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Evaluation of Antimitogenic and Cytotoxic Potential of *Anacardiumoccidentale* Leaf Extracts in *Allumcepa* Root Tip Cells and Against Sarcoma-180 Cells

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Abstract: ***Anacardium occidentale of family Anacardiaceae is a tropical tree and used traditionally for the treatment of different diseases. In the present study, we have evaluated the anti-mitotic and cytotoxic activities of ethanolic (EEAOL), methanolic (MEAOL) and water (WEAOL) extracts of Anacardium occidentaleleaf in Allium cepa root tip cells and against Sarcoma-180 cell lines. Ethanol, methanol, and water extracts were prepared from pulverized powder obtained from shed-dried leaves of Anacardium occidentale. The antimitotic activity was measured using Allium cepa root meristematic cells. The studies were extended to Sarcoma-180 cell lines to obtain the cytotoxic potential of the plant. Anacardium occidentaleextracts were found to be antimitotic for Allium cepa root tip cells at the concentration of 100mg/ml and also exhibited in vitro cytotoxicity against Sarcoma-180 cell lines at100 µg/ml concentration. These findings reveal that Anacardium occidentale leaf possesses pronounced antimitotic and cytotoxic potential in Alliumcepa root and against Sarcoma-180 cell lines.***

Keywords: *Anacardium occidentale*; *Allium cepa*; S-180 cell; Antimitotic; Cytotoxic.

1. Introduction

Cancer is characterized by uncontrolled cell growth and malignant behavior. It is thought that cancer is mainly caused by the interaction between genetic susceptibility and environmental factors [1]. The mitosis occurs in both somatic and gamet cells and is responsible for the multiplication of cell number [2]. The use of plant-based medicines are believed to date back to prehistoric medicine[3].Antimitotic agents constitute a major class of cytotoxic drugs and among them plant-derived compounds such as paclitaxel, vincristine, and combretastatin are included [4].Current anticancer therapeutic strategy comprises multiple ways of intervention and cytotoxic drugs remain a mainstay in cancer chemotherapy for the next future[5]. Most of the plant derived anti-cancer drugs affect the microtubule dynamics of the cell and induce modification of biological processes and signaling pathways that ultimately lead to apoptotic death [6].

Anacardium occidentale. belongs to the family *Anacardiaceae*. It is popularly known as cashew. In folk medicine the extracts of the leaf, bark and root of the plant were widely used. Cashew is a tropical tree native to Brazil and now it is also widely grown in other tropical countries like India. Several studies reported this tree's action as an antiseptic, anti-diarrheal [7], anti-ulcerative [8]anti-diabetic [9], cough suppressant, diuretic, febrifuge, refrigerant, astringent and antibacterial agent [10]. The active principles are thought to be tannins, anacardic acid, and cardol.

Anti-mitotic drugs are used extensively for the treatment of cancer. *Allium* has proven a rapid, reliable, and inexpensive system by which the antimitotic effects of various chemical compounds may be monitored [11, 12].Characterized by rather homogenous meristematic cells, very large chromosomes and only sixteen chromosome numbers, the *Allium cepa* species (common onion) is ideal for use in bioassays [13]. It has also been widely used for detection of cytostatic, cytotoxic and mutagenic properties of different compounds, including anticancer drugs of plant origin [14].

Thus, the present study is aimed to search out the antimitotic activity and cytotoxic potential ofethanolic, methanolic and water extract of *Anacardium occidentale* leaf in *Allium cepa* root tip cells and against Sarcoma-180 cell lines.

Allium test could be useful in correlating the antimitotic effect of the plant in *Allium cepa* with that of mammalian cells as it is reported that Allium test shows good correlation with mammalian test systems [15].

2. Materials and Methods

2.1. Plant Material

At first, leaves of the *Anacardium occidentale* were collected from Vidyasagar University campus, Midnapore, West Bengal, India, during morning period in the month of March, and authenticated by the Department of Botany,Vidyasagar University, West Bengal, India.

2.2. Preparation of Extract

At first, leaves of the *Anacardium occidantale* were dried in the shade at room temperature. Then it was allowed to crush and 500 g of powder was suspended in 750 ml of each solvent (ethanol, methanol and water) for 72 hours in room temperature. The extracts were then filtered through filter paper separately. The filtrate was concentrated with a rotary evaporator at 40^0C under reduced pressure.The concentrated

filtrates were poured in petridishes and then were incubated at 37^0C for drying to produce crude ethanolic and methanolic extracts. Water extract was obtained by lyophilizing the filtrate for the removal of water. The extracts were then used for the antimitotic and ***in vitro*** cytotoxicity assay.

