www.ijrps.pharmascope.org

ISSN: 0975-7538
Research Article

Anticancer activity of methanol extract of *Cucurbita maxima* against Ehrlich ascites carcinoma

Prerona Saha[1,2], U. K. Mazumder[1]*, P. K. Haldar[1], Sagar Naskar[1], Sriparna Kundu[1], Asis Bala[1], Biswakanth Kar[1]

[1]Department of Pharmaceutical Technology, Jadavpur University, Kolkata- 700 032, India
[2]Guru Nanak Institute of Pharmaceutical Science and Technology, Nilgunj Road, Panihati, Kolkata-700114, India

ABSTRACT

One of the largest causes of mortality worldwide is cancer. Increasing interest and research on herbal medicine have revealed its importance in treating many diseases including cancer. The present study was carried out to evaluate the antitumor activity of methanol extract of *Cucurbita maxima* Duchesne aerial parts (MECM) on Ehrlich Ascites Carcinoma (EAC) model in mice. After inoculation of EAC cells into mice, treatment with MECM (200 and 400 mg/kg) and standard drug 5-Fluorouracil (20 mg/kg) were continued for 9 days. Evaluation of the effect of drug response was made by the study of tumor growth response including increase in life span, study of hematological parameters, biochemical estimations, antioxidant assay of liver tissue and *in vitro* cytotoxicity. Experimental results revealed that *C.maxima* possesses significant anticancer activity which may be due to its cytotoxicity and antioxidant properties. Further research is going on to find out the active principle(s) of MECM for its anticancer activity.

Keywords: Anticancer; EAC; *Cucurbita maxima;* Cucurbitaceae; cytotoxicity.

INTRODUCTION

Over the past few decades, cancer has remained as the largest cause of mortality worldwide and the number of individuals living with cancer is steadily expanding. Hence, a major portion of the current pharmacological research is involved with the anticancer drug design customized to fit new molecular targets (Xia *et al.*, 2004). Due to the enormous propensity of plants, which synthesize a variety of structurally diverse bioactive compounds, the plant kingdom is a potential source of chemical constituents with antitumor and cytotoxic activities. Traditionally various plants have long been used in the treatment of cancer (Kim *et al.*, 2005; Kintzios, 2006; Indap *et al.*, 2006; Bufalo *et al.*, 2009).

The plant *Cucurbita maxima* Duchesne (commonly known as pumpkin) belongs to the family Cucurbitaceae and is widely cultivated throughout the world for use as vegetable as well as medicine. Both of its fruits and the aerial parts are commonly consumed as vegetable. It is a large climbing herb, annual or perennial. Its aerial part consists of flexible succulent stem with trifoliate leaves (Kirtikar and Basu, 2003). The plant has been used traditionally as medicine in many countries such as China, India, Yugoslavia, Brazil and America (Popovic , 1971; Jia *et al.*, 2003; Adolfo and Michael, 2005). Traditionally it is used in most countries as antidiabetic, antitumor, antihypertensive, anti-inflammatory, immunomodulatory and antibacterial agents (Caili *et al.,* 2006). Several *in vitro* and *in vivo* studies with crude pumpkin fruit extract as well as various purified fractions, including proteins and polysachharides, have shown anticancer activity against melanoma, Ehrlich ascites carcinoma and leukaemia (Ito *et al.*, 1986). Proteins from pumpkin seeds were reported to inhibit melanoma proliferation (Xie , 2004). However, in spite of traditional use, pharmacology of its aerial parts has not yet been explored scientifically. As such, the present investigation was carried out to evaluate the anticancer activity of the methanol extract of *Cucurbita maxima* Duchesne aerial parts (MECM) against *in vivo* and *in vitro* Ehrlich Ascites Carcinoma (EAC) tumor model.

* Corresponding Author
Email: prerona_saha@rediffmail.com
Contact: +91-9836152837
Received on: 12-12-2010
Revised on: 26-12-2010
Accepted on: 06-01-2011

MATERIALS AND METHODS

Plant material

The aerial parts of *C.maxima* were collected in June 2009, from Khardah, West Bengal, India and identified by the Botanical Survey of India, Howrah, India. A voucher specimen (P/CM/2/09) was retained in our laboratory for further reference.

Preparation of plant extract

The aerial parts were dried and powdered in a mechanical grinder. The powdered material was extracted with methanol using Soxhlet apparatus. This extract was

filtered and concentrated in *vacuo* and kept in a vacuum desiccator for complete removal of solvent. The yield was 11.49% w/w with respect to dried powder. Aqueous suspension of MECM was prepared using 2 % (v/v) Tween-80 and used for the treatment.

Animals

Healthy Swiss albino mice (20 ± 2 g) were used for the study. The animals were kept in polypropylene cages with sawdust bedding and maintained under standard laboratory conditions. Standard pellet diet (Hindustan Lever, Kolkata, India) and water were given *ad libitum*. The mice were acclimatized to laboratory condition for one week before commencement of experiment. The experiments were performed based on animal ethics guidelines of University Animals Ethics Committee.

