Antitumor Activity and Antioxidant Role of *Ichnocarpus frutescens* Against Ehrlich Ascites Carcinoma in Swiss Albino Mice

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Abstract - The plant *Ichnocarpus frutescens* (Linn) R.Br. (Family-Apocynaceae) has been indicated for the treatment of various diseases, one amongst it is cancer. The purpose of this study was to investigate experimentally the possible antitumor activity and antioxidant role of *Ichnocarpus frutescens* in the mice transplanted with Ehrlich ascites carcinoma (EAC). The chloroform and methanol extract of whole plant of *Ichnocarpus frutescens* (CEIF and MEIF) were administered intraperitoneally at the dose of 150 mg/kg and 300 mg/kg, body weight per day for 7 days after 24 h of tumor inoculation in mice. Treatment with CEIF at the dose of 150 mg/kg and 300 mg/kg remarkably decreased the tumor volume, packed cell volume, viable cell count and increased the nonviable cell count of EAC tumor bearing mice when compared to the effect of MEIF at 150 mg/kg and 300 mg/kg. Further the EAC mice treated with CEIF and MEIF showed significant decrease in the level of lipid peroxidation and significant increase in the level of antioxidant enzymes such as glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT), however the decreasing and increasing capacity of CEIF was less in both doses as compared to MEIF. Based on these results, it can be concluded that the chloroform and methanol extract of *Ichnocarpus frutescens* exhibit significant antitumor and antioxidant activity in EAC bearing mice.

Key words - *Ichnocarpus frutescens*, Ehrlich ascites carcinoma, antitumor activity, *in vivo* antioxidant activity.

Introduction

Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives each year (Abdullaev et al., 2000). Prevention is the sensible maneuver towards the ultimate goal of cancer control (Suffness and Pezzuto, 1991). Several methods exist for the treatment of cancer in modern medicine. These include chemotherapy, radiotherapy and surgery. Chemotherapy is now considered as the most effective method of cancer treatment. Intervention with chemo-preventive agents at the early stage in carcinogenesis is theoretically more rational than attempting to eradicate fully developed tumors with chemotherapeutic drugs. However, most cancer chemotherapeutic agents severely affect the host normal cells (Mascarenhas, 1994). Hence the use of natural products now has been contemplated of exceptional value in the control of cancer and its eradication program (Suffness and Pezzuto 1991).

Medicines derived from plants have played a pivotal role in health care of ancient and modern cultures. Ayurveda, the Indian system of medicine mainly uses plant based drugs or formulations to treat various ailments including cancer. Recent surveys suggest that one in three Americans uses dietary supplements daily and the rate of usage is much higher in cancer patients, which may be up to 50% of patients treated in cancer centers (Richardson et al., 2000).

The plant *Ichnocarpus frutescens* (Linn) R.Br. (Family-Apocynaceae) popularly known as “Dudhi”, “Shyamalata” in Bengali “Black Creeper” in English and “Ananta”, “Sariva” in Sanskrit is a large much branched twining shrub; young branches finely fulvous-tomentose. Leaves 4.5-7.5 by 2-3.8 cm, elliptic-oblong, acute or acuminate, glabrous above, glabrous or slightly pubescent and pale beneath, base usually rounded (Kirtikar and Basu, 1998), occasionally found in village surrounding and hedges throughout India. It is locally called as *Botlai* and the plant is used by the local peoples of Mohuda, Berhampur,
Orissa, India for simple fevers and to treat against liver disorder. The whole plant is used as tribal medicine in atrophy, bleeding gums, cough, dysentery, hematuria, splenomegaly (Chatterjee and Pakrashi, 1995) and in abdominal, glandular tumors (Asolkar et al., 1992) leaves and stem decoction in fever, and root as antipyretic, demulcent, diuretic, hypoglycemic and as tonic in anorexia, leucorrhea, syphilis, urinary calculi (Chatterjee and Pakrashi, 1995). Stalk and leaves is used in decoction in the treatment of skin eruptions. A decoction of the roots of Colocynth, Anantamul, Sariva (Sanskrit: Ichnocarpus frutescens) and Hedyotis biflora prepared in the usual way is administered with the addition of powdered long pepper and bedellium in chronic skin diseases, syphilis, loss of sensation and hemiplegia (Nadkarni, 1976). Studies on chemical constituents of the plant revealed the presence of urosolic acid and kaempferol in the leaves (Khan et al., 1995), α-amyrin, α-amyrin acetate, lupeol, lupeol acetate, fridelin, epi-friedelinol, β-sitosterol from stems (Lakshmi et al., 1985) and quercetin, quercetin-3-O-β-D-glucopyranoside from flowers of the plant (Singh and Singh, 1987).

