



# EVALUATION OF THE ANTICANCER ACTIVITY OF ADIANTUM INCISUM EXTRACT

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## ABSTRACTS

*Malignant diseases like cancer are characterised by the uncontrolled and rapid proliferation of aberrant cells that can either cluster into growths or tumours or spread throughout the body. Cancer is the second leading cause of death in humans, after heart disease. Adiantum incisum Don will be subjected to an initial phytochemical screening and anticancer assessment in this study against an animal model of Ehrlich ascites carcinoma. The results show that A. incisum Don's ethanolic extract has strong anticancer action and lowers increased levels of lipid peroxidation because it contains terpenoids and flavonoids. As a result, A. incisum Don's ethanolic extract may have numerous therapeutic uses for cancer. The paper represents the anticancer activity of methanol extract of Adiantum incisum (MEAI) entire plant was evaluated using the EAC cells which are known as (Ehrlich Ascites Carcinoma) on Swiss albino mice.*

**Keywords:** Trypan blue, MTT assay, EAC, Antioxidant, Tumor, Histopathology.

## I. Introduction

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Cancer, the second leading cause of death worldwide next to cardiovascular diseases, is a group of more than 100 different diseases, characterized by uncontrolled cellular growth, local tissue invasion and distant metastases. (Dashora NV *et al.*, 2010). In most organs and tissues of a mature animal, a balance is usually maintained between cell renewal and cell death. The various types of mature cells in the body have a given lifespan; as these cells die, new cells are generated by the proliferation of new cells is regulated so that the number of any particular type of cell remains constant. However under certain some abnormal cells (*AL-Bala et al.*, 2012) are able to evade this controlled or programmed process of cell death (apoptosis). It can spread to almost any part of the living system. There are four characteristics of cancer cells that distinguish them from normal cells are uncontrolled proliferation, dedifferentiation and loss of function, invasiveness and metastasis. Altered cells divide uncontrollably to form lumps or masses of tissue called tumors. A tumor that is not capable of indefinite growth and does not invade the healthy surrounding tissue extensively is benign. A tumor that continues to grow and becomes progressively invasive is malignant; the term cancer refers specifically to a malignant tumor. In addition to uncontrolled growth, malignant tumors exhibit metastasis (Kuby *et al.*, 2003) in this process, small clusters of cancerous cells from the initial or primary tumor have been released known as secondary tumor, have reached other sites through lymphatic's and blood vessels, and are carried to other tissues, where they continue to proliferate. Radiation and Chemotherapy are the best method of cancer treatment. Chemotherapy is curative for some cancers, such as some leukemia's, ineffective in some brain tumors, and needless in others, such as most non-melanoma skin cancers too. A major challenge for medical



oncology is to develop therapeutic treatment that will prevent toxicity induced by antitumor treatments without impairing their antitumor effects (Das *et al.*, 2012). In the new way now researchers have recently focused on the uses of Ehrlich's ascites carcinoma (EAC) cells on the local plants which can cure cancer. The Ehrlich tumor was rapidly growing, very aggressive behavior and can grow in almost everywhere in the mice's strains. In the ascetic form, it has been used as a transplantable tumor model which is investigate the effects of several antitumor substances (Segura *et al.*, 2000). Adiantum incisum is used in the treatment of chest affections, cough, diabetes, fever and skin diseases. Fresh or dried leaves are ground to make paste or powder. 50 g of this paste powder is mixed in coconut oil 200 ml. This oil is applied on scalp to check hair fall (Anupama., 2016). The Whole and Juice of fronds are used to treat diabetes. The leaves powder used in liver diseases (Sapna Rai., 2016). Leaves are external remedy for skin disease (Ambasta., 1986). The leaves are used in malaria treatment (Chatterjee and Pakrashi., 1991). Large dose of this plant use in emetic. The roots are used in bone fracture. For fever the juice of plant is given with pepper (Anupama., 2016).