2.3. Tumour Cells

Sarcoma-180 (S-180) cells were collected from the Department of Biotechnology, Indian Institute of Technology, Kharagpur. Cells were maintained by intraperitoneal inoculation of 1×10^6 cells/mice [16] and developed a milky white fluid containing rounded tumor cells.

2.4. Study of Mitotic index[17]

The red variety of *Allium cepa* were collected from local market, the bulbs of *Allium cepa*were sprouted in water saturated sand tub for 3 to 4 day at room temperature. The roots thus developed were treated by dipping these in the aqueous, methanolic and ethanolic solution of *Anacardium occidentale* leaf extract at the concentration 100mg/ml for 24 hours. Treatment of roots with distilled water served as control. The roots thus treated with above solutions were then cut to separate root tips and the root tips are transferred to fixing solution, 1:3v/v aceto-alcohol for 1 hour in room temperature. Then the root tips were taken out and preserved in 70% alcohol in refrigerator. At the time of staining, the root tips were taken out from 70% alcohol and immerged in 2% acetorcine and 1NHCl and boiled at the smearing point. Then the root tips were placed on graze free slide added with 2%acetic acid, covered with cover slip then squashed to prepare smear and observed under microscope. The numbers of cells in each stage of cell division were counted in four fields for each group.

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Number of Total cells}} \times 100$$

2.5. Study of Short-Term in Vitro Cytotoxicity: [18]

Short term cytotoxic activity of MEAOL, EEAOL, and WEAOL were assayed by determining the percentage viability of Sarcoma-180 cells using the trypan blue dye exclusion technique (by Modified Dongre S H. et al). Sarcoma-180 cells were cultured in healthy albino mice weighing between 20-28 g by serial intraperitoneal inoculation of a suspension of Sarcoma-180 cell (1×10^6 cells/ml). The cells were aspirated aseptically from the peritoneal cavity of mice on 18^{th} day of inoculation and washed with PBS and centrifuged for 5 min. at 1000 rpm in a cooling centrifuge. The pellet was re-suspended with RPMI 1640 medium (10% FBS and antibiotic solution) and seeded in flat bottom culture plate with MEAOL EEAOL and WEAOL at the concentration of 100 μg/1×10^6cell/ml. The culture plates were incubated for 3hrs at 37^oC and 5% CO_2.Then trypan blue dye exclusion technique was performed to determine the percentage of viability at the end of 1^{st}, 2^{nd} and 3^{rd} hrs. The dead cells failed to exclude the stain and appeared in blue colour. The percentage viability was calculated by counting number of viable cells/100 cells in each microscopic field.

2.6. Statistical Analysis

The values are given as mean ±SEM and the data was analyzed by Student's t-test. P values less than 0.05 were considered statistically significant.

3. Results

The effects of EEAOL, MEAOL and WEAOL on percentage of cells in different stages of mitosis and mitotic index in *Allium cepa* root tips areshown in Fig-1(A, B). All the treated group shows significantly reduced number of dividing cell compared to water treated group. The mitotic index of EEAOL, MEAOL and WEAOL treated group and water control group were 40, 30, 30, and 51.58 respectively.

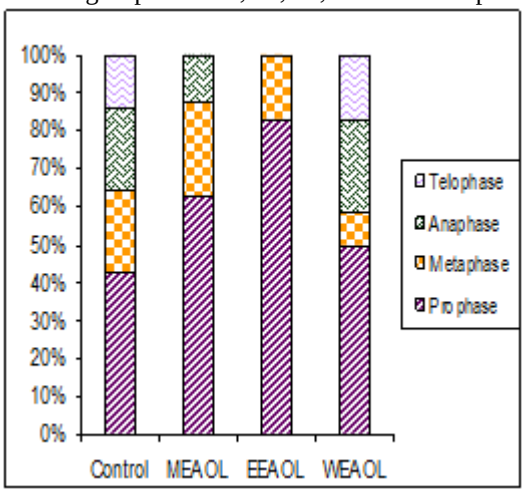

Figure 1A

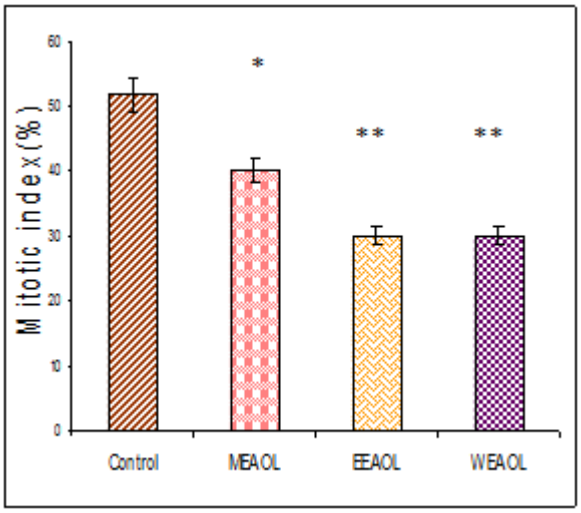

Figure 1B

Fig-1. The bar diagram shows the effect of EEAOL, MEAOL and WEAOL on % of cells in different stages of mitosis (Fig.1, A) and mitotic index (Fig.1, B) in *Allium cepa* root tips.Data are expressed as mean ± SEM. Probability values are given in asterisks. * indicates P< 0.05, ** indicates p<0.01; values are taken in respect of control.

