World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Evaluation of *in- vivo* antitumor and antioxidant activity of *Ervatamia coronaria* leaves

Gomathi Periasamy, Pharmacognosy Course and Research Team, Department of Pharmacy, College of Health Sciences, Mekelle University, Mekelle, Ethiopia

Received: 12-05-2014 / Revised: 08-06-2014 / Accepted: 19-06-2014

ABSTRACT

The present study was designed to determine the antitumor and antioxidant properties of crude methanol extract from the leaves of *Ervatamia coronaria* (MEEC) against Ehrlich Ascites Carcinoma (EAC) bearing Swiss albino mice. The effect of MEEC on the growth of transplantable murine tumor, life span of EAC bearing host, viable and non-viable cell count, packed cell volume, hematological profile and biochemical parameters such as lipid peroxidation (LPO), reduced glutathione content (GSH), superoxide dismutase (SOD) and catalase (CAT) activities were estimated. MEEC caused significant ($P<0.01$) decrease in tumor volume, packed cell volume and viable count; and it prolonged the life span of EAC-tumor bearing mice. Hematological studies reveal that the Hb content and RBC count were restored to near normal levels in extract treated animals. MEEC significantly ($P<0.05$) decreased the levels of LPO and significantly increased the levels of GSH, SOD and CAT. Moreover the MEEC was found to be devoid of conspicuous short-term toxicity in the mice when administered daily for 14 days at the doses of 50, 100 and 200 mg/kg. The results suggested that the methanol extract of *Ervatamia coronaria* leaves exhibited antitumor effect by modulating lipid peroxidation and augmenting antioxidant defense system in EAC bearing Swiss albino mice.

Key words: *Ervatamia coronaria*, Antitumor, antioxidant, Ehrlich Ascites Carcinoma

INTRODUCTION

Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives every year (Abdullaev *el al*., 2000). An extremely promising strategy for cancer prevention today is chemoprevention, which is defined as the use of synthetic or natural agents (alone or combination) to block the development of cancer in humans. Plants, vegetables and herbs used in the folk and traditional medicine have been accepted currently as one of the main source of cancer chemoprevention drug discovery and development (Abdullaev, 2001). There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine.

Plant derived natural products such as flavonoids, terpenoids and steroids (Osawa *el al*., 1990; Di Carlo *et al*., 1999; Keith *et al*., 1990) etc. have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and chemopreventive effects (Roja and Heble, 1994). Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against infection and degenerative diseases, in the cases of inflammation, cancer and diabetes (De Feudis *et al*., 2003).

Ervatamia coronaria Stapf., (Synonym : *Tabernaemontana divaricata*) (Van Beek *et al*., 1984) belongs to the family Apocynaceae, is a glabrous, evergreen tree indigenous to China and India and is cultivated in gardens for its ornamental and fragrant flowers. This species has been extensively investigated and a number of chemical constituents such as alkaloids, triterpenoids, steroids, flavonoids, phenyl propanoids and phenolic acids (Pawelka and Stoeckigt, 1983; Atta-Ur-Rahman *et al*., 1985; Yu and Liu, 1999; Rastogi *et al*., 1980; Sharma and Cordell, 1988; Van Der Heijden, 1989) were isolated from leaves, roots and stems of the plant. In Indian traditional systems of medicine the plant material is widely used as a purgative, tonic to the brain, the spleen and the liver; in the treatment of cancer, wounds and inflammations (Kirtikar and Basu, 1975; Hsu, 1967). The plant extract was also found to possess analgesic, antipyretic, vasodilator and CNS depressant effects, antispasmodic, hypotensive activity, antiinflammatory, uterine stimulant effect

***Corresponding Author Address: Dr. Gomathi Periasamy,** Assistant Professor, Pharmacognosy Course and Research Unit, Department of Pharmacy, College of Health Sciences, P.O. Box No. 1871, Mekelle University, Mekelle, Ethiopia; Email: pgoms@yahoo.com*

and cytotoxic activity (Taesotikul *et al*., 1989; Dhar *et al*., 1968; Da Silva *et al*., 1984).

Furthermore, literature survey of *Ervatamia coronaria* revealed that no researchers have reported *in vivo* antitumor and antioxidant activities of this plant so far. Therefore, it is worth conducting an investigation on *in vivo* antitumor and antioxidant activities of methanol extract of *Ervatamia coronaria* leaves (MEEC) in EAC tumor bearing mice.

