its cytotoxic effect on the roots by a reduction in the rate of cell division (Table 2). This shows its mito-depressive activity and its cytotoxicity in plant test system. These aspects were observed mainly with the increment of the concentrations of the extracts, where the mitotic index showed a significant decrease in relation to the control. The correlation analysis between the concentrations and the mitotic index revealed that there was a high negative correlation of r= -0.971 at 24 hrs and r= -0.950 at 48 hrs of extract treatment. Further, the root length also showed a high negative correlation after 24 hrs (r= -0.986) and 48 hrs r= -0.964).

Discussion

The result of this study indicates that the cytotoxic effect of *C. longa* rhizome extract depend on both time and concentration.This may be due to the presence of the active constituent, curcumin, in the rhizome extracts. Previously, our own group standardized and quantified the percentage of curcumin in selected species of *Curcuma* L. (Seema

Table 1: Effect of *C. longa* rhizome extracts on *A.cepa* root growth

Concentration of rhizome extract (g/ml)**treated**	**Mean root length** (cm) **24 hrs**	**Mean root length** (cm) **48 hrs**	**Mean root length** (cm) **72 hrs**	**Mean root length** (cm) **120 hrs**
Control	4.18 ± 0.24	5.28 ± 0.38	5.86 ± 0.36	7.56 ± 0.36
0.01	3.64 ± 0.16	3.9 ± 0.34	4.94 ± 0.15	5.88 ± 0.28
0.02	3.9 ± 0.14	3.72 ± 0.19	3.82 ± 0.13	4.18± 0.27
0.03	2.92 ± 0.25	3.3 ± 0.34	3.28 ± 0.20	3.64 ± 0.22
0.04	2.42 ± 0.25	2.78 ± 0.18	2.9 ± 0.18	3.34 ± 0.17
0.05	2.2 ± 0.25	2.3 ± 0.31	2.74 ± 0.25	3.1 ± 0.20

Table 2: Effect of *C. longa* rhizome extracts on mitotic index of *A. cepa* root tip cells

Treatment time	**Treatment Concentration (g/ ml)**	**No .of cells analysed**	**No. of dividing cells**	**M I**	**% M I**	**%Decrease compared to control**
24hrs	Control	1522	146	9.56	100	-
	0.01	1438	124	8.62	89.8	10.25
	0.02	1278	70	5.47	57.03	42.97
	0.03	1346	62	4.60	47.9	52.1
	0.04	1420	51	3.59	37.4	62.6
	0.05	1292	36	2.78	28.9	71.1
48hrs	Control	1513	138	9.12	100	-
	0.01	1524	126	8.26	90.5	9.5
	0.02	1450	64	4.41	48.35	51.65
	0.03	1281	48	3.7	40.57	59.4
	0.04	1374	39	2.83	31.0	69
	0.05	1424	32	2.2	24.12	75.8

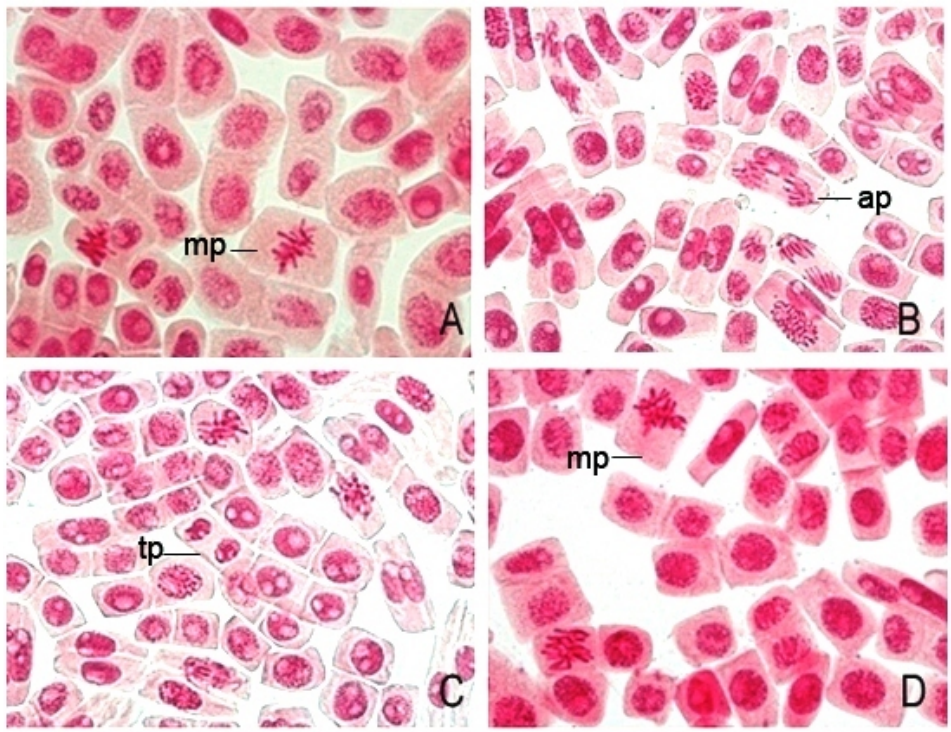

Figure 1: Mitotic cell division of *Allium cepa* root tip cells at 24 hrs, (**A**), Control; (**B-D**), test solutions. **pp**, prophase; **mp**, metaphase; **ap**, anaphase; **tp**, telophase.