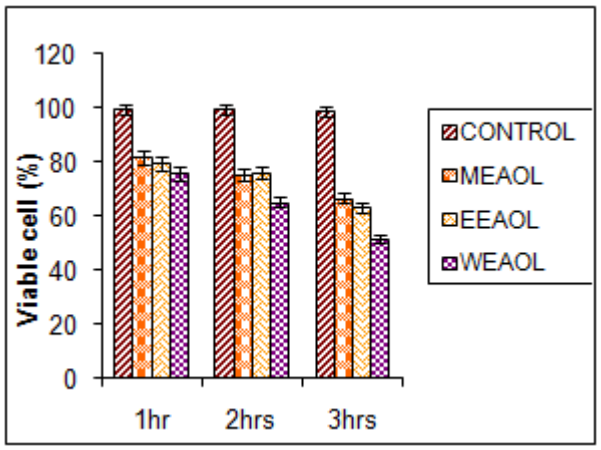

Fig-2. The bar diagram shows the effect of MEAOL, EEAOL, and WEAOL on in vitro short term cytotoxicity against S-180 cells. Data are expressed as mean ± SEM. Probability values are given in asterisks. * indicates P< 0.05, ** indicates p<0.01; values are taken in respect of tumour control.

The cytotoxic activity of MEAOL, EEAOL and WEAOL against S-180 cells is shown in Fig-2. The MEAOL, EEAOL and WEAOL exhibited a time-dependent cytotoxic effect on the S-180 cells at the concentration of 100µg/ml. Less numbers of viable S-180 cells were observed in MEAOL, EEAOL and WEAOL treated groups compared to S-180 control (tumourcontrol) group. Among the three extracts, WEAOL showed maximum cytotoxic effect after three hours of treatment.

4. Discussion

The present study showed that MEAOL, EEAOL and WEAOL had prominent antimitotic activity. The maximum percentage of root tips cells of the treated groups were observed to be in prophase indicating inhibition of transition from prophase to metaphase and subsequent phases. Maximum numbers of non-dividing cells were observed in the extract treated group compared to the control group. The extracts of *Anacardiumoccidentale* leaf seem to stay at prophase stage of cell division. Mitotic index of root tips of *Allium cepa*treated with EEAOL, MEAOL and WEAOL showed significantly lower mitotic index compared to control group, among the three extracts the EEAOL showed the highest degree of suppression of cell division. A group of anticancer drugs competitively inhibit enzyme that participates in DNA and RNA synthesis, and thus cytotoxic drugs suppress the cell cycle [19]. Logically, theymay possessa greater toxiceffect on rapidly dividing cells such as malignant and myeloid cells [20].*Anacardium*as a medicinal plant contain various flavonoids, alkaloids, etc. These chemicals may exhibit antimitotic property [21], which may suppress the cell division of another species like *Allium cepa*.

In the present study the cytotoxic property of MEAOL EEAOL, and WEAOL was assayed on S-180 cells by trypan blue dye exclusion technique. The extracts were also effective in decreasing the cell viability of Sarcoma-180 cells. The result showed that WEAOL exhibited more cell death compared to MEAOL and EEAOL in time-depended manner. The extracts may possess some active components, which showed prominent anti-tumour effect. Anti-tumor drugs that interact with microtubules and tubulin are known to block mitosis and induce cell death by apoptosis [22].The extracts may mediatethe effects through other mechanisms also. Those include restriction in RNA and or DNA synthesis, DNA damage, loss of membrane integrity, breakdown of cytoskeleton etc. [23]. Moreover the activecompounds present in extracts may bind with different cell proteins, which are responsible for cell division and may make them inactive[24].

Apoptosis is a critical molecular target for the prevention of cancer [25]. The potential use of *Anacardiumoccidentale*leaves as therapeutic agent holds great hope for the isolation of one or more cytotoxic chemicals from crude extracts and the judicious use of such chemicals can control the progression of cancer and also can prevent the formation of tumour in susceptible individuals.

Declaration of Interest

Authors declare that there are no conflicts of interests.

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