Acute toxicity study

Healthy Swiss albino mice (20 ± 2 g) of either sex, starved overnight, were divided into five groups (n=4). Group I-IV animals were orally fed with MECM in increasing dose levels of 0.5, 1.0, 1.5 and 2.0 g/kg b.wt, while group V (untreated) served as control. The animals were observed continuously for first 2 h for any gross change in behavioral, neurological and autonomic profiles or any other symptoms of toxicity and mortality if any, and intermittently for the next 6 h and then again after 24 h , 48 h and 72 h for any lethality or death. One-tenth and one-fifth of the maximum safe dose of the extract tested for acute toxicity were selected for the *in vivo* experiment. (Ghosh, 1984).

Tumor cells

Ehrlich ascites carcinoma (EAC) cells were obtained from Chittaranjan National Cancer Institute (CNCI), Kolkata, India. The EAC cells were maintained in Swiss albino mice, by intraperitoneal (i.p.) transplantation on every 9th days (Dagli *et al.*, 1992). The ascitic fluid was collected by syringe and the tumor cell count was performed in a Neubauer hemocytometer and 2×10^7 cells/ml were obtained by dilution with normal saline (Orsolic *et al.*, 2005). Tumor cell suspension showing more than 90 % viability (checked by trypan blue dye (0.4%) exclusion assay) was used for transplantation.

EXPERIMENTAL PROTOCOL

Healthy Swiss albino male mice were weighed and divided into five groups (n=12). EAC cells (2×10^6 cells/ mouse) were injected i.p. to each mouse of each group except normal saline group. This was taken as Day 0. Extract and reference drug (5-fluorouracil) treatment were continued for subsequent 9 days starting from Day 1. On 10th day, 24 h after the last dose six mice were sacrificed from each group and the rest were kept for the life span study of the tumor hosts. After sacrificing the animals, blood was collected to evaluate the hematological and biochemical parameters. Liver tissue was collected from the animals for the evaluation of *in vivo* antioxidant status.

The groups and the design of the experiment were as follows (Mazumder *et al*., 1997):

Group I: 2% Tween-80 (5ml /kg b.wt, i.p.)

Group II: EAC ($2X10^6$ cells/mouse) + 2% Tween-80 (5ml/kg b.wt, i.p.)

Group III: EAC ($2x10^6$ cells/mouse) + MECM (200mg/kg b.wt, i.p.)

Group IV: EAC ($2x10^6$ cells/mouse) + MECM (400mg/kg b.wt, i.p.)

Group V: EAC ($2x10^6$ cells/mouse) + 5-fluorouracil (20mg/kg b.wt, i.p.)

Antitumor activity of MECM was assessed by observation of changes with respect to the following parameters.

Tumor growth response

The effect of MECM on tumor growth and host's survival time were examined by studying the following parameters such as tumor volume, packed cell volume, tumor cell count, viable tumor cell count, nonviable tumor cell count, median survival time and increase in lifespan.

Tumor volume and Packed cell 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. Packed cell volume was determined by centrifuging the ascetic fluid at 1000 rpm for 5 min.

Tumor cell count

The ascetic 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 and nonviable 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 and those which took the stain were nonviable. These viable and nonviable cells were counted.

Percentage increase in life span

The effect of MECM on tumor growth was monitored by recording the mortality daily for 6 weeks and percentage increase in life span (%IMST) was calculated. An enhancement of life span by 25% or more was considered as effective antitumour response (Mazumder *et al.,* 1997; Gupta *et al.,* 2000)

IMST (%) = [(Median survival time of treated group/ Median survival time of control group)-1]X100

Median Survival Time (MST) = [Day of first death + Day of last death]/ 2

Hematological studies

RBC, WBC counts and estimation of hemoglobin was done by standard procedures from the blood obtained intracardially (Wintrobe , 1961; D'Armour , 1965).

Hemoglobin estimation

0.1ml of heparinized blood was taken in Sahli's Hemoglobinometer and diluted with 0.1N HCl until the color matched with standard. The reading was then taken from the graduated cylinder and expressed as g/100ml of blood.

Counting of erythrocytes

The blood sample was diluted (1:200) with the diluting fluid using Thoma pipette. After vigorous mixing, a drop of resultant mixture was discharged under the cover glass of Neubauer hemocytometer and the corpuscles were allowed to settle for 3 minutes. The number of erythrocytes in 80 small squares was counted under light microscope. The number of cells in 1 cumm of undiluted blood was calculated.

Total count of leukocytes

Blood was diluted 1:20 with a diluting fluid. The Neubauer hemocytometers were filled with the mixture and the number of cells in four corner blocks (each block subdivided into 16 squares) was determined and the total leukocyte count per cumm of blood was calculated.