## II. Classification:

Cancers are classified by the type of cell that the tumor cells resemble and are therefore presumed to be the origin of the tumor. These types include:

- Carcinoma: Cancers derived from epithelial cells. This group includes many of the most common cancers, particularly in the aged, and includes nearly all those developing in the breast, prostate, lung, pancreas, and colon.
- Sarcoma: Cancers arising from connective tissue (i.e. bone, cartilage, fat, nerve), each of which develops from cells originating in mesenchymal cells outside the bone marrow.
- Lymphoma and leukemia: These two classes of cancer arise from hematopoietic (blood-forming) cells that leave the marrow and tend to mature in the lymph nodes and blood, respectively. Leukemia is the most common type of cancer in children accounting for about 30%.
- Germ cell tumor: Cancers derived from pluripotent cells, most often presenting in the testicle or the ovary (seminoma and dysgerminoma, respectively).
- Blastoma: Cancers derived from immature "precursor" cells or embryonic tissue. Blastomas are more common in children than in older adults.

## III. Causes of Cancer:

There is no single cause of cancer. Scientists believe that, it is interaction of many factors together that produces cancer (Loechler. 2003 and Turker, 2009). The majority of cancers, some 90–95% of cases, are due to genetic mutations from environmental factors. The remaining 5–10% are due to inherited genetics. Environmental, as used by cancer researchers, means any cause that is not inherited genetically, such as lifestyle, economic and behavioral factors and not merely pollution. Common environmental factors that contribute to cancer death include tobacco (25–30%), diet and obesity (30–35%), infections (15–20%), radiation (both ionizing and non-ionizing, up to 10%), stress, lack of physical activity and pollution.

#### IV. Pathophysiology:

In order for a normal cell to transform into a cancer cell, genes which regulate cell growth and differentiation must be altered. Genetic changes can occur at many levels, from gain or loss of entire chromosomes to a mutation affecting a single DNA nucleotide. There are two broad categories of genes which are affected by these changes. Expression of these oncogenes promotes the malignant phenotype of cancer cells. Tumor suppressor genes are genes which inhibit cell division, survival, or other properties of cancer cells. Tumor suppressor genes are often disabled by cancer-promoting genetic changes. Typically, changes in many genes are required to transform a normal cell into a cancer cell.

