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EVALUATION OF ANTIMITOTIC EFFECT OF *CALOTROPIS PROCERA* L ON *ALLIUM CEPA* L

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ABSTRACT

Calotropis procera is a tropical medicinal plant known for its multiple curative effects on wide range of diseases. Current study has evaluated the antimitotic activity of the extracts of leaf and latex of *C. procera* on root meristem cells of *Allium cepa.* Onion bulbs were allowed to grow roots on moist cotton imbibed with solutions of test sample with known concentration of the extracts. Mitotic index was estimated in squash preparation of root tips collected after 72 hrs and compared with control. Reduction in mitotic index of the target cells was observed to varying extent among the treatments. Chloroform extract of the latex showed highest level of inhibition of cell division and ethyl acetate extract of the latex showed least inhibition, while chloroform extract of the leaf did not interfered with cell division. Outcomes of the study have indicated presence of potential anticancer component in *C. procera.*

Keywords: *Allium cepa*, *Calotropis*, extract, mitotic index.

INTRODUCTION

Medicinal plants are source for innumerable number of bioactive compounds, inducing diverse responses among living organisms. Plant extracts are used for the treatment of wide range of ailments[1,2]. Crude extracts, purified bioactive compounds or their synthetic versions are being used as therapeutics. For the last few decades, phytochemistry has been making rapid progress and herbal products are becoming popular owing to their efficiency and safer mode of action. Anti cell proliferative activity is one of the effects studied for probing the anticancer potential of prospective crude drugs [3,4]. Root meristem of onion (*Allium sepa*) is a model system often adopted for evaluating the cytotoxicity and antiproliferative activity of plant extracts [5,6,7].

Calotropis procera is a tropical plant of the family, Apocynaceae and native to North Africa, Tropical Africa, Western Asia, South Asia, and Indochina. *C. procera* produces milky latex abundantly, which contains combination of enzymes and glycosides [8]. These are concerned with defense mechanism of the plant against pathogens and pests. Latex of the plant has been reported to exhibit cytotoxic effect on different model systems [3,8,9,10]. Current investigation has been carried out to compare the effect of latex and leaf extracts of *C. procera* on root meristem of *A. cepa*.

MATERIALS AND METHODS

Leaves of *C. procera* were collected from AIT, Bangalore campus, washed in tap water and shade dried for about two weeks at room temperature. The dried leaves were powdered in a mixer grinder and kept in air tight containers for further use. Latex from *C. procera* was collected from the wound created on the plants while plucking the leaves. Fresh latex was stored in a refrigerator until further use.

Preparation of extracts: Methanol, chloroform and ethyl acetate extracts of the leaf and latex were prepared as described below.

Methanol extract: 100g of dried powdered leaf and latex were percolated in 300 ml methanol (100%) separately in 1 liter conical flask and shaken well for 4 hours. The mixtures were filtered through Whatmann filter paper No. 1 The filtrate was poured into porcelain crucibles and kept on a water bath at 80°C, until all the solvent was evaporated. The residues were collected weighed and stored at room temperature. These samples are called hereafter as Leaf Methanol Extract [LFM] and Latex Methanol Extract [LTM].

Chloroform extract: 5g of LFM and LTM were taken in separate conical flasks (250 ml) and 100 ml of chloroform was added to each. This mixture was shaken well for 30 min and allowed to settle in separating funnel. Two layers were formed. The lower layer was discarded and the upper layer was collected into a porcelain crucible. The solvent was allowed to evaporate at room temperature and the acquired fractions are called as Leaf Chloroform Extract [LFC] and Latex Chloroform Extract [LTC] respectively hearafter.

Ethyl acetate extract: 5g of LFM and LTM were taken in separate conical flasks (250 ml) and 100 ml of ethyl acetate was added to each. This mixture was shaken well for 30 min and allowed to settle in separating funnel. Two layers were formed. The lower layer was discarded and the upper layer was collected into a porcelain crucibles. The crucibles were heated on a water bath set at 80°C until the solvent evaporated. No residue was available in LFM. Residue obtained from fractionation of LTM is called as Latex Ethyl acetate Extract [LTE].