Biochemical estimation

Blood samples were collected from the animals intracardially and serum was separated for the biochemical estimations of serum glutamic pyruvate transaminase (SGPT), serum glutamic oxaloacetate transaminase (SGOT) (Reitman and Frankel 1957) and alkaline phosphatase (ALP) (Kind and King, 1954).

***In vivo* antioxidant assay**

The antioxidant assay was performed with liver tissue and evaluation was carried out by measuring the level of lipid peroxidation (Ohkawa *et al*., 1979) and the amount of enzymatic (catalase; CAT) and nonenzymatic antioxidant system (reduced glutathione; GSH) by the methods of Luck (1963) and Ellman (1959) respectively.

Assay for *in vitro* cytotoxicity

The *in vitro* short term cytotoxicity of MECM was assayed using EAC cell lines. Briefly $1x10^6$ viable cells of EAC cell line, suspended in 0.1 ml of phosphate buffered saline (PBS) (0.2 M, pH 7.4) with various concentrations of extract (100- 800 µg/ml) and phosphate buffer saline in a final volume of 1 ml were incubated at 37°C for 30 minutes. Cell suspension in in phosphate buffer saline without extract served as control. After the incubation, the viability of the cells was determined using trypan blue by the method of Boyse *et al*. (1964). The percentage of cytotoxicity was determined by calculating % inhibition and IC_{50} values.

Statistical Analysis

Values were presented as mean ± S.E.M. Data were statistically evaluated by one-way analysis of variance (ANOVA) followed by post hoc Dunnett's test using SPSS software. $P< 0.05$ was considered as statistically significant and $p<0.01$ as highly significant.

RESULTS

In acute toxicity study, MECM did not show any toxic effect upto the dose of 2 g/kg b.wt, accordingly 200 and 400 mg/kg b.wt were taken as low and high dose of MECM for the experiment.

In case of tumor growth response study, MECM treatment significantly reduced tumor volume, packed cell volume and viable cell count compared to those of EAC control mice, while nonviable cell count was found to be increased significantly in the treated groups. These results were summarized in Table.1. Table.2 depicts the effects of MECM on prolongation of life span. In life span study, the median survival time for the control group was 19.5 days, whereas it was 27 and 33 days for low dose and high dose of MECM treated groups. The median survival time for the group treated with standard drug 5-FU was 34 days. Increase in life span at low dose and high dose of MECM treated groups (38.46% and 69.23% increase) as well as for the standard drug treated group (74.36%) were found to be significant with respect to the EAC control mice and it reflects the antitumor property of the extract MECM.

Administration of MECM significantly reduced WBC count in both the groups of III and IV with respect to that of EAC control group. RBC count and hemoglobin content, which were decreased after EAC inoculation, were found to be significantly restored to the normal levels in the animals treated with MECM of both 200 and 400 mg/kg b.wt, as well as standard drug 5-FU.The results (Fig.1) implies the protective role of MECM on the hematological profile of EAC bearing mice.

Biochemical estimation as shown in Table.3 indicates the elevated level of liver functional enzymes in serum in EAC treated group with respect to normal animals, while these were significantly reduced to near normal value in the drug treated groups.

Fig.2 illustrates the effects of MECM on the antioxidant status of EAC bearing mice. The level of lipid peroxide in liver tissue (expressed as nM lipid peroxide/mg of wet liver tissue) was significantly increased in EAC control mice when compared to normal control animals. After administration of MECM (200 and 400 mg/kg b.wt), lipid peroxide levels were significantly reduced when compared with EAC control mice. Similarly, reduced glutathione level which was GSH level which was reduced in EAC control group was restored to the near normal values by treatment with MECM as well as 5-FU. In EAC control mice there were significant reduction in antioxidant enzyme catalase activity, which was significantly improved by the treatment with MECM, as

Table 1: Effect of Methanol extract of *C.maxima* (MECM) on Tumor growth response of EAC bearing mice

Groups	Ascitic tumor volume (ml)	Packed cell volume (ml)	Tumor cell count ($X10^7$ /ml)		Total cell count ($X10^7$ /ml)
			Viable [% cell count]	Non viable [% cell count]	
EAC Control	4.51±0.35	2.17±0.22	14.00±0.53 [98.18%]	0.26±0.18 [1.82%]	14.26±0.45
MECM (200 mg/kg)	1.82±0.10*	0.60±0.14*	6.92±0.50* [87.64%]	0.98±0.58* [12.36%]	7.90±0.55*
MECM (400 mg/kg)	1.04±0.08*	0.32±0.08*	4.25±0.75* [62.62%]	2.54±0.98* [37.38%]	6.79±0.94*
5Fluorouracil (20 mg/kg)	0.83±0.16*	0.40±0.06*	4.15±0.46* [60.10%]	2.75±0.32* [39.90%]	6.90±0.30*

Values are Mean±S.E.M.; n=6 in each group. Drug treatment was done for 9 days. * $p < 0.01$for treated groups *vs* EAC control group; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett's test.