MATERIAL AND METHODS

Plant material: The leaves of the plant *Ervatamia coronaria* (Family: Apocynaceae) were collected from Erode district of Tamilnadu, India. The plant material was taxonomically identified by Botanical Survey of India, Kolkata. A voucher specimen (No. GMG 01) has been preserved in our laboratory for future reference. The leaves were dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried powder material of the leaves was defatted with petroleum ether and the marc thus obtained was then extracted with methanol in a soxhlet apparatus. The solvent was completely removed under reduced pressure and a semisolid mass was obtained (MEEC, yield 14.51 %). Phytochemical screening of the extract revealed the presence of flavonoids, tannins, alkaloids, glycosides and steroids. The dried MEEC was suspended in normal saline and used for the present study.

Chemicals: The following chemicals were obtained from the indicated commercial sources: Phenazonium methosulphate (PMS) and Nicotinamide adenine dinucleotide (NADH), thiobarbituric acid (TBA), nitroblue tetrazolium chloride (NBT) (Loba Chemie, Bombay, India); 1-chloro-2,4-dinitro benzene (CDNB), bovine serum albumin (Sigma chemical co., St. Luis, MO, USA); Folin-Ciocalteau phenol, reduced Glutathione and 5,5'-dithio bis-2-nitro benzoicacid (DTNB) (SISCO Research Laboratory, Bombay, India). All the reagents used were of analytical reagent grade.

Animals: Studies were carried out using male Swiss albino mice weighing 21 ± 2 g. They were obtained from the animal house of Jadavpur University, Kolkata. The mice were grouped and housed in poly acrylic cages (38x23x10 cm) with not more than 12 animals per cage and maintained under standard laboratory conditions (temperature 25 ± 20C) with 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 10 days before commencement of the experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee.

Tumor Cells: 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 2x106 cells per mouse after every 10 days. EAC cells 9 days old were used for the screening of antitumor activity of MEEC.

PHARMACOLOGY

Acute Toxicity Test: The animals were divided into six groups containing eight animals in each group. MEEC was suspended in normal saline and administered orally as a single dose to groups of mice at different concentrations (500, 750, 1000, 1250, 1500 and 2000 mg/kg body weight). These animals were observed for a 72 h period. The number of deaths was expressed as a percentile and the LD50 was determined by probit a test using the death percentage versus the log dose (Thompson and Weil, 1952).

Antitumor Activity: Male Swiss Albino mice were divided in to 6 groups (n=12). All the groups were injected with EAC cells (0.2 ml of 2x106 cells per mouse) intraperitonelly except the normal group. This was taken as day zero. On the first day, 5 ml/kg of normal saline was administered to group 1 and 2 (normal and EAC control). MEEC at different dose (50, 100 and 200 mg/kg) and the standard drug 5-Fluorouracil (20 mg/kg) (Kavimani and Mani Senthilkumar, 2000) were administered in group 3, 4, 5 and 6 respectively for 14 days orally. After the last dose and 18 hr fasting, six mice from each group were sacrificed for the study of antitumor activity, hematological and liver biochemical parameters. The rest of the animal groups were kept to check the survival time of EAC-tumor bearing hosts. The antitumor activity of the methanol extract of Ervatamia coronaria was measured in EAC animals with respect to the following parameters:

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 100 rpm per 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's counting chamber and the number 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 didn't take up the dye were viable and those that took the dye were non-viable. These viable and non-viable cells were counted.

Cell count = Number of cells x dilution/ Area x thickness of liquid film

Percentage increase life span (% ILS): The effect of MEEC on tumor growth was monitored by recording the mortality daily for a period of 6 weeks and percentage increase in life span (% ILS) was calculated.

% ILS = (Mean survival of treated group/ Mean survival of control group)-1 x 100

Mean survival = (Day of first death + day of last death)/2

Body weight: Body weight of the experimental mice were recorded both in the treated and control group at the beginning of the experiment (day 0) and sequentially on every 5th day during the treatment period.

Hematological Parameters: At the end of the experimental period, the next day after an overnight fast blood was collected from freely flowing tail vein and used for the estimation Hemoglobin (Hb) content, red blood cell count (RBC) (D'Armour *et al*., 1965) and white blood cell count (WBC) (Wintrobe *et al*., 1961). WBC differential count was carried out from Leishman stained blood smears (Dacie and Lewis, 1958).

Biochemical Assays: After the collection of the blood samples, the mice were sacrificed. Then their liver was excised, rinsed in ice-cold normal saline solution followed by cold 0.15 M Tris-HCl (pH 7.4)**,** blotted dry and weighed. A 10 % w/v homogenate was prepared in 0.15 M Tris-HCl buffer and was used for the estimation of lipid peroxidation (LPO) and reduced glutathione (GSH). The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 40 C. the supernatant thus obtained was used for the estimation of superoxide dismutase (SOD), catalase (CAT) and total protein.