Bioassay on onion: Fresh onions purchased from local market were used for the experiment. Onions were kept on cotton pads soaked in tap water (CONTROL) and in known dilution (.05mg/ml) of individual extracts (TEST) under room temperature in the laboratory and monitored for root development. Newly formed roots were cut carefully from the control and experimental sets of onions separately after 72 hrs [0.5 -1.0cm] and dipped in 1N HCL for 5 minute, stained with aceto-orecin and heat fixed. The heat fixed samples were kept undisturbed for about 15-20 minutes. Root tips were placed on clean slide. Extreme tips were cut using forceps and needle. The root tips were pressed by thumb after placing cover slip. The slides were viewed carefully under microscope to identify dividing cells. Mitotic index was calculated as the percent of meristem cells undergoing mitosis using the following formula:

Mitotic index [MI] = (No. of cells dividing / Total No. of cells) * 100

RESULTS AND DISCUSSION

Effect of the test samples on root meristem of *A. cepa* is illustrated in Fig. 1. Root meristem cells in the control (Figure – 1a) were of normal shape, vigor and exhibiting different stages of mitosis. Among the five extracts tested, no visible change on cells or nuclei was observed in treatment with LFC (Figure – 1c) to that of control. Root meristem exposed to LFM (Figure – 1b) showed slightly elongated cells with many of them continue to remain in prophase stage of mitosis. Cells of meristem treated with LTM (Figure – 1d) also exhibited change in their shape with elongated appearance. Number of dividing cells was comparatively less and most of the dividing cells remained at early stage of mitosis for prolonged time. Effect of treatment of the root meristem with LTE (Figure – 1e) showed slight change in cell shape with elongated appearance. Treatment with LTC showed (Figure – 1f) marked difference in cell morphology with highly elongated cells. Dividing cells were rarely observed in this treatment. Comparative evaluation of the antimitotic effects of the leaf and latex extracts is illustrated in Fig.5. Among the five extracts tested, LTC recorded lowest mitotic index indicating highest level interference with cell division of the target plant while LFC recorded mitotic index similar to that of control, thereby indicating lack of influence on cell division of the plant.

Current study has confirmed the negative influence of four out of five extracts of *C. procera* on mitotic division of *A. cepa*. From the average mitotic index values obtained, it can be inferred that LTC has maximum inhibitory effect followed by LTM, LFM and LTE. LFC recorded no inhibitory effect on cell division of the plant. Sehgal *et al* [5] had recorded 20-30% reduction in mitotic index of the root meristem of *A. cepa* using dried latex of *C. procera* at a concentration of 1mg/ml at 48 and 96 hrs of treatment. In another similar investigation Kumar and Singhal[11] have reported significant reduction in mitotic index of the radical tip meristem of *Vigna radiata* by treating with LTM at concentration of 0.1mg/ml for 48 hrs. Current study has recorded higher levels of mitotic inhibition at concentration (0.05mg/mL) below that of the above studies using chloroform extract of the fresh latex (LTC). Further, the study has also confirmed antimitotic activity of the methanol extract of the leaf extract (i. e. LFM,).

CONCLUSION

It can be concluded that the antimitotic component ~~of~~ present in *C. procera* exists in both leaves and latex and the intensity of activity of the latex is more when compared to leaf. The component can be partially purified through sequential extraction of the latex with methanol and chloroform. Further studies on identification of the active component would help in revealing a pharmaceutically important compound with potential use in cancer treatment.

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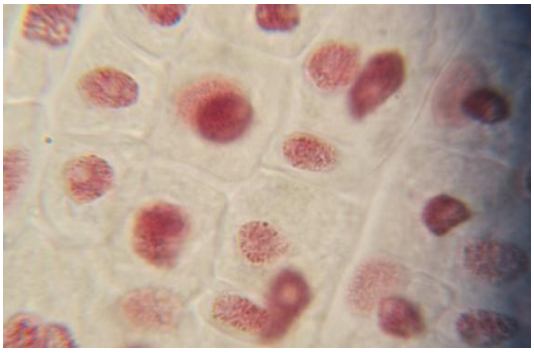
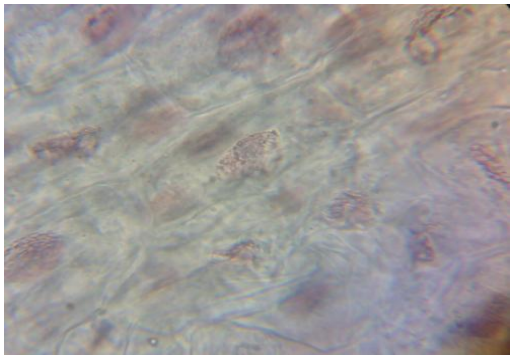
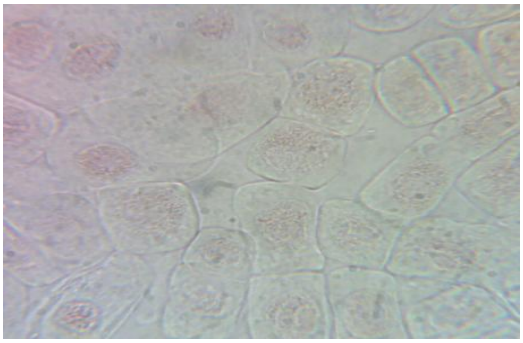