Table 2: Effect of Methanol extract of *C.maxima* (MECM) on prolongation of life span of EAC bearing mice

Groups	MST (days)	%IMST
EAC Control	19.5	-
MECM (200 mg/kg)	27.0	38.46
MECM (400 mg/kg)	33.0	69.23
5Fluorouracil (20 mg/kg)	34.0	74.36

MST: Median survival time; % IMST : % Increase in MST= [(T/C)-1] x100 where T is median survival time of treated group and C that of control group

was observed in standard drug treated mice in group V as well.

In the *in vitro* cytotoxicity study, MECM showed direct cytotoxicity on the EAC cell line, in a concentration dependent manner in the dose range of 100-800 µg/ml, as was revealed in Fig.3 and the IC_{50} value was found to be 378.5 µg/ml.

DISCUSSION

Cancer is a pathological state involving uncontrolled proliferation of tumor cells. The present study was carried out to investigate the antitumor potential of MECM against EAC bearing mice. EAC (Ehrlich Ascites Carcinoma) is a very rapidly growing carcinoma with very aggressive behavior (Segura *et al.,* 2000). It is able to grow in almost all strains of mice. The Ehrlich Ascitic tumor implantation induces *per se* a local inflammatory reaction, with increasing vascular permeability, which results in an intense edema formation, cellular migration, and a progressive ascitic fluid formation and accumulation (Fecchio *et al.,* 1990). The ascitic fluid is essential for tumor growth, since it constitutes a direct nutritional source for tumor cells (Shimizu *et al.,* 2004). MECM treatment significantly reduced tumor volume probably by lowering the ascitic nutritional fluid volume. Further, the packed cell volume and the number of viable EAC tumor cells in peritoneum were significantly lower in the mice treated with MECM when compared to the tumor control group. These results could indicate either a direct cytotoxic effect of MECM on tumor cells or an indirect local effect, which may involve macrophage activation and vascular permeability inhibition.

Prolongation of life span of the animals is a reliable criterion for judging the value of any anticancer drug (Gupta *et al.,* 2004). The increase of life span of tumor bearing mice by MECM treatment is a positive result and supports the antitumor effect of MECM.

In cancer chemotherapy the major problem are myelosuppression and anemia (Price and Greenfield 1958; Maseki *et al.,* 1981). The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions (Fenninger and Mider, 1954). Treatment with MECM brought back the hemoglobin content, RBC and WBC cell count near to normal values. This indicates that MECM possesses protective action on the heamopoietic system.

Significant elevation in the levels of SGOT, SGPT, SALP reflects the hepatocellular damages caused by a number of agents. Biochemical measurements of these parameters showed that to some extent hepatotoxicity was associated after 9 days of inoculation with EAC. Treatment with the MECM restored the elevated bio-

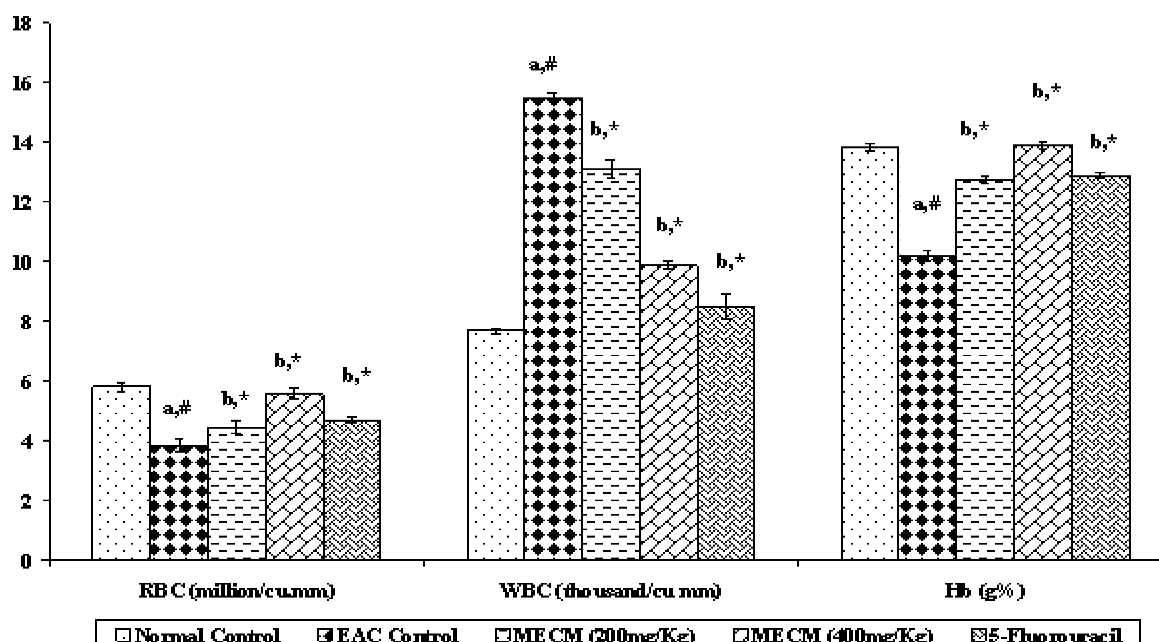

Figure 1: Effect of Methanol extract of *C.maxima* (MECM) on hematological parameters of EAC bearing mice

