**Natural Product Sciences**
13(1) : 54-60 (2007)

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

**Deepak K Dash[1], Siva S Nayak[1], Soma Samanta[2], Tirtha Ghosh[3], Tarun Jha[2], Bhim C Maiti[2], and Tapan K Maity[2]***

*[1]College of Pharmaceutical Sciences, Mohuda, Berhampur, Orissa, India.*
*[2]Division of Pharmaceutical Chemistry, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032, India.*
*[3]Institute of Pharmacy and Technology, Salipur, Orissa-754202, India.*

**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 extact 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 chemotheraputic 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 *Botilai* and the plant is used by the local peoples of Mohuda, Berhampur,

*Author for correspondence
Fax: +913324146074; E-mail: jutkmaity@yahoo.com

Orissa, India for simple fevers and to treat against liver disorder. The whole plant is used as tribal medicine in atropy, 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, leucorrhoea, 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*) and Hedyotis biflora prepared in the usual way is administered with the addition of powdered long pepper and *bdellium* 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 Dao, 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/I-I (98)/2005/Tech.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 10days before commencement of experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee.

**Drugs and chemicals** – Thiobarbituric 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 $2 \times 10^6$ 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 $2 \times 10^6$ 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

buffer ($p^H$ 7.2) and Tween 80 (2%) and standard drug 5-flurouracil at 20 mg/kg were administered intraperitoneally to each mouse at 24 h interval in groups III, IV, V, VI, and VII respectively. Food and water were withheld 18 h before sacrificing the animals. On 9$^{th}$ day, all the animals were sacrificed for the study of antitumor activity and *in vivo* antioxidant activity (Gayen *et al.*, 2003).

**Tumor growth response** – The antitumor activity of CEIF and MEIF was assessed by the following parameters such as

**Body weight:** Body weight the experimental mice was recorded both in the treated and control group at the beginning of the experiment (day 0) and on the final day before sacrifice in order to evaluate the relative changes.

**Tumor volume:** The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and packed cell volume was determined by centrifuging at 1000 rpm for 5 min.

**Tumor cell count:** The ascitic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the numbers of cells in the 64 small squares were counted.

**Viable/non viable tumor cell count:** The cells were then stained with trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable and those that took the stain were nonviable. These viable and non viable cells were counted.

$$\text{Cell count} = \left(\frac{\text{No. of cells} \times \text{dilution}}{\text{Area} \times \text{thickness of liquid film}}\right)$$

**Acute toxicity study** – Male Albino mice of 10 animals per group and weighing between 20 - 25 g were administered with graded doses of (500 - 3000 mg/kg, body weight, i.p) the chloroform and methanol extract of whole plant of *Ichnocarpus frutescens*. After administration of the CEIF and MEIF they were observed for deaths after 48 h treatment. The toxicological effects were observed in terms of mortality and expressed as $LD_{50}$ (Ghosh, 1984). During the experiment on abovesaid dose regimen no death was observed. So it can be concluded that the CEIF and MEIF extracts were safe upto 3000 mg/kg, body weight i.p.

**Estimation of *in vivo* antioxidants** – After collection of blood samples the mice were sacrificed and their livers were excised immediately and washed in ice cold normal saline, followed by 0.15 M Tris-Hcl (pH 7.4), blotted dry and weighed. A 10% w/v of homogenate was prepared in 0.15 M Tris-Hcl buffer and processed for the estimation of lipid peroxidation (LPO) (Ohkawa *et al.*, 1979). A part of homogenate after precipitating proteins with Trichloroacetic acid (TCA) was used for estimation of glutathione (GSH) (Beutler and Kelly, 1963). The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 4 °C. The supernatant thus obtained was used for estimation of superoxide dismutase (SOD) and catalase (CAT) activities (Kakkar *et al.*, 1954; Aebi, 1974).

**Statistical analysis** – The experimental results were expressed as the Mean ± SEM for six animals in each group. The results were analysed statistically using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. P values < 0.05 were considered as statistically significant.