Estimation of Lipid Peroxidation (LPO): The levels of Thiobarbituric acid reactive substances (TBARS) in the liver was measured by the method of Ohkawa *et al*., 1979 as a marker for lipid peroxidation. A mixture of 0.4 ml of 10 % liver homogenate, 1.5 ml of 8.1 % sodium dodecyl sulphate (SDS), and 1.5 ml of 0.8 % TBA solution was heated at 950 C for 1 h. After cooling, 5.0 ml of n-butanol-pyridine (15:1) was added, and the absorbance of the n-butanol-pyridine layer was measured at 532 nm.

Estimation of Reduced Glutathione (GSH): The tissue GSH was determined by the method of Beutler and Kelly, 1963. Virtually all the non-protein sulfhydryl groups of tissues are in the form of reduced GSH. 0.2 ml of tissue homogenate was mixed with 1.8 ml of EDTA solution. To this 3.0 ml precipitation reagent (after precipitating proteins with TCA) was added, mixed thoroughly and kept for 5 min before centrifugation. To 2.0 ml of the filtrate, 4.0 ml of 0.3 M disodium hydrogen phosphate solution and 1.0 ml of DTNB reagent were added and the absorbance read at 412 nm.

Estimation of Superoxide dismutase (SOD): The activity of SOD in tissue was assayed by the method of Kakkar, 1984. The assay mixture contained 1.2 ml sodium pyrophosphate buffer (pH 8.3, 0.025 mol/L), 0.1 ml PMS (186 mmol/L), 0.3 ml nitroblue tetrazolium (300 mmol/L, 0.2 ml NADH (780 mmol/L) and diluted enzyme preparation and water in a total volume of 3 ml. After incubation at 300 C for 90 sec, the reaction was terminated by the addition of 1.0 ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 ml n-butanol. The color intensity of the chromogen in the butanol layer was measured at 560 nm against n-butanol.

Estimation of Catalase (CAT): Catalase was assayed according to the method of Maehly and Chance, 1954. The estimation was done spectrophotometrically following the decrease in absorbance at 230 nm. The tissue was homogenized in M/150 phosphate buffer (pH 7.0) at 40 C and centrifuged at 5000 rpm. The reaction mixture contained 0.01 M phosphate buffer (pH 7.0), 2 mM H2 O2 and the enzyme extract. The specific activity of catalase is expressed in terms of units/mg protein. A unit is defined as the velocity constant per second.

Estimation of Total Proteins: The protein content of tissue homogenates was measured by the method of Lowry *et al*., 1951. 0.5 ml of tissue homogenate was mixed with 0.5 ml of 10 % TCA and centrifuged for 10 min. The precipitate obtained was dissolved in 1.0 ml of 0.1 N NaOH. From this an aliquot was taken for protein estimation. 0.1 ml of aliquot was mixed with 5.0 ml of alkaline copper reagent and allowed to stand at room temperature for 10 min. 0.5 ml of Folin's phenol reagent was added and the blue color developed was read after 20 min at 640 nm.

Short-term Toxicity: To determine short-term (14 days) toxicity, healthy Swiss albino mice were

divided into 4 groups of 8 animals in each. Group 1 received normal saline (5 ml/kg), orally once daily for 14 days (vehicle control). Groups 2,3 and 4 received MEEC at the doses of 50, 100 and 200 mg/kg respectively, orally once daily for 14 days. At twenty-four hours after the last dose, and after 18-h fasting, the mice were sacrificed. Blood and liver were collected and important internal organs were removed, weighed and observed for pathological changes. Hematological parameters were determined as described above. Serum glutamate pyruvate transaminase (SGPT) and glutamate oxaloacetate transaminase (SGOT) were determined (Bergmeyer *et al*., 1978). Urea was estimated by the enzymatic method and calcium was estimated by the O-cresolphthalein complexone method (Tietz, 1987). Phosphorous was estimated by the colorimetric method (Henry, 1974). Liver biochemical parameters were estimated by the standard methods described above.

STATISTICAL ANALYSIS

The experimental results were expressed as mean ± S.E.M. Data were assessed by ANOVA followed by the Student t-test; P value <0.05 was considered as statistically significant.

RESULTS

Acute Toxicity study: In the acute toxicity assay no deaths were observed during the 72 h period at the doses tested. At these doses, the animals showed no stereotypical symptoms associated with toxicity, such as convulsion, ataxy, diarrhoea or increased diuresis. The median lethal dose (LD_{50}) was determined to be higher that highest dose tested i.e., 2.0 g/kg.