Plant derived natural products such as flavonoids, terpenoids, and steroids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity (Defeudis et al., 2003; Takeoka and Doo, 2003). Antioxidants play an important role in inhibiting and scavenging free radicals thus providing protection to humans against infection and degenerative diseases. Realizing these facts, this work was carried out to evaluate the antitumor activity and antioxidant status of chloroform and methanol extract of whole plant of *Ichnocarpus frutescens* in the mice transplanted with Ehrlich ascites carcinoma (EAC) cells.

**Experimental**

**Plant material** – The plant *Ichnocarpus frutescens* was collected from Mohuda forest area, Ganjam district, Berhampur, Orissa, India in the month of September. The plant material was taxonomically identified by the taxonomists of Botanical Survey of India, Govt. of India, Shibpur, Howrah, India. A voucher specimen (NO.CNH/1-I-198/2005/Itech.II/1448) has been preserved in our laboratory for the future references.

**Extraction** – The whole plant was dried under shade and then powered with a mechanical grinder to obtain course powder, which was then subjected to successive extraction in a Soxhlet apparatus using petroleum ether (60 - 80°C), chloroform and methanol. Solvent elimination under reduced pressure afforded the chloroform extract (2% w/w) and methanol extract (17% w/w) with respect to the dried plant material respectively.

**Experimental animals** – Studies were carried out using male Swiss albino mice weighing 20 ± 2 g. They were obtained from the animal house, Indian Institute of Chemical Biology (IICB), Kolkata, India. The animals were grouped and housed in polyacrylic cages (38 × 23 × 10 cm) with not more than twelve animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (12/12 h). The animals were fed with standard pellet diet supplied by Hindustan Lever Ltd., Kolkata, India and water *ad libitum*. All the animals were acclimatized to laboratory condition for 10 days before commencement of experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee.

**Drugs and chemicals** – Thio barbituric acid (TBA), Nitro blue tetrazolium chloride (NBT), Phenazine methosulphate were procured from Central Drug House, New Delhi, India and 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), Reduced Glutathione (GSH), Nicotinamide adenine dinucleotide (NADH) and the rest of the chemicals utilized were of analytical grade and were obtained from Sisco research laboratories, Mumbai, India.

**Tumor cells** – Ehrlich ascites carcinoma (EAC) cells were obtained from Chittaranjan National Cancer Institute, Kolkata, India. The EAC cells were maintained *in vivo* in Swiss albino mice by interperitoneal inoculation of 2 × 10^6 cells/mouse after 10 days. EAC cells of 9 days old were used for the screening of CEIF and MEIF (Rajeshwar et al., 2005).

**Experimental protocol** – Male Swiss albino mice were divided into seven groups of six animals (n = 6) each. Ehrlich Ascites Carcinoma (EAC) cells were collected from the donor mice and were suspended in sterile isotonic solution (0.9% w/v NaCl). The numbers of tumor cells per ml of this suspension were counted under microscope with the help of hemocytometer. A definite number (about 2 × 10^6 cell/0.2 ml) of these living viable cells were implanted into the peritoneal cavity of each mouse of each group except the normal group. This was taken as day zero. One day for incubation was allowed for multiplication of tumor cells in the body before starting the drug administration. From the second day up to eighth day only vehicle (sterile phosphate buffer and tween 80) in 5 ml/kg/mouse/day was administered intraperitoneally to group-I (Normal) and group-II (EAC control) respectively. Similarly CEIF, MEIF at the doses 150 mg/kg and 300 mg/kg were prepared suspension in sterile phosphate