Values are Mean±S.E.M.; n=6 in each group. Drug treatment was done for 9 days. [a] EAC control group *vs* normal control group, [#] $p < 0.01$; [b] Treated groups *vs* EAC control group, [*] $p< 0.01$; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett's test.

Table 3: Effect of Methanol extract of *C.maxima* (MECM) on biochemical parameters of EAC treated mice

Groups	SGPT (IU/L)	SGOT (IU/L)	SALP (IU/L)
Normal Control	111.00±1.65	97.79±2.99	59.50±2.00
EAC Control	229.70±4.52[a,#]	210.54±6.01[a,#]	149.12±4.05[a,#]
MECM (200 mg/kg)	169.90±5.60[b,*]	120.55±7.12[b,*]	72.50±7.12[b,*]
MECM (400 mg/kg)	146.00±5.00[b,*]	95.50±4.50[b,*]	60.00±5.80[b,*]
5-Fluorouracil (20 mg/kg)	143.15±3.39[b,*]	99.96±4.16[b,*]	61.05±3.20[b,*]

Values are Mean±S.E.M.; n=6 in each group. Drug treatment was done for 9 days. [a] EAC control group *vs* normal control group, [#] $p < 0.01$; [b] Treated groups *vs* EAC control group, [*] $p< 0.01$; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett's test.

chemical parameters more or less to normal range, indicating the protection of the tumor cell induced hepatotoxicity by MECM.

The improper balance between ROMs (Reactive Oxygen Metabolites) and antioxidant defences results in 'oxidative stress', which deregulates the cellular functions leading to various pathological conditions including cancer (Bandyopadhyay *et al.,* 1999; Adesegun *et al.,* 2009). The oxidative stress may lead to damage of the macromolecules such as lipids and can induce lipid peroxidation *in vivo* (Yagi, 1991). In EAC bearing mice the level of lipid peroxide in liver was significantly elevated, which was however reduced to near normal level in the MECM treated group animals. This reflects the decrease in free radical production and the subsequent reduction in oxidative stress, one of the main risk factors for the disease.

Glutathione, a potent inhibitor of neoplastic process plays an important role as an endogenous antioxidant system that is found particularly in high concentration in liver and is known to have key function in the protective process (Sinclair *et al.,* 1990). The level of reduced glutathione was depleted in cancer bearing mice which may be due to its utilization by the excessive amount of free radicals. Treatment with MECM was found to increase the GSH content in the liver as compared to EAC control animals.

On the other hand the free radical scavenging enzyme catalase is present in all oxygen-metabolizing cells and its function is to provide a direct defense against the