Antitumor activity

Effect of MEEC on Mean Survival Time: The effects of MEEC at the doses of 50, 100 and 200 mg/kg on the Mean survival time of EAC bearing mice is shown in Table 1. In the EAC control group the mean survival time was 21.0 ± 0.74, while it increased to 24.2 ± 0.22 (50 mg/kg), 27.4 ± 0.15 (100 mg/kg) and 30.2 ± 0.26 (200 mg/kg) days respectively in the MEEC treated groups. The group treated with the standard drug 5-Fluorouracil (20 mg/kg) shows 35.6 ± 0.23 days for the same.

Effect of MEEC on tumor growth: The tumor volume, packed cell volume and viable cell count were found to be significantly (P<0.01) increased and non-viable cell count was significantly (P<0.01) low in EAC control animals when compared with normal control animals. Administration of MEEC at the doses of 50, 100 and 200 mg/kg significantly (P<0.01) decreased the tumor volume, packed cell volume and viable cell count. Further more, non-viable tumor cell count at different doses of MEEC were significantly (P<0.01) increased in a dose dependent manner. Finally, the change in body weights of the animals suggests the tumor growth inhibiting property of MEEC. All these results clearly indicate that the MEEC has a remarkable capacity to inhibit the growth of solid tumor induced by EAC cell line in a dose dependent manner in experimental animals (Table 1).

Effect of MEEC on hematological parameters: The effect of MEEC on hematological parameters of EAC treated animals were shown in Table 2. Hematological parameters of tumor bearing mice on day 14 were found to be significantly altered compared to the normal group. The hemoglobin content and RBC count in the EAC control group was significantly (P<0.001) decreased as compared to the normal group. Treatment with MEEC at the dose of 50, 100 and 200 mg/kg significantly (P<0.01) increased the hemoglobin content and RBC count to more or less normal levels. The total WBC counts and protein was found to be increased significantly in the EAC control group when compared with normal group (P<0.001). Administration of MEEC at the doses of 50, 100 and 200 mg/kg to EAC bearing mice significantly (P<0.01) reduced the WBC count and protein as compared with the EAC control animals. In differential count of WBC the percentage of neutrophils increased while the lymphocyte count decreased in the EAC control group. Treatment with MEEC at different doses changed these altered parameters towards more or less normal values.

Effect on lipid peroxidation: As shown in Fig 1, the levels of lipid peroxidation in liver tissue were significantly increased by 38.81 % in the EAC control group as compared to the normal group (P<0.001). After administration of MEEC at different doses (50, 100 and 200 mg/kg) to EAC bearing mice, the level of lipid peroxidation was reduced by 18.64 %, 45.76 % and 83.05 % respectively in comparison to the EAC control group (P<0.05). Results were expressed as nmoles MDA/mg protein/ml.

Effect of MEEC on reduced glutathione: The effect of MEEC on reduced glutathione content of EAC bearing mice were summarized in Fig 2. Inoculation of EAC drastically decreased the GSH content to 26.74 % in the EAC control group when compared with the normal group (P<0.001). The administration of MEEC at the doses of 50, 100 and 200 mg/kg to the EAC bearing mice increased

GSH levels by 16.92 %, 29.23 % and 36.92 % respectively, as compared with EAC control group ($P<0.05$).

Effect of MEEC on SOD level: Fig 3 shows the activity of SOD in liver tissue of experimental groups. The levels of SOD in the liver of EAC bearing mice decreased by 38.49 % ($P<0.01$) in comparison with normal group. Administration of MEEC at the doses of 50, 100 and 200 mg/kg increased the levels of SOD by 13.24 %, 25.98 % and 36.92 % respectively ($P<0.05$) as compared to EAC control animals.

Effect of MEEC on CAT levels: Fig 4 illustrates the activity of catalase in experimental animals. The CAT levels in EAC control group decreased by 57.11 % ($P<0.01$) compared with normal group. Treatment with MEEC at the doses of 50, 100 and 200 mg/kg increased the CAT levels significantly ($P<0.05$) by 15.34 %, 27.83 and 33.55 % respectively when compared to that of EAC control mice.

Short-term toxicity: When the mice were observed for the behavioural changes after oral administration of a single dose of the extract none of the mice were exhibited any abnormal behavioural responses at the doses of 50, 100 and 200 mg/kg. Administration of repeated daily doses of 50, 100 and 200 mg/kg for 14 days did not influence the bodyweight of the mice. The weights of liver, kidney, brain and spleen were also not altered by the treatment. Hematological parameters like hemoglobin and RBC count remained unaltered at the dose of 50, 100 and 200 mg/kg. But there was a marginal increase in WBC count. The results were summarized in Table 3.

