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Antimitotic activity of hydroethanolic extract of *Coffea arabica* seeds by using *Allium cepa* root tip assay

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Abstract
The present study was carried out to evaluate the antimitotic potential of *Coffea arabica* seeds by standard assay method using *Allium cepa* root meristem model. The result of the study showed that the *Coffea arabica* seeds extract had excellent anti-mitotic activity that was comparable to the standard methotrexate. In Allium assay, aqueous extract of seeds of *Coffea arabica* and methotrexate showed significant concentration dependent inhibitory influence against the dividing cells of Allium roots and decreased root growth, length and mitotic index as compared to control distilled water. Maximum numbers of non-dividing cells were observed in higher dose. As a result of this cells arrest in mitosis and eventually die by apoptosis. There was a marked decrease in the mitotic index. Thus, it can assume the possible mechanism of the anticancer activity of *Coffea arabica* seeds extract may be due to the presence of phenolic compounds in the extract may contribute in cell growth inhibition. Our findings support the reported therapeutic use of *Coffea arabica* seeds as an antimitotic agent in the Indian system of medicine.

Keywords: *Coffea arabica* seeds extract, dividing and non-dividing cells, mitotic index, methotrexate

Introduction

The antimitotic activity was screened using *Allium cepa* root meristematic cells which have been used extensively in screening of drugs with antimitotic activity. *Allium cepa* species (common onion) is ideal for use in bioassays. It has also been widely used for detection of cytostatic, cytotoxic and mutagenic properties of different compounds, including anticancer drugs of plant origin (Iwasaki, 1993) [1]. The roots of all plants have distinguished regions, one of them being the region of cell division that lies beyond the root cap and extends a few mm after that. Cells of this region undergo repeated divisions (Laiha *et al*., 1998) [2]. The rate of cell division is higher in this region compared to that of the other tissues. This region is called the meristematic region (meristos: divided). This division is similar to the cancer division in humans. Hence, these meristematic cells can be used for preliminary screening of drugs with anticancer activity. Even though doubts can be raised about extrapolation of results from plant tissue to animals and finally to humans (Williams and Omoh, 1996) [3]. The search for an appropriate plant for the study is considered not only the phytochemical component but also its traditional use. The present study was carried out to evaluate the antimitotic potential of *Coffea arabica* seeds by standard assay method using *Allium cepa* root meristem model.

2. Materials and methods

Collection and Authentication of *Coffea arabica*

The fresh *Coffea arabica* seeds was collected from Thirupalaikudi, Ramanadhapurm District on June, 2018 and authenticated in ICAR by Dr. N. Kaliaperumal M.Sc., Ph.D., Scientist- in-charge, CMFRI. The collected seeds was washed thoroughly and dried in shade. Then, the dried *Coffea arabica* seeds were powdered and preserved in an airtight container.

Preparation of *Coffea arabica* seeds Extraction

1kg of dried, powdered plant material is extracted with 30:70 proportion of hydro-ethanol for maceration periods (24hrs). The extraction was carried at room temperature with 150 rpm agitation. The extracts were filtered through Whatman filter paper after the macretion period. The extracts were concentrated by using the Rotary Evaporator and the dry weight of the crude extracts was weighed and stored at 4°C in a dark place for further analysis.

Evaluation of antimitotic activity using *Allium cepa* roots

Antimitotic activity study was conducted as per the methods reported by previous workers with modifications (Grant, 1982; Fiskesjo, 1988; Shweta *et al*., 2014) [4, 5, 6].

Exposure to Text Samples

The bulbs with root tips grown up to 2-3 cm were removed from the water and placed on a layer of tissue paper to remove excess of water. The bulbs were divided into four groups. The first group served as control (tap water). Second group: *Allium cepa* roots were dipped in the *Coffea arabica* seeds extract (10 mg/mL). Third group: *Allium cepa* roots were dipped in the *Coffea arabica* seeds extract (20 mg/mL). Fourth group: *Allium cepa* roots were dipped in the

Coffea arabica seeds extract (40 mg/mL). Fifth group: *Allium cepa* roots were dipped in the *Coffea arabica* seeds extract (80 mg/mL). Sixth group: *Allium cepa* roots were dipped in the Methotrexate (0.80 mg/mL), used as a standard control. All the groups were incubated at 25±2°C for 96 hours away from direct sunlight. The test samples were changed daily with fresh ones. The length of roots grown during incubation (newly appearing roots not included), root number and the mitotic index were recorded after 96 hours.

