Original Research Paper

Volume-7 | Issue-12 | December-2017 | ISSN - 2249-555X | IF : 4.894 | IC Value : 86.18

Biochemistry

EVALUATION OF ANTIMITOTIC ACTIVITY OF *APLOTAXIS AURICULATA* RHIZOMES USING *ALLIUM CEPA ROOT* MERISTAMATIC CELLS

R Shalini — Research Scholar, PG and Research Department of Biochemistry, Marudupandiyar College, Tamil Nadu, India

S Velavan* — Associate professor, PG and Research Department of Biochemistry, Marudupandiyar College, Tamil Nadu. India *Corresponding Author

ABSTRACT The aim of this work was investigate the antimitotic activity of *Aplotaxis auriculata* rhizomes. The inhibitory effect of methanolic extract of *Aplotaxis auriculata* rhizome was evaluated on the growth and mitotic activity of *A. cepa* root meristems and the effect was compared with standard anticancer drug Methotrexate. A progressive increase in root length (32.40mm), root numbers (18) and mitotic index (85.17%) observed in control group after 96 hrs of experimental period. The methanolic extract of *Aplotaxis auriculata* rhizome and Methotrexate produced root decay and decreased the root length and root number significantly at 96 h as compared to control ($p<0.05$). Maximum numbers of non-dividing cells were observed. *Aplotaxis auriculata* rhizome has significant activity in root length, number and mitotic index and near to the standard.

KEYWORDS : *Aplotaxis auriculata* rhizome, *Allium Cepa*, Antimitotic activity.

INTRODUCTION

Medicinal herbs have been used in folk medicine for millennia. Simply, in recent times, scientific study of their effects has flourished. Nevertheless, some of them can cause adverse effects or have the potential to interact with other medications; moreover, there is little information on the potential risk to health of such herbs. Based on their longterm use by humans, one might expect herbs used in traditional medicine to have low toxicity (Celik et al. 2010).

The general principles of the mechanisms of mitosis are best and most easily studied in the actively growing regions of plants such as a shoot or root apex. Frequently, such studies involve the use of chemicals which modify the normal course of mitosis (Nwakanma et al.2010). A wide variety of secondary metabolites obtained from plants are tested for their ability to treat cancer. Various anticancer drugs from plants are known to be effective against proliferating cells. They exhibit cytotoxic effect by interfering with cell-cycle kinetics. These drugs are effective against cells that are proliferating and produce cytotoxic effect either by damaging the DNA during the S-phase of the cell cycle or by blocking the formation of the mitotic spindle in M-phase. However most of the cytotoxic drugs exhibit side effects, and hence, there is a need for drugs that are efficient and have less side effects (Sehgal et al. 2006).

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). The aim of this work was investigate the antimitotic activity of Aplotaxis *auriculata* rhizomes.

MATERIALS METHODS

Plant materials

The *Aplotaxis auriculata* rhizomes were collected in January 2015 from Kolli hills, Nammakal District, Tamil Nadu, India from a single herb. The rhizomes were identified and authenticated by Dr. S. John Britto, The Director, the Rabiant Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of alcoholic extract

The collected *Aplotaxis auriculata* rhizomes were washed several times with distilled water to remove the traces of impurities from the rhizomes. Then examined carefully old, infected and fungus damaged portion of the rhizomes were removed. Healthy rhizomes were spread out in a plain paper and shade dried at room temperature for about 10 days and ground in to fine powder using mechanical grinder. 25gms of the powder of rhizomes was transferred into different conical flask (250ml). The conical flask containing 100ml of Alcoholic solvents. The conical flask containing rhizomes powder and solvent was shaked it well for 2 hours by free hand. After 1 day, the extracts were filtered using Whatmann filter paper No.1. and was transferred into china dish. The supernatant was completely removed by keeping the china dish over water bath at 45°C.The obtained extracts were stored at 4°C in air tight bottle until further use.

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).

***Allium cepa* Bulbs:** Approximately equal size bulbs (40±10 g) of the onions (*Allium cepa* L.) were obtained from the local vegetable market at Thanjavur, Tamil Nadu, India. Any onions that were dry, moldy or have started shooting green leaves were discarded.

Growing *Allium cepa* Meristems: The outer scales were removed from the healthy onion bulbs leaving the root primordia intact. These bulbs were grown in dark for 48 h over 100 ml of tap water at ambient temperature until the roots have grown to approximately 3 cm. The water was changed daily during this period. The viable bulbs were then selected and used for subsequent studies.

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 *Aplotaxis auriculata* rhizome extract (10 mg/mL). Third group: *Allium cepa* roots were dipped in the *Aplotaxis auriculata* rhizome extract (20 mg/mL). Fourth group: *Allium cepa* roots were dipped in the *Aplotaxis auriculata* rhizome extract (30 mg/mL). Fifth group: *Allium cepa* roots were dipped in the *Aplotaxis auriculata* rhizome extract (40 mg/mL). Sixth group: *Allium cepa* roots were dipped in the Methotrexate (0.10 mg/mL) was used as a standard control. All the groups were incubated at 25±2°C for 96 h 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 h.

Microscopic Studies and Determination of Mitotic Index:

After 96 h, 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). 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 as Mitotic Index = Number of dividing cells/Total number of cells x 100.

RESULTS

Antimitotic activity of *Aplotaxis auriculata* rhizome using *Allium cepa* root meristamatic cells

The antimitotic activity was screened using *Allium cepa* root meristamatic cells which have been used extensively in screening of drugs with antimitotic activity. The inhibitory effect of methanolic extract of *Aplotaxis auriculata* rhizome was evaluated on the growth and mitotic activity of *A. cepa* root meristems and the effect was compared with standard anticancer drug Methotrexate. A progressive increase in root length (32.40mm), root numbers (18) and mitotic index (85.17%) observed in control group after 96 hrs of experimental period.