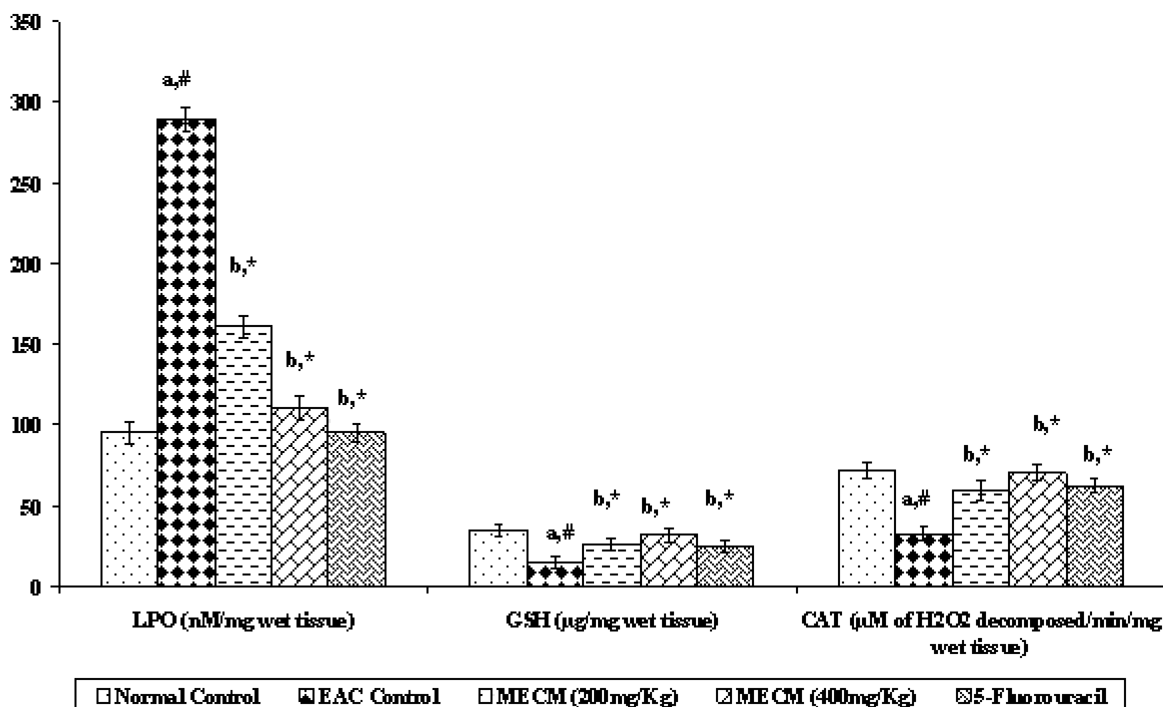

Figure 2: Effect of Methanol extract of *C.maxima* (MECM) on antioxidant status of EAC bearing mice

LPO: Lipidperoxide; GSH: Reduced Glutathione; CAT: Catalase. Values are Mean±S.E.M.; n=6 in each group. Drug treatment was done for 9 days. ^{a} EAC control group *vs* normal control group,$^{\#}$ $p < 0.01$; ^{b} Treated groups *vs* EAC control group, * $p< 0.01$; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett's test.

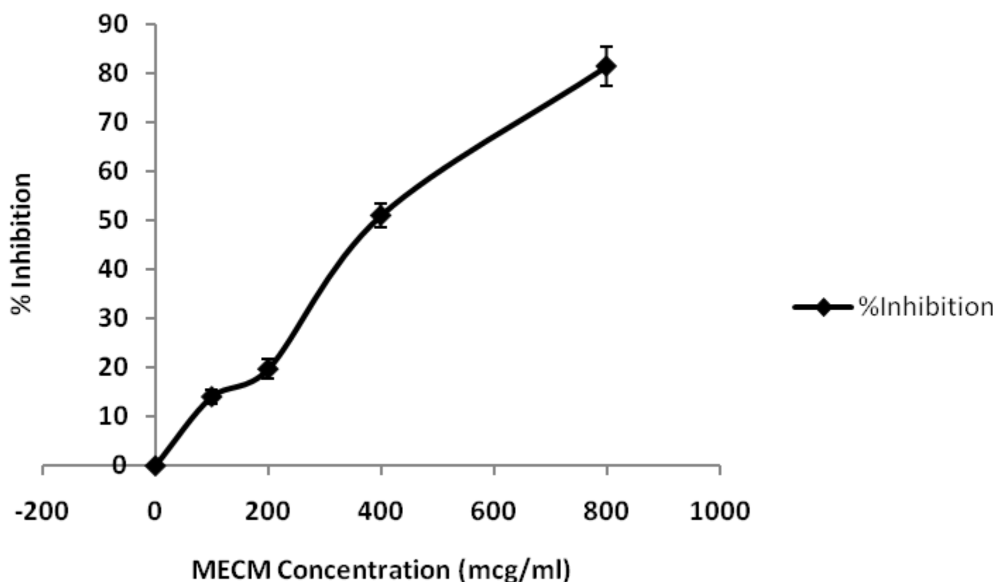

Figure 3: Effect of Methanol extract of C.maxima (MECM) on in vitro EAC cell lines

Values are Mean ± S.E.M.; where n=3.

potentially damaging reactivities of superoxide and hydrogen peroxide. The inhibition of CAT activities as a result of tumor growth has also been reported (Marklund *et al.,* 1982). Similar findings were observed in the present investigation with EAC bearing mice. The administration of MECM at different doses increased the CAT levels in a dose dependent manner, which along with the restoration of lipid peroxide and GSH content to near normal indicates the antioxidant and free radical scavenging property of MECM.

Cytotoxicity is one of the main targets by chemicals to produce antitumor activity, number of anticancer drugs possess significant cytotoxic activity (Suffness and Pezzuto, 1991). In present study on *in vitro* EAC

cell line, MECM also has shown to possess direct cytotoxic activity, when compared to EAC control group and it potentiates the direct cytotoxicity as one of the most probable mechanisms for the anticancer activity of the extract.