**Table 1.** Effect of CEIF and MEIF on body weight, tumor volume, packed cell volume and viable and nonviable tumor cell count of EAC bearing mice

| Groups | Dose (mg/kg) | Body weight (g) | | Tumor volume (ml) | Packed Cell volume (ml) | Tumor cell count ($\times 10^7$ cells/ ml/mouse) | |
|---|---|---|---|---|---|---|---|
| | | Initial | Final | | | Viable | Nonviable |
| Group I – Normal control (Sterile phosphate buffer solution) | – | 21.35 ± 0.47 | 21.98 ± 0.08 | – | – | – | – |
| Group II – EAC ($2 \times 10^6$ cells/ml/mouse) | – | 21.46 ± 0.40 | 25.13 ± 0.26[a] | 3.68 ± 0.13 | 1.62 ± 0.09 | 8.63 ± 0.25 | 0.38 ± 0.01 |
| Group III – CEIF + EAC | 150 mg/kg | 21.55 ± 0.25 | 22.75 ± 0.28[b] | 0.92 ± 0.06[b] | 0.30 ± 0.02[b] | 1.27 ± 0.08[b] | 1.10 ± 0.02[b] |
| Group IV – CEIF + EAC | 300 mg/kg | 21.68 ± 0.31 | 23.18 ± 0.17[b] | 1.19 ± 0.09[b] | 0.44 ± 0.02[b] | 1.54 ± 0.02[b] | 0.74 ± 0.01[b] |
| Group V – MEIF + EAC | 150 mg/kg | 21.51 ± 0.41 | 23.66 ± 0.06[c] | 1.41 ± 0.08[c] | 0.56 ± 0.01[c] | 1.84 ± 0.02[c] | 1.32 ± 0.02[c] |
| Group VI – MEIF + EAC | 300 mg/kg | 21.33 ± 0.31 | 23.81 ± 0.12[c] | 2.01 ± 0.10[c] | 0.78 ± 0.02[c] | 2.17 ± 0.03[c] | 0.82 ± 0.01[c] |
| Group VII – 5-FU + EAC | 20 mg/kg | 21.86 ± 0.27 | 21.91 ± 0.57[c] | – | – | – | – |

Values are Mean ± SEM. n = 6 animals in each group.
[a] $P < 0.01$ is considered significant when compared with group I.
[b] $P < 0.01$ is considered significant when compared with group II.
[c] $P < 0.05$ is considered significant when compared with group II.

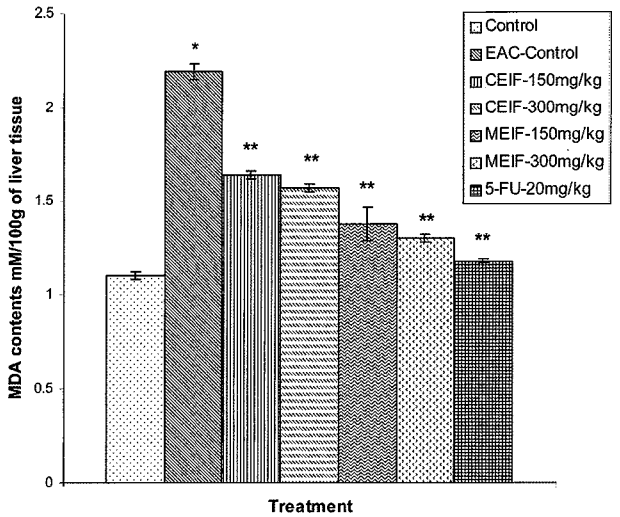


**Fig. 1.** Effect of CEIF and MEIF on TBARS levels in mice liver tissues. Values are mean ± SEM, 6 animals in each group (n = 6). * $P < 0.05$ Values are considered statistically significant when compared with normal control. ** $P < 0.05$ Values are considered statistically significant when compared with induced control.