Microscopic Studies and Determination of Mitotic Index

After 96 hours, the root tips were fixed with fixing solution of acetic acid and alcohol (1:3). Squash preparations were made by staining the treated roots with acetocarmine stain (Badria *et al*., 2001) [7]. For each root tip, the numbers of mitotic cells and total meristematic cells were counted manually in 5-8 fields of view using high resolution (100x) bright field light microscopy. The mitotic index was calculated: Mitotic Index = Number of dividing cells/Total number of cells x 100.

Results and discussion

The hydroethanolic extract of *Coffea arabica* seeds produced root decay and decreased the root length and root number significantly at 96 h as compared to control ($p<0.05$). The average root length in control, 10, 20, 40 and 80µg/ml of *Coffea arabica* seeds was 7.30, 6.70, 5.20, 4.70 and 3.20mm at 96 hr respectively. The root numbers in control 10, 20, 40 and 80µg/ml of *Coffea arabica* seeds was 7, 6, 5, 4 and 3 at 96 hr respectively. The mitotic index at 10, 20, 40 and 80µg/ml of *Coffea arabica* seeds was 74.65, 54.43, 42.16 and 31.28% at 96 hr respectively. The highest dose as 80mg/ml of *Coffea arabica* seeds has significant activity in root length and number and near to the standard. These antimitotic activities were supported by mitotic index (Table 1, 2 and Figure 1).

Table 1: Effect of sample on root length and root number of *Allium cepa* roots

Groups	Mean root length (mm)				Mean root Number (s)		
	Before treatment	After treatment	Average root growth	% of root growth inhibition	Before Treatment	After Treatment	Average root number
Group I (Water control)	47.00	54.30	7.30	-	18	25	7
10 µg/ml (T1)	27.10	33.80	6.70	8.21	14	20	6
20 µg/ml (T2)	33.00	38.20	5.20	28.76	21	26	5
40 µg/ml (T3)	41.90	46.60	4.70	35.61	15	19	4
80 µg/ml (T4)	46.50	49.70	3.20	56.16	21	24	3
Std. Methotrexate (0.1mg/ml)	54.40	56.70	2.30	68.49	26	28	2

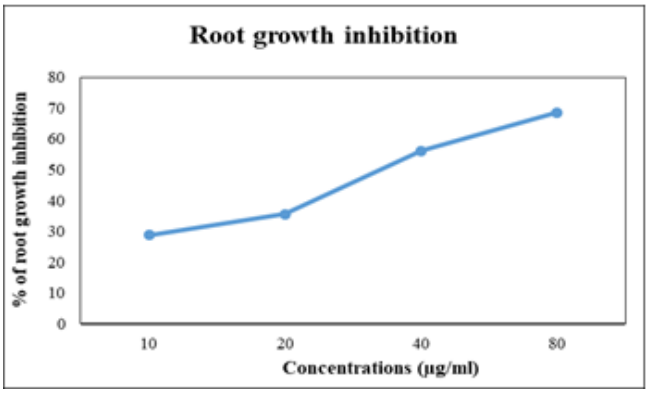

Fig 1: % of growth inhibition on treatment with different concentrations of extract

Table 2: Effect of sample on mitotic index of *Allium cepa* roots

Groups	Dividing cells	Non dividing cells	Total number of cells	Mitotic index (%)
Group I (Water control)	126	16	142	88.73
10 µg/ml (T1)	109	37	146	74.65
20 µg/ml (T2)	86	72	158	54.43
40 µg/ml (T3)	78	107	185	42.16
80 µg/ml (T4)	61	134	195	31.28
Std. Methotrexate (0.1mg/ml)	55	139	194	28.35

Morphometric study on *Allium cepa* roots with different extracts of *Coffea arabica* seeds

The water control shows normal growth with greater root length and numbers. Treatment with different concentrations (10, 20, 40 and 80mg/ml) of *Coffea arabica* seeds extract shows decreased the growth gradually in dose dependent manner. The highest dose (80mg/ml) and standard has significantly reduced the root length and number compared to other doses and near to the standard (Plate 1).