The result of the present investigation is quite encouraging and it explores the potent anticancer activity of MECM probably because of its direct cytotoxic effect which is further potentiated by its antioxidant properties (Jiau-Jian and Larry, 1977). Preliminary phytochemical study showed the presence of flavonoid, polyphenolics, saponin, protein and carbohydrate in MECM. Many such compounds are known to possess potent antitumor properties, particularly some proteins and polysachharide fractions in *C. maxima* fruits and seeds have been reported to possess anticancer activities (Kintzios, 2006; Ito *et al.,* 1986; Xie 2004). Hence the potent anticancer activity of MECM may be due to any of these phytoconstituents. Further research is ongoing in our laboratory to find out the bioactive principle(s) for the anticancer activity of the extract.

ACKNOWLEDGEMENT

Necessary support and cooperation from Dr. Abhijit Sen Gupta, Director-cum-Principal & Prof. Dipankar Chakraborty, Registrar, Guru Nanak Institute of Pharmaceutical Sciences and Technology, Kolkata are hereby gratefully acknowledged.

REFERENCES

Adesegun, S.A., Fajana, A., Orabueze, C. I., and Coker, H.A.B. 'Evaluation of Antioxidant Properties of *Phaulopsis fascisepala* C.B.Cl. (Acanthaceae)'. eCAM, vol.6, no.2, 2009 pp. 227–231.

Adolfo, A.C. and Michael, H. 'Mexican plants with hypoglycaemic effect used in the treatment of diabetes'. Journal of Ethnopharmacology, vol.99, 2005 pp.325–348.

Bandyopadhyay, U., Das, D. and Banerjee, R.K. 'Reactive oxygen species: Oxidative damage and pathogenesis'. Current science, vol.77, 1999 pp.658.

Boyse, E., Old, E. and Chouroubnkov, I. 'Cytotoxic test of determination of mouse antibody', in Eisen, M. (ed.) Methods in Medical Research, vol. I, Year Book Medical Publishers, Chicago:IL. 1964 pp.39-40.

Bufalo, M.C, Candeias, J.M.G. and Sforcin, J.M. '*In vitro* Cytotoxic Effect of Brazilian Green Propolis on Human Laryngeal Epidermoid Carcinoma (HEp-2) Cells.' eCAM, vol.6, no.4, 2009 pp. 483–487.

Caili, F.U., Shi, Huan. and Quanhong, L.I. 'A review on pharmacological activities and utilization technologies of pumpkin'. Plant Foods for Human Nutrition, vol.61, 2006 pp.73-80.

Dagli, M.L.J., Guearra, J.L. and Saldiva, P.H.N. 'An experimental study on the lymphatic dissemination of the solid Ehrlich tumor in mice'. Braz J Vet Res Anim Sci, vol.29, 1992 pp.97-103.

D'Armour, F.E., Blood, F.R., and Belden, D.A. in 'The Manual for laboratory work in mammalian physiology', 3rd ed. Illinois Chicago, The University of Chicago Press. 1965 pp.4-6.

Ellman, G.L. 'Tissue sulphydryl groups'. Arch Biochem Biophys vol.82, 1959 pp.70-72.

Fecchio, D., Sirois, P., Russo, M. and Janear, S. 'Studies on inflammatory response induced by Ehrlich tumor in mice peritoneal cavity'. Inflammation vol.14, 1990 pp.125–131.

Fenninger, L.D., and Mider, G.B. in 'Advances in cancer research'. Grenstein, J.P., and Haddow, A., (eds.) vol II. New York: Academic Press. 1954 p.244.

Ghosh, M.N. 'Fundamentals of Experimental Pharmacology', 2nd Ed. Scientific Book Agency, Calcutta. 1984 pp.192-194.

Gupta, M., Mazumder, U.K., Rath, N. and Mukhopadhyay, D.K. 'Antitumor activity of methanolic extract of *Cassia fistula* L. seed against Ehrlich ascites carcinoma'. Journal of Ethnopharmacology, vol. 72, 2000 pp.151-156.

Gupta, M., Mazumder, U.K., Sambath Kumar, R., Sivakumar, T., and Vamsi, M.L.M. 'Antitumour activity and antioxidant status of Caesapinia bonducella against Ehrlich ascites carcinoma in Swiss albino mice'. J Pharmacol Sci vol.94, 2004 pp.177–184.

Indap, M.A., Radhika, S., Motiwale, L. and Rao, K.V.K. 'Quercetin: antitumour activity and pharmacological manipulations for increased therapeutic gains'. Indian J Pharm Sci vol.68, 2006 pp.465–469.

Ito, Y., Maeda, S. and Sugiyama, T. 'Suppression of 7, 12- dimethylbenz[a]anthracene-induced chromosome aberrations in rat bone marrow cells by vegetable juices'. Mutat Res, vol.172, no.1,1986 pp. 55–60.

Jia, W., Gao, W. and Tang, L. 'Antidiabetic herbal drugs officially approved in China'. Phytother Res vol.17, no.10, 2003 pp.1127–1134.