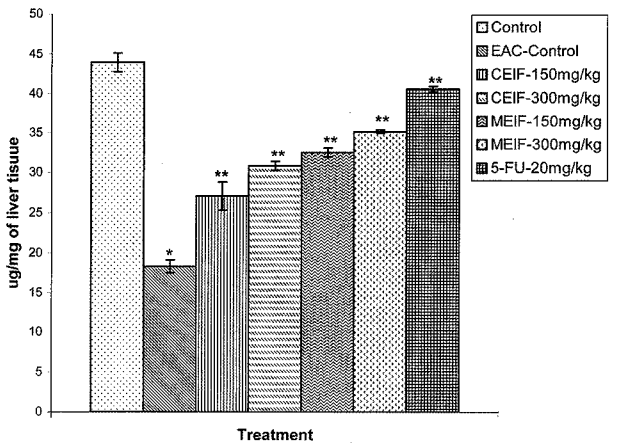


**Fig. 2.** Effect of CEIF and MEIF on GSH levels mice liver tissues. Values are mean ± SEM, 6 animals in each group (n = 6). * $P < 0.05$ Values are considered statistically significant with normal control. ** $P < 0.05$ Values are considered statistically significant with induced control.

## Results

The antitumor activity of CEIF and MEIF extracts against EAC tumor bearing mice were summarized in Table 1. The effect of CEIF and MEIF extracts on the EAC tumor mice were assessed by different parameters such as body weight, tumor volume, packed cell volume and tumor cell count (viable and nonviable cell). Induction of EAC cells to the animals significantly increased the body weight. Administration of MEIF (150 and 300 mg/kg) significantly ($P < 0.05$) decreased the body weight, tumor volume, packed cell volume and cell

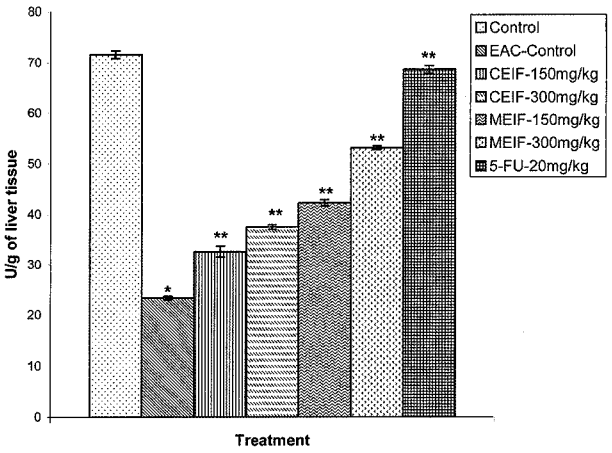


**Fig. 3.** Effect of CEIF and MEIF on SOD levels in mice liver tissues. Values are mean ± SEM, 6 animals in each group (n = 6). * $P < 0.05$ Values are considered statistically significant with normal control. ** $P < 0.05$ Values are considered statistically significant with induced control.

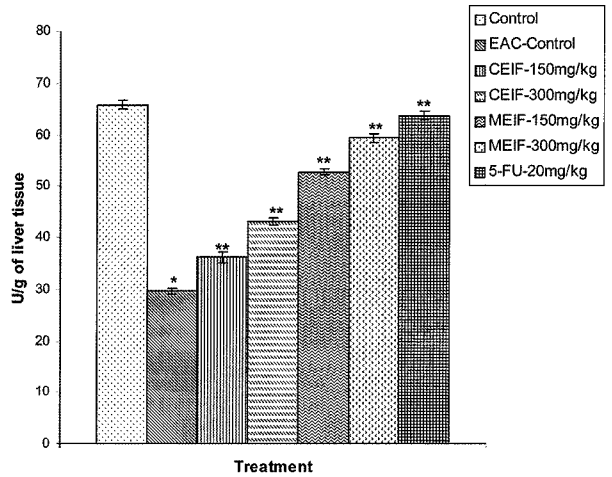


**Fig. 4.** Effect of CEIF and MEIF on CAT levels in mice liver tissues. Values are mean ± SEM, 6 animals in each group (n = 6). * $P < 0.05$ Values are considered statistically significant with normal control. ** $P < 0.05$ Values are considered statistically significant with induced control.

count (viable and nonviable) and the effect was more pronounced ($P < 0.01$) in the group of animals treated with CEIF at the dose of 150mg/kg and 300 mg/kg body weight.