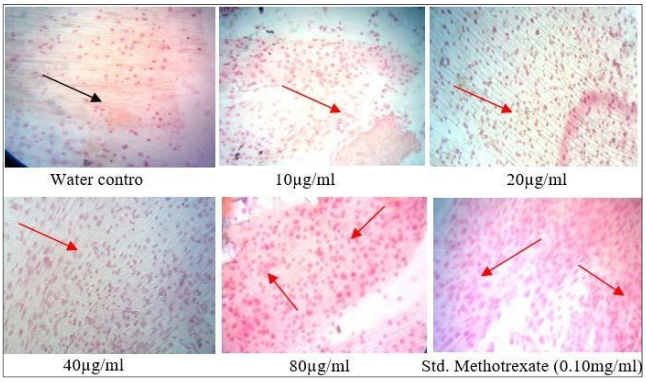

Plate 1: Photomicrograph of extract on mitotic index of Allium cepa root (Red arrow indicates non-dividing cells; Block arrow indicates dividing cells

In *Allium cepa L*. root tip model root system of plant cells is commonly used as a test for investigating environmental pollution factors, toxicity of chemical compounds and evaluating potential anticancer properties. It has been used since 1938. It is very comfortable as it is easy to make preparations of onion roots. They contain rather homogenous meristematic cells, having only 16 chromosomes, which are very long, well visible and get stained easily. The test is a fast and inexpensive method, allowing the investigation of universal mechanisms for meristematic plant cells and extrapolation on animal cells (Kuras *et al*. 2006) [8].

The antimitotic activity was screened using *Allium cepa* root meristamatic cells which have been used extensively in screening of drugs with anticancer activity (Abhang *et al*., 1991; Latha *et al*., 1998) [9, 10]. The roots of all plants have distinguished regions, one of them being the region of cell division that lies beyond the root cap and extends a few mm after that. Cells of this region undergo repeated divisions. The fate of cell division is higher in this region compared to that of the other tissues. This region is called the meristamatic region (meristos: divided) (Dutta, 1971) [11]. This division is similar to the cancer division in humans. Hence, these meristamatic cells can be used for preliminary screening of drugs with anticancer activity. Even though doubts can be raised about extrapolation of results from plant tissue to animals and finally to humans, Khilman has noted that plant cells are 1000 times more resistant to colchicines which is a potent anticarcinogen and which acts by inhibiting the microtubule formation. Thus, it is possible that chemicals that affect plant chromosomes will also affect animals (Williams and O moh, 1996) [3]. *Allium* assay is a rapid, highly sensitive and reproducible bioassay for detecting cytotoxicity.

The antimitotic activity was screened using *Allium cepa* root meristamatic cells which have been used extensively in screening of drugs with anticancer compounds. *Allium* assay is a rapid, highly sensitive and reproducible bioassay for detecting cytotoxicity. Cytotoxicity at all concentrations test extract were evidenced by evaluating macroscopic parameters, i.e., reduction in root number and root length both of which were indicative of root growth inhibition. In the present study mitotic index of different concentrations of extract clearly indicates the efficiency in the inhibition of growth of cancer cells either by affecting microtubules or encouraging microtubule formation, and thus stopping the microtubules from being broken down. This makes the cells become so clogged with microtubules that they cannot continue to grow and divide. As a result of this cells arrest in mitosis and eventually die by apoptosis. Growth inhibition effect may be due to diminished cell division (Fiskesjo, 1988) [5]. Therefore, it is evident that the plant extracts possess antimitotic activity. Similar reports were observed in Shweta *et al*. (2013, 2014) [12, 6] and Shalini and Velavan, (2017) [13].

Several authors demonstrated that reduction in MI may be the result of their cytotoxic effects; either disturbances or blockage during the synthesis of DNA or G2 phase of the cell cycle or the insufficient synthesis of ATP during spindle fiber formation and elongation therefore can inhibit the cells from further division (Sreeranjini and Siril 2011) [14]. Earlier reports also indicated that plant extracts having MI reducing effect can be regarded as cyto-genotoxic (Frescura *et al*. 2013, Khanna and Sharma 2013) [15, 16]. Therefore, reduction in the mitotic index is an important parameter that can be implemented to examine the antimitotic as well as cyto-genotoxic effects of biochemicals. The present study revealed that treatment of *Allium cepa* root meristems with extracts containing both polar and non-polar fractions of *Coffea arabica* seeds extract had a de trimental effect on the test material *Allium cepa*. Treatment not only brought down the frequency of dividing cells but also produced a good number of anomalies in the mitoticcells. There was a marked decrease in the mitotic index.

Conclusion

From result, it was conclude that hydroethanolic extract of seed of *Coffea arabica* inhibits cell division in *Allium cepa* assays and suggests that the seeds may exhibit inhibitory influence on abnormal cell growth as like in cancer. Though the present study validates the traditional use of extract in the treatment of cancer. Further studies could be carried out using cancer cell lines and mammalian cancer cells are necessary to encourage its consumption as a medicine.further studies in cancer cell lines is necessary.

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