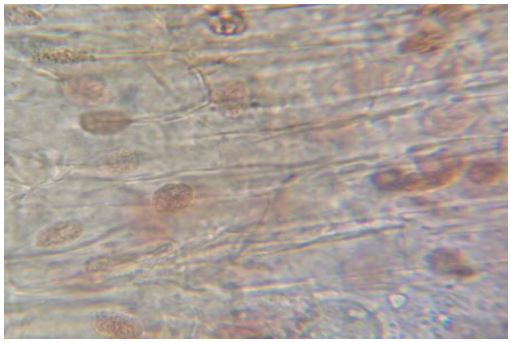
a. Control



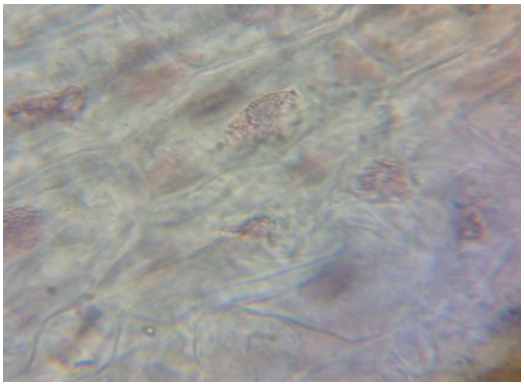
b. Effect of LFM



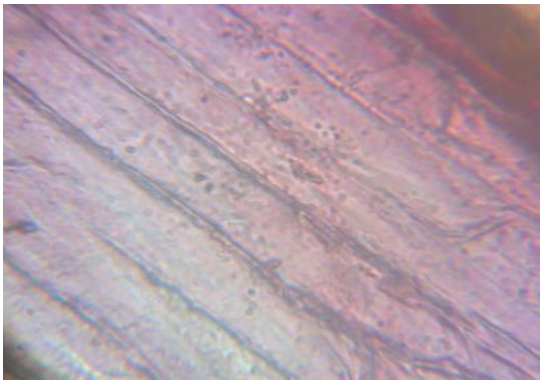
c. Effect of LFC.



d. Effect of LTM



e. Effect of LTC LTE.



f. Effect of LTC

Fig.1. Microscopic view of root meristem cells of *A. cepa*.

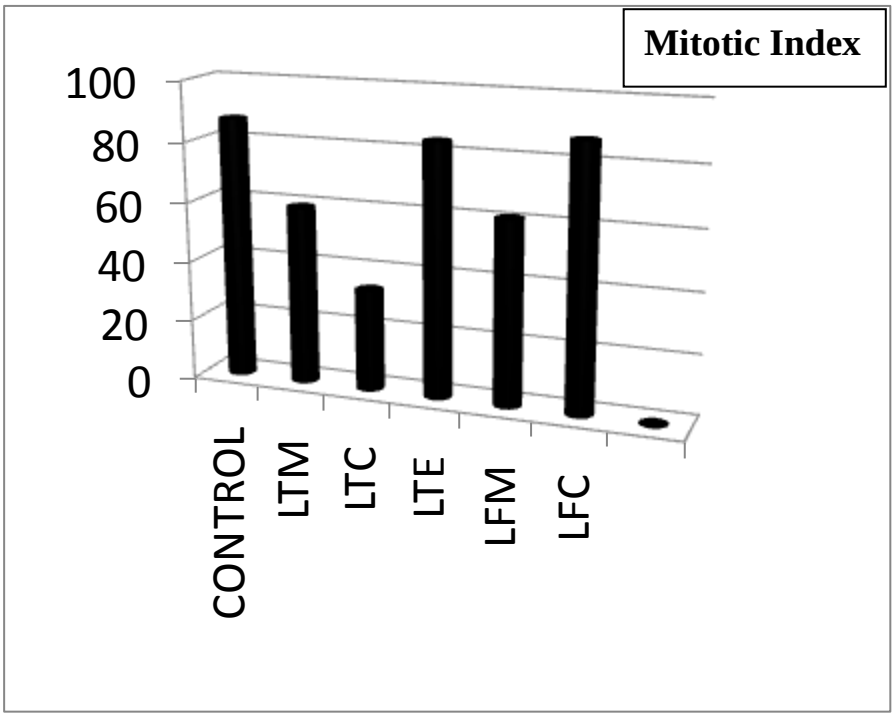


Fig.2: Comparison of average mitotic indices of control and test samples.

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