The level of lipid peroxidation in liver tissue was significantly increased in the EAC control group as compared to the normal group ($P < 0.05$). After administration of CEIF and MEIF at 150 mg and 300 mg/kg and 5-FU at 20 mg/kg to EAC bearing mice, the level of lipid peroxidation was reduced significantly ($P < 0.05$) when compared with EAC control animals and the effect was less pronounced in the group of animals treated with CEIF at the dose of 150 mg/kg and 300 mg/kg (Fig.1).

The changes in the concentration of reduced glutathione and the activities of superoxide dismutase and catalase in liver on the treatment of EAC control mice with CEIF, MEIF and 5-FU are shown in Fig. 2, 3 and 4 respectively. There was a significant ($P < 0.05$) reduction in glutathione and the activities of liver superoxide dismutase and catalase in the EAC control group when compared with normal group. Treatment with CEIF, MEIF (150 and 300 mg/kg) and 5-FU (20 mg/kg) to EAC bearing mice significantly ($P < 0.05$) increased the liver glutathione and restored the activities of liver superoxide dismutase and catalase to near normal status when compared with EAC control animals however the effect was less pronounced in the group of animals treated with CEIF at the dose of 150 mg/kg and 300 mg/kg.

## Discussion

Human have always relied on nature for survival since ancient times, which has been their main source of food, protection, clothing, transportation and remedies (Farnsworth *et al.*, 1985; Cragg *et al.*, 1997). Natural products have been regarded as important sources that could produce potential chemotherapeutic agents (Kim and Park, 2002). Plant-derived compounds, in particular have a special place in anticancer therapy and some of the new chemotherapeutic agents currently available for use in a clinical setting include paclitaxel, vincristine, podophyllotoxin and camptothecin, a natural product precusor for water-soluble derivatives (Kinghorn and Balandrin, 1993; Gerzon, 1980; Jardine, 1980; Wall and Wani, 1993). Obviously natural products are extremely important as sources of medicinal agents. Although there are some new approaches to drug discovery, such as combinatorial chemistry and computer-based molecular modeling design, none of them can replace the importance of natural products in drug discovery and development. *Ichnocarpus frutescens* in both the extract (CEIF and MEIF) has been reported to be non-toxic in acute toxicity studies *in vivo* with almost no side effects. The studies on the *in vivo* anticancer activity of *Ichnocarpus frutescens* are lacking. Therefore, it was desired to screen the anticancer activity of different extract of *Ichnocarpus frutescens* in swiss albino mice transplanted with Ehrlich ascites carcinoma.

The results of the present investigation demonstrate the significant antitumor activity of chloroform and methanol extract of whole plant of *Ichnocarpus frutescens* against EAC bearing mice. Although, the methanol extract is significantly effective against ascites tumor model, the chloroform extract is more effective antitumor agent in both the doses. The antitumor activity revealed that treatment with CEIF and MEIF at the doses 150 mg and 300 mg/kg reduced the increase in body weight, tumor volume, packed cell volume and tumor cell count. The extract also restored the hepatic lipid peroxidation and free radical scavenging enzyme GSH as well as antioxidant enzymes such as SOD and CAT in tumor bearing mice to near normal levels.

In EAC-bearing mice, a regular rapid increase in ascites tumor volume was noted. Ascites fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells (Prasad and Giri, 1994). Treatment with CEIF and MEIF inhibited the tumor volume, tumor cell count and increased the percentage of trypan blue positive stained dead cells in tumor bearing mice. So it may be concluded that CEIF and MEIF by decreasing the nutritional fluid volume and arresting the tumor growth can act as antineoplastic agent.