The methanolic extract of *Aplotaxis auriculata* rhizome and Methotrexate produced root decay and decreased the root length and root number significantly at 96 h as compared to control ($p<0.05$). The root length at 10, 20, 30 and 40mg/mL of *Aplotaxis auriculata* rhizome was 28.50 ± 5.98mm, 25.30 ± 1.56mm, 22.21±1.32mm and 19.35±1.21mm at 96 hr respectively while standard shows 20.20 ± 1.62mm. The root numbers at 10, 20, 30 and 40mg/mL of *Aplotaxis auriculata* rhizome was 16, 13, 10 and 09 at 96 hr respectively while standard shows 10 numbers. The mitotic index at 10, 20, 30 and 40mg/mL of *Aplotaxis auriculata* rhizome was 72.45, 58.24, 43.92 and 34.13% at 96 hr respectively while standard shows 36.06%. The highest dose as40mg/mL of *Aplotaxis auriculata* rhizome has significant activity in root length, number and mitotic index and near to the standard.

Table.1: Effect of *Aplotaxis auriculata* on root length, root number and mitotic index of *Allium cepa* roots

Groups	Mean root length (mm)	Mean root Number (s)	Mitotic index (%)
Group I (Water control)	32.40 ±2.26	18±1.25.	85.71
10 mg/ml of *Aplotaxis auriculata* rhizome extract	28.50 ± 5.98	16±1.02	72.45
20 mg/ml of *Aplotaxis auriculata* rhizome extract	25.30 ± 1.56	13±0.85	58.24
30 mg/ml of *Aplotaxis auriculata* rhizome extract	22.21±1.32	10±0.70	43.92
40 mg/ml of *Aplotaxis auriculata* rhizome extract	19.35±1.21	09±0.63	34.13
Group IV (Std. Methotrexate (0.1mg/ml))	20.20 ± 1.62	10±0.70	36.06

Values are expressed as Mean± SD for triplicates

4.12 Morphometric study on *Allium cepa* roots with different extracts of *Aplotaxis auriculata*

The water control shows normal growth with greater root length and numbers. Treatment with different concentrations (10, 20, 30 and 40mg/mL) of *Aplotaxis auriculata* rhizome extract shows decreased the growth gradually in dose dependent manner. The highest dose (40mg/mL) and standard has significantly reduced the root length and number compared to other doses and near to the standard (Plate 1).

Plate.1: Treatment of *Allium cepa* roots with different extracts of *Aplotaxis auriculata*

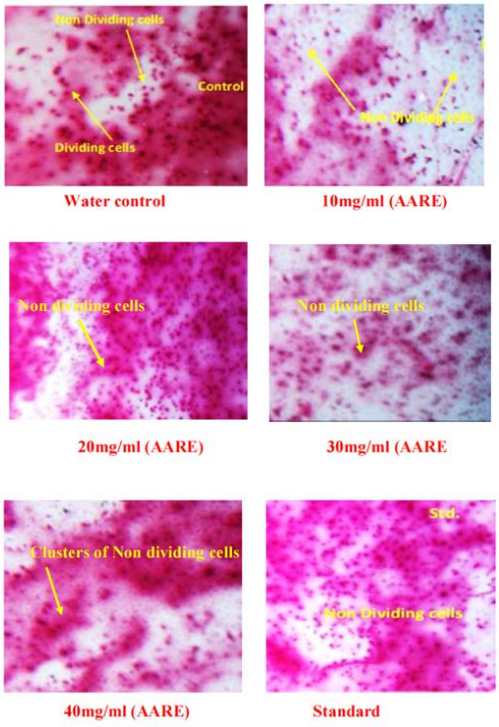

Plate.2: Photomicrograph of *Aplotaxis auriculata* extract on mitotic index of *Allium cepa* roots.

DISCUSSION

Antimitotic activity of *Aplotaxis auriculata* rhizome using *Allium cepa* root meristamatic cells

The antimitotic activity was screened using *Allium cepa* root meristamatic cells which have been used extensively in screening of drugs with antimitotic activity (Abhang et al., 1991; Latha et al., 1998) 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) This division is similar to the above mentioned 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). *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. 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. Phytosterol has effect on apoptosis. The rate of tumor growth is dependent upon a balance between the rates of cell proliferation and apoptosis. Apoptosis is a programmed cell death, as influenced by phytosterol (Awad et al., 2000). Hence, the sterols from *Aplotaxis auriculata* rhizome must be contributing to the anticancer potential of the herb.

The result from the study showed that the extract of *Aplotaxis auriculata* rhizome had excellent anti-mitotic activity that was comparable to the activity of methotrexate. Maximum numbers of

non-dividing cells were observed. As a result of this cells arrest in mitosis and eventually die by apoptosis. Similar reports were observed in Shweta et al. (2013, 2014) and Thenmozhi and Mahadeva Rao, (2011). Thus, it can assume the possible mechanism of the anticancer activity of *Aplotaxis auriculata* rhizome may be due to the presence of triterpenoids and phenolic compounds in the extracts. It may be further concluded that the presence of Kaempferol, Quercetin, Ellagic acid, Gentisic acid, Epicatechin and other similar phytoconstituents present in the extract may contribute in cell growth inhibition. Our findings support the reported therapeutic use of this plant as a antimitotic or anticancer agent in the Indian system of medicine.

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