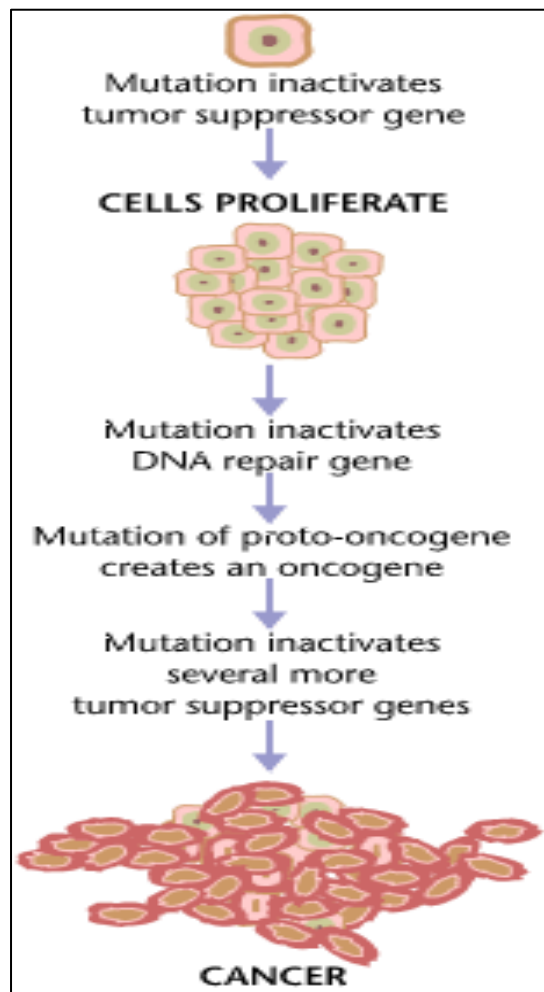


Figure 1: Genesis of Cancer

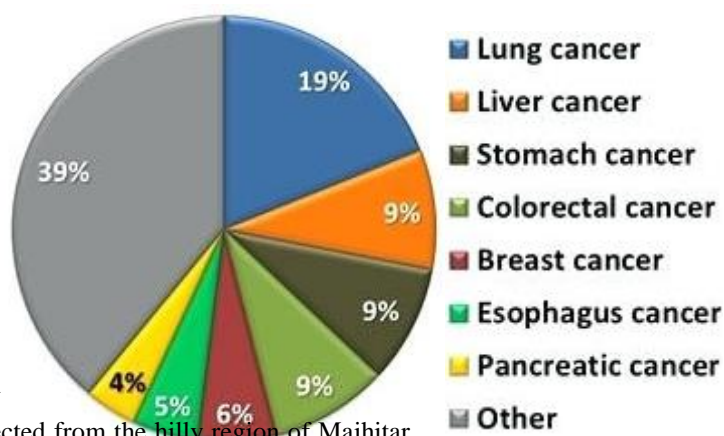
**V. Ethnomedicinal evidence:**

*Adiantum incisum* is used in the treatment of chest affections, cough, diabetes, fever and skin diseases. Fresh or dried leaves are ground to make paste or powder. 50 g of this paste powder is mixed in coconut oil 200 ml. This oil is applied on scalp to check hair fall (Anupama., 2016).

The Whole and Juice of fronds are used to treat diabetes. The leaves powder used in liver diseases (Sapna Rai., 2016). Leaves are external remedy for skin disease (Ambasta., 1986). The leaves are used in malaria treatment (Chatterjee and Pakrashi., 1991). Large dose of this plant use in emetic. The roots are used in bone fracture. For fever the juice of plant is given with pepper (Anupama., 2016).

In 2018, according to the World Health Organization (WHO) an estimated 17.3 million people death for the cancer. The number of cancer survivors is expected to increase to 27.3 million by 2036.

1. Lung (1.63 million deaths)
2. Liver (746 200 deaths)
3. Stomach (723 500 deaths)
4. Colorectal (693 700 deaths)
5. Breast (522 400 deaths)
6. Esophageal cancer (400 225 deaths)
7. Cervical cancer (265 800 deaths)
8. Urinary bladder cancer (165 300 deaths)



**VI. Plant collection and extraction**

*Adiantum incisum* dried plants (2 kg) were collected from the hilly region of Majhitar, Sikkim, India. The month of November 2018. Then dried full plant was powdered in a mechanical grinder and the extraction of the powder (300 gm) was carried out in soxhlet apparatus by using methanol (21.6% w/w, yield). The solvent were completely removed under reduced pressure in a rotary vacuum evaporator. The concentrated extracts were stored in vacuum desiccators for further use.



**Fig.2 A** Whole plant of *Adiantum incisum*



**Fig.2 B** Leaf of *Adiantum incisum*

**VII. Animals**

Male Swiss albino mice weighing 20–22 g were taken. They were obtained from the animal house, B. N. Ghosh& Co. Kolkata, India. The mice were grouped and housed in poly acrylic cages (38 × 23 × 10 cm) with



not more than 6 animals per cage and maintained under standard laboratory conditions (temperature  $25 \pm 2$  °C and dark/ light cycle 14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. The mice were acclimatized to laboratory conditions for 7 days before commencement of the experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee.