Preliminary chemical examinations of pet. ether, chloroform and methanol extracts showed detectable amounts of pentacyclic triterpenoids, steroids and flavonoids. Triterpenoids like α-amyrin, α-amyrin acetate, lupeol, lupeol acetate, friedelin, epi-friedelinol (Lakshmi *et al.*, 1985) and flavonoids like quercetin, quercetin-3-O-β-D-glucopyranoside (Singh and Singh, 1987) are reported to be present in the plant. The anticancer activity is more significant in case of CEIF, which can be attributed to the presence of different phytochemical constituents such as α-amyrin, α-amyrin acetate, lupeol, lupeol acetate etc. It has been reported that α-amyrin acetate and lupeol possesses potential anticancer effect on JB6 murine epidermal cell line assay and by the inhibition of cyclooxygenase-2 (COX-2) enzyme (Blanco *et al.*, 2006). Even though α-amyrin acetate, lupeol, lupeol acetate like triterpenoids present in chloroform extract of *Ichnocarpus frutescens*, it can be inferred that there may be some other important constituents in the chloroform extract, which might have contributed to the enhanced antitumor activity of *Ichnocarpus frutescens* than the methanol extract.

The exact mechanism of action of CEIF and MEIF is not known. The antineoplastic activity cannot be attributed to a single mechanism but several mechanims may be operational simultaneously for effective tumor cell death.

Lipid peroxidation mediated by free radicals is considered as a primary mechanism of cell membrane destruction and cell damage (Plaa and Wistshi, 1976). The oxidation of unsaturated fatty acids in biological membranes leads to reduction in membrane fluidity and

disruption of membrane structure and function (Campo *et al.*, 2001). Malondialdehyde (MDA), the end product of lipid peroxidation was also reported to be higher in carcinomatous tissue than in non-diseased organs (Yagi, 1987). Increase in the level of TBARS indicate enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defence mechanisms to prevent the formation of excess free radicals (Camporti, 1985). Glutathione, a potent inhibitor of the neoplastic process, plays an important role in the endogenous antioxidant system. It is found in particularly high concentration in the liver and is known to have a key function in the protective process. Excessive production of free radicals resulted in oxidative stress, which leads to damage of macromolecules, for example, lipid peroxidation in vivo (Sinclair *et al.*, 1990). It was also reported that the presence of tumors in the human body or in experimental animals is known to affect many functions of the vital organs, especially in the liver, even when the site of the tumor does not interfere directly with organ function (Dewys, 1982). Administration of CEIF and MEIF in different doses significantly reduced the elevated levels of lipid peroxidation and increased the glutathione content in EAC-treated mice. However the reducing activity of elevated lipid peroxidation and increasing capacity of glutathione content of CEIF in both the doses is less as compared to MEIF.

Cells are also equipped with enzymatic antioxidant mechanisms that play an important role in the elimination of free radicals. SOD, CAT, and glutathione peroxides are involved in the clearance of superoxide and hydrogen peroxide ($H_2O_2$). SOD catalyses the diminition of superoxide into $H_2O_2$, which has to be eliminated by glutathione peroxidase and/ or catalase (Rushmore and Picket, 1993). Consistent with this, it has been reported that a decrease in SOD activity in EAC-bearing mice may be due to loss of $Mn^{2+}$-containing SOD activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver. The inhibitions of SOD and CAT activities as a result of tumor growth were also reported (Sun *et al.*, 1989). Similar findings were observed in the present investigation with EAC-bearing mice. The administration of CEIF and MEIF at different doses significantly increased the SOD and CAT levels. However the increasing activity of SOD and CAT levels of CEIF at both doses is less as compared to MEIF.

From our study it is clear that the CEIF and MEIF inhibited the growth of EAC in the mice. The exact mechanism of action of CEIF and MEIF is not known. It may be due to operation of multiple events. Currently, efforts are being made to identify and isolate the cytotoxic priniciple(s) from the crude extracts of active samples and also to investigate the mode of action of the active compounds in these cell lines.