Jiau-Jian, L. and Larry, W.O. 'Over expression of manganese-containing superoxide dismutase confers resistance to the cytotoxicity of tumor necrosis factor α and/or hyperthermia'. Cancer Res vol.57, 1977 pp.1991-98.

Kim, J.B., Koo, H.N., Joeng, H.J., Lyu, Y.S., Park, S.G., Won, J.H., et al. 'Induction of apoptosis by Korean medicine Gagam-whanglyunhaedoktang through activation of caspase-3 in human leukemia cell line, HL-60 cells'. J Pharmacol Sci, vol.97, 2005 pp.138–145.

Kind, P.R.N. and King, E.J. 'Estimation of plasma phosphatase by determination of hydrolysed phenol with antipyrin'. J Clin Pathol, vol.7, 1954 pp.322-331.

Kintzios, S.E. 'Terrestrial plant derived anticancer agents and plants used in anticancer research'. Crit Rev Plant Sci, vol.25, 2006 pp.79–113.

Kirtikar, K.R. and Basu, B.D. 'Indian Medicinal Plants'. Vol V, 2nd Ed. Oriental Enterprises. India: 2003 pp.1606-1608.

Luck, H. 'Methods of Enzymatic Analysis' Bergmeyer, H.V. (ed.), vol. III, Academic press, New York. 1963 pp.886-888.

Marklund, S.L., Westman, N.G., Lundgren, E. and Roos, G. 'Copper and zinc containing superoxide dismutase, manganese-containing superoxide dismutase, catalase, and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues'. Cancer Res, vol.42, 1982 pp.1955-61

Maseki, M., Nishiagaki, I., Hagishara, M., Tomoda, Y. and Yagi, K. 'Lipid peroxidation levels and lipid content of serum lipoprotein fractions of pregnant subjects with or without preeclampsia'. J Clin Chim Acta, vol.41, 1981 pp.424-426.

Mazumder, U.K., Gupta, M., Maiti, S. and Mukherjee, M. 'Antitumor activity of *Hygrophilaspinosa* on Ehrlich ascites carcinoma and sarcoma-180 induced mice'. Indian J Expt Biol, vol.35, 1997 pp.473-477.

Ohkawa, H., Oishi, N. and Yagi, K. 'Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction'. Anal Biochem, vol.95, 1979 pp.351-358.

Orsolic, N., Kosalec, I. and Basic, I. 'Effects of local administration of propolis and its polyphenolic compounds on tumor formation and growth'. Bio.Pharm. Bull, vol.28, no.10, 2005 pp.1928-1933.

Popovic, M. 'On growing squash and pumpkin (Cucurbita sp.) in Yugoslavia'. Savremena Poljoprivreda, vol.11, 1971 pp.59–71.

Price, V.E and Greenfield, R.E. 'Anemia in cancer', in: Greenstein, J.P., Haddow, A., (eds.) Advances in cancer research. vol V. New York: Academic Press. 1958 pp.199-200.

Reitman, S. and Frankel, A.S. 'A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase'. Am J Clin Path, vol.28, 1957 pp.53-56.

Segura, J.A., Barbero, L.G. and Marquez, J. 'Ehrlich ascites tumour unbalances splenic cell population and reduces responsiveness of T cells to Staphylococcus aureus enterotoxin B stimulation'. Immunology Lett., vol.74, 2000 pp.111–115.

Shimizu, M., Azuma, C., Taniguchi, T. and Murayama, T. 'Expression of cytosolic phospholipase A2α in murine C12 cells, a variant of L929 cells, induces arachidonic acid release in response to phorbol myristate acetate and Ca^{2+} ionophores, but not to tumor necrosis factor-α'. J Pharmacol Sci, vol.96, 2004 pp.324–332.

Sinclair, A.J., Barnett, A.H. and Lunie, J. 'Free radical and auto-oxidant systems in health and disease. Br J Hosp Med, vol.43, 1990 pp.334-44.

Suffness, M. and Pezzuto, J.M. 'Methods in plant Biochemistry', vol. VI, Academic Press, New York. 1991 p.71.

Wintrobe, M.M., Lee, G.R., Boggs, D.R., Bithel, T.C., Athens, J.W. and Foerester, J. Clinical Hematology, 5th ed. Philadelphia, Les and Febiger, PA. 1961 p.326.

Xia, M., Wang, D., Wang, M., Tashiro, S., Onodera, S., Minami, M., et al. '*Dracorhodin percholarate* induces apoptosis via activation of caspases and generation of reactive oxygen species'. J Pharmacol Sci, vol.95, 2004 pp.273–283.

Xie, J.M. 'Induced polarization effect of pumpkin protein on B16 cell'. Fujian Med Univ Acta, vol.38, no.4, 2004 pp.394–395.

Yagi, K. 'Lipid peroxides and human diseases'. Chem Physiol Lip, vol.45, 1991 pp.337-51.