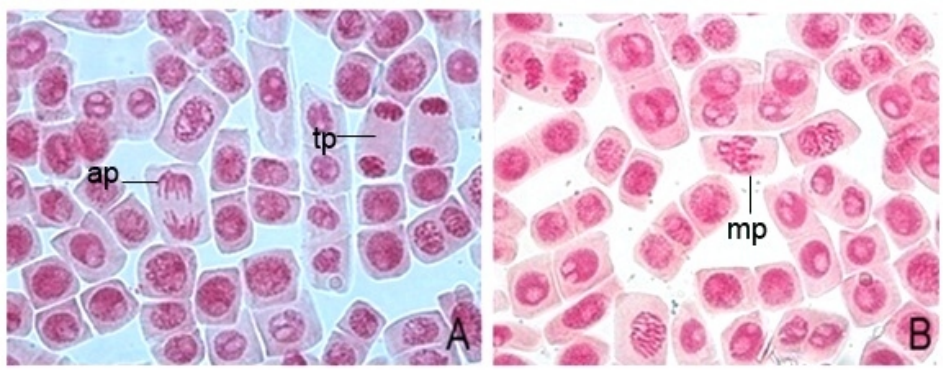

Figure 2: Mitotic cell division of *Allium cepa* root tip cells at 48hrs, (**A**), Control; (**B**), test solution. **mp**, metaphase; **ap**, anaphase; **tp**, telophase.

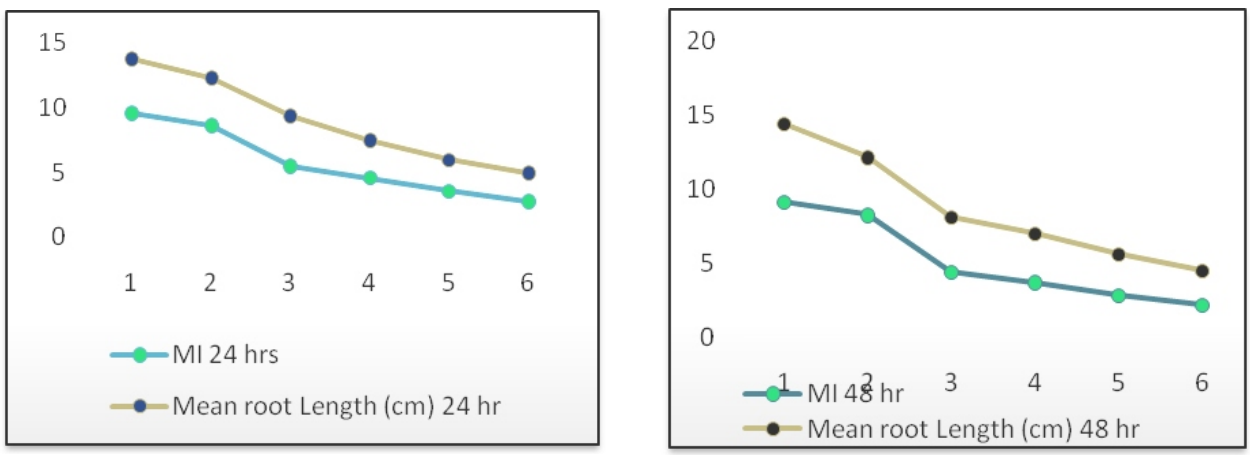

Figure 3: Decline in the mean root length and mitotic index at 24 hrs and 48hrs

R *et al.* 2015) including *Curcuma longa* L. The cells of *Allium cepa* root tips after treatment with extracts of *C. longa* showed decreased in mitotic index with increasing concentration of the test solution containing curcumin. This may due to the concentration dependent effect of curcumin in the test solution. Ragunathan and Panneerselvam (2007) evaluated the anti-mutageneic effect of curcumin in *Allium cepa* root meristem cells. They showed that the percentage of mitotic index decreased with increasing concentrations of curcumin, which explains its cytotoxicity in plant test system, but there was reduction in the number of chromosomal aberrations significantly suggesting the anti-mutagenic activity of curcumin. Similar results were observed in previous studies about other medicinal plant extracts and the cell death was considered the major depressor of the mitotic index. Since *Allium cepa* test is known to have a good correlation with mammalian test systems (Teixeira *et al.* 2003) further studies on the therapeutic effects of *C. longa* and other related species are of great significance.

Conclusion

The study reveals that rhizome extract of *Curcuma longa* L. not only showed reduction in the root growth but also exhibited mito-depression. It may be concluded that the mitodepressive effect of the rhizome extract is due to the presence of biologically active curcumin stored in the rhizome of this plant extensively used in traditional medicine. It is further revealed that mitodepressive effect is time and concentration dependent, but is safe for therapeutic applications since no chromosome abnormalities were encountered.

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