## Acknowledgements

One of the authors Deepak Kumar Dash, Senior Research Fellow is grateful to the authority of College of Pharmaceutical Sciences, Mohuda, Berhampur, Orissa for sanctioning leave and the authority of Jadavpur University for providing all facilities.

## References

Abdullaev, F.I., Rivera, L.R., Roitenburd, B.V., and Espinosa, A.J., Pattern of childhood cancer mortality in Mexico. *Arch. Med Res.*, **31**, 526-531, (2000).

Aebi, H., Catalase, In Bergmeyer, H.U (ed.), *Methods of Enzymatic Analysis*, Vol. II, Academic Press, New York, 1974, pp. 673-684.

Asolkar, L.V., Kakkar, K.K., and Chakre, O.J., *Second suppliment to Glossary of Indian Medicinal Plants with active principles (1965-81)*, National institute of science communication, Dr. K. S. Krishnan Marg, New Delhi, India, 1992, pp. 366.

Beutler, E., and Kelly, B.M., The effect of sodium nitrate on red cell glutathione. *Experientia.* **18**, 96-97 (1963).

Blanco, E.J.C., Cuendet, M., Park, E.J., Su, B.N., Riverocruz, J.F., Farnsworth, N.R., Pezzuto, J.M., and Kinghorn, A.D., Potential cancer chemopreventive agents from *Arbutus unedo*. *Nat. Prod. Res.* **20**, 327-334 (2006).

Campo, G.M., Squadrito, F.S., Ceccarelli, N., Calo, A., Avenoso, S., Campo, G, Squadrito, D., Aitavilla. Reduction of carbon tertrachloride-induced rat liver injury by IRFI 042, a novel duel vitamin E-like antioxidant. *Free. Radical. Res.* **34**, 379-393 (2001).

Camporti, M., Biology of Disease. Lipid peroxidation and cellular damage in toxic liver injury. *Lab. Invest.* **53**, 599-623 (1985).

Chatterjee, A. and Pakrashi, SC., *The Treatise on Indian Medicinal Plants,* Vol. IV, Publications and information Directorate, New Delhi, India, 1995, pp. 110-112.

Cragg, G.M., Newman, D.J., and Snader, K.M., Natural products in drug discovery and development. *J. Nat. Prod.* **60**, 52-60 (1997).

DeFeudis, F.V., Papadopoulos, V., and Drieu, K., *Ginkgo biloba* extracts and cancer: a research area in its infancy. *Fundam. Clin. Pharmacol.* **17**, 405-17 (2003).

DeWys, W.D., Pathophysiology of cancer cachexia: current understanding and areas for future research. *Cancer. Res.* **42**, 721s-726s (1982).

Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarto, D.D., and Guo, Z., Medicinal plants in therapy. *World Health Organization Bulletin*, **63**, 965-981 (1985).

Gerzon, K., Anticancer Agents Based on Natural Product Models, In Cassady, J.M., and Douros, J.D. (eds.), Academic Press, New York, 1980, pp. 271-317.

Gayen, S., Debnath, B., Samanta, S., Ghosh, B., Basu, A., Jha, T., 1, 5, *N, N'*- Disubstituted -2- (Substituted Benzenesulphonyl)- Glutamamide analogues as anticancer agents - 3: Synthesis, biological screening and QSAR study. *Int. Electro. J. Mol. Des.* **4**, 556-578 (2005).

Ghosh, M.N., *Fundamentals of Experimental Pharmacology*, Second edition, Scientific Book Agency, Calcutta, India, 1984, pp. 177-211.

Jardine, I., Anticancer Agents Based on Natural Product Models, In

Cassady J.M., and Douros, J.D. (eds.), Academic Press, New York, 1980, pp. 319-351.

Kakkar, P., Dos, B., Viswanathan, P.N., Maehly. A.C., and Chance, B., In Glick, D. (ed.), *Methods of biomedical analysis*, Vol. I, Interscience, New York, 1954, p.357.