### VIII. Transplantation of tumor cell

Ehrlich ascites tumor cells were maintained in our laboratory according to the standard protocol. EAC cells were obtained from Chittaranjan National Cancer Institute (CNCI), Kolkata, India. The EAC cells were maintained in vivo in Swiss albino mice by intraperitoneal transplantation of  $2 \times 10^6$  cells per mouse after every 10 days. Ascitic fluid was drawn out from EAC tumor bearing mouse at the log phase (day 7 – 8 of tumor bearing) of the tumor cells. Each animal received 0.1 ml of tumor cell suspension containing  $2 \times 10^6$  EAC cells intra peritoneally.

### Results:

In MEAI treated groups (50 and 100 mg/kg b.w.) tumor volume, tumor weight, viable cell count was significantly decreased as compared to that of the EAC control group. Life span increased by 29% and 36% in a dose dependant manner as compared to EAC control group. The histopathology of the liver and kidney was observed. The hematological, biochemical and liver tissue antioxidant parameter are significantly ( $p < 0.05$ ) restored towards the normal level after treatment with MEAI.

### Materials and Methods

#### Chemicals

Trypan blue, MTT (MERCK Limited, Mumbai, India)

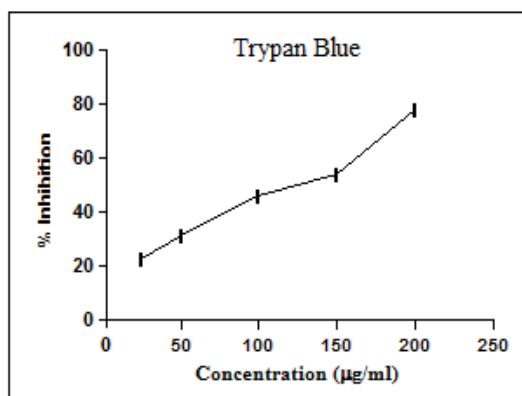
#### Determination of *in vitro* cytotoxicity

In vitro cytotoxicity studies of MEAI were done by standard trypan blue (0.4%) exclusion method and MTT assay. The experiment was performed in triplicate.

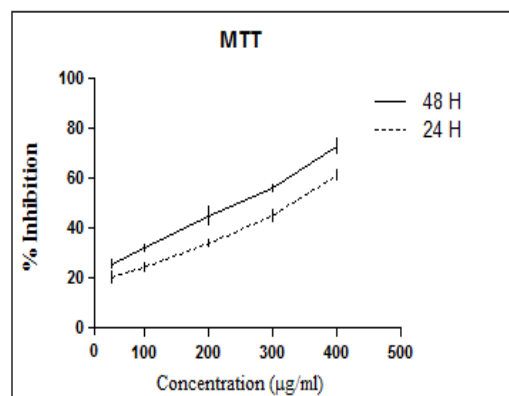
### IX. RESULTS

#### Determination of *in vitro* cytotoxicity

In the assay for in vitro cytotoxicity study, Trypan blue exclusion assay and MTT assay (for 24 hr. & 48 hr.) on MEAI showed the direct cytotoxic effect on the EAC cell line in a dose dependent manner with IC50 value of 112  $\mu\text{g/ml}$ , 242  $\mu\text{g/ml}$  & 181  $\mu\text{g/ml}$  respectively in (Fig. 3 and Fig. 4).



**Fig. 3:** Cytotoxic effect of MEAI on *in vitro* EAC cell line by Trypan blue assay. Values are mean  $\pm$  SEM obtained, where n = 6



**Fig. 4:** Cytotoxic effect of MEAI on *in vitro* EAC cell line by MTT assay. Values are mean  $\pm$  SEM obtained, where n = 6

## X. Conclusions:

The *in vivo* anticancer study delineates that the methanol extract of *Adiantum incisum* does have some potential to fight cancer. Also 100mg/kg MEAI showed better response compared to 50mg/kg MEAI suggesting a possible dose dependent influence of the extract on cancer. However the compound or molecules in the extract that might be responsible for its anticancer activities are still to be identified. Also a through mechanistic study remains to be undertaken to establish constituents of MEAI as potential candidates for cancer therapy.

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