Khan, M.S.Y., Javed, K., and Khan, M.H., Chemical constituents of the leaves of *Ichnocarpus frutescens* R. Br. *J. Ind. Chem. Soc.* **72**, 65-66 (1995).

Kim, J., and Park, E.J., Cytotoxic anticancer candidates from natural resources. *Curr. Med. Chem. Antican. Agent*, **2**, 485-537 (2002).

Kinghorn, A.D. and Balandrin, M.F., *Human Medical Agents from Plants*, American Chemical Society Symposium Series 534, American Chemical Society, Washington, DC, 1993, pp. 80-95.

Kirtikar, KR. and Basu, BD., *Indian Medicinal Plants*, Vol.II, Lalit Mohan Basu Publications, Allahabad, India, 1998, pp. 1590-1592.

Lakshmi, D.K.M., Rao, E.V., and Rao, D.V., Triterpenoid constituents of *Ichnocarpus frutescens*. *Indian Drugs* **22**, 552-553, (1985).

Mascarenhas, M., Structure-activity characterization, a quick method to screen mushrooms for the presence of antitumor glucans. *Mushroom Res.* **3**, 77-/80 (1994).

Nadkarni, KM., *The Indian Materia Medica*, Vol. I, Bombay Popular Prakashan, Bombay, India, 1976, p. 674.

Ohkawa, H., Onishi, N., and Yagi, K., Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. *Anal. Biochem.* **95**, 351-58 (1979).

Plaa, G.L. and Wistshi, H., Chemicals, drugs and lipid peroxidation. *Annu. Rev. Pharmacol. Taxicol.* **16**, 125-41 (1976).

Prasad, S.B. and Giri, A., Antitumor effect of cisplatin against murine ascites Dalton's lymphoma. *Ind. J. Expt. Biol.* **32**, 155-162 (1994).

Rajeshwar, Y., Gupta, M., and Mazumder, U.K., Antitumor activity and *in vivo* antioxidant status of *Mucuna pruriens* (Fabaceae) seeds against Ehrlich ascites carcinoma in swiss albino mice. *I.J.P.T.*, **4**, 46-53 (2005).

Richardson, M.A., Sanders T., Palmer, J.L., Greisinger, A., and Singletary, S.E., Complementary/ Alternative medicine use in a comrehensive cancer center and the implications for oncology. *J. Clin. Oncol.*, **18**, 2505-2514 (2000).

Rushmore, T.H. and Picket, C.B., Glutathione-S-transferase, structure, regulation, and therapeutic implication. *J. Biol. Chem.* **268**, 11475-11478 (1993).

Sinclair, A.J., Barnett, A.H., and Lunie, J., Free radicals and antioxidant systems in health and disease. *Brit. J. Hospl. Med.* **43**, 334-344 (1990).

Singh, RP. and Singh RP., Flavonoids of the flowers of *Ichnocarpus frutescens*. *J.Indian. Chem. Soc.* **64**, 715-716 (1987).

Suffness, M. and Pezzuto, J.M., In: *Methods in Plant Biochemistry*, Vol. VI, Academic Press, New York, p. 71, 1991.

Sun, Y., Oberley, L.W., Elwell, J.H., and Sierra, R.E., Antioxidant enzyme activities in normal and transformed mice liver cell. *Int. J. Cancer.* **44**, 1028-1033 (1989).

Takeoka, GR. and Dao, LT., Antioxidant constituent of almond [*Prunus dulcis* (Mill.) D.A. Webb.] hulls. *J. Agric. Food. Chem.* **51**, 496-501 (2003).

Wall, M.E. and Wani M.C., *Human Medicinal Agents from Plants*, In Kinghorn, A.D., and Balandrin, M.F. (eds.), American Chemical Society Symposium Series 534, 1993, pp. 149-169.

Yagi, K., Lipid peroxide and human disease. *Chem. Physiol. Lip.* **45**, 37-51 (1987).

(Accepted December 20, 2006)