DISCUSSION

In the present manuscript we have shown the *in vivo* antitumor and antioxidant status of *Ervatamia coronaria* extract in EAC bearing mice. The MEEC treated animals at the doses of 50, 100 and 200 mg/kg significantly inhibited the tumor volume, packed cell volume, tumor cell count and brought back the hematological parameters to more or less normal levels. 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. Short-term toxicity studies indicate that MEEC at the doses of 50, 100 and 200 mg/kg for 14 days did not exhibit any adverse effects.

In EAC bearing mice a regular rapid increase in ascites tumor volume was noted. Ascites fluid is the direct nutritional source to tumor cells and rapid increase in ascitic fluid with tumor growth could be a means to meet the nutritional requirements of tumor cells (Prasad and Giri, 1994). Treatment with MEEC inhibited the tumor volume, tumor cell count and increased the percentage of trypan blue positive stained dead cells in tumor bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of life span of animals (Clarkson and Burchenal, 1965) and disappearance of leukemic cells from blood (Feninger and Mider, 1954a). A decrease in tumor volume and viable tumor cell count as mentioned above finally reduced the tumor burden and enhanced the life span of EAC bearing mice.

The major problems encountered in cancer chemotherapy are of myelosupression and anemia (Price ans Greenfild, 1958; Hogland, 1982). 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 (Feninger and Mider, 1954b). Perturbation of hematological parameters in tumor bearing animals is partly due to the toxic effects produced in them. In addition myelosupression in cancer chemotherapy is a common phenomenon, which is responsible for poor prognosis (Donehower, 1990). Treatment with MEEC brought back the hemoglobin content, RBC and WBC cell count near to normal levels. This indicates that MEEC possess protective action on the hemopoietic system.

Excessive production of free radicals resulted in oxidative stress, which leads to damage of macromolecules such as lipids can induce lipid peroxidation *in vivo* (Yagi, 1987). Increased lipid peroxidation would cause degeneration of tissues. Lipid peroxidase formed in the primary site would be transferred through the circulation and provoke damage by propagating the process of lipid peroxidation (Sinclair *et al*., 1990). Malondialdehyde (MDA), the end product of lipid peroxidation was reported to be higher in cancer tissues than in non-diseased organs (Yagi, 1987). Glutathione, a potent inhibitor of neoplastic process plays an important role as an endogenous antioxidant system. It is found particularly in high concentration in liver and is known to have a key function in the protective process (Sinclair *et al*., 1990). MEEC reduced the elevated levels of lipid peroxidation and increased the glutathione content in EAC bearing mice.

SOD and CAT, the free radical scavenging system are present in all oxygen-metabolizing cells and

their function is to provide a defense against the potentially damaging reactivities of superoxide and hydrogen peroxide. A decrease in SOD activity in EAC bearing mice which might be due to loss of Mn SOD activity in EAC cells and the loss of mitochondria leading to a decrease in total SOD activity in the liver (Sun *et al*., 1989).

The inhibition of SOD and CAT activities as a result of tumor growth was also reported (Marklund *et al*., 1982). Similar findings were observed in the present investigation with EAC bearing mice. The administration of MEEC at different doses increased the SOD and CAT levels in a dose dependent manner, which may indicate the antioxidant and free radical scavenging property of MEEC.

It was reported that plant-derived extracts containing antioxidant principles showed cytotoxicity towards tumor cells (Jiau-Jian and Larry, 1977) and antitumor activity in experimental animals (Ruby *et al*., 1995). Antitumor activity of these antioxidants in either through induction of apoptosis (Ming *et al*., 1998) or by inhibition of neovascularization (Putul *et al*., 2000). The lowering of lipid peroxidation and increase in levels of GSH, SOD and CAT in MEEC treated group indicates its potential as an inhibitor of EAC induced intracellular oxidative stress.

CONCLUSION

The present study demonstrates the methanol extract of *Ervatamia coronaria* (MEEC) increased the life span of EAC tumor bearing mice and decreased lipid peroxidation and thereby augmented the endogenous antioxidant enzymes in the liver. All these parameters suggest that the methanol extract of *Ervatamia coronaria* leaves exhibits potential antitumor and antioxidant activities. Further investigation are in progress in our laboratory to identify the active principles involved in this antitumor and antioxidant activity.

Table 1. Effect of methanol extract of *Ervatamia coronaria* (MEEC) on body weight, mean survival time, % ILS, tumor volume, packed cell volume and viable and non-viable tumor cell count of EAC bearing mice

Parameters	EAC control ($2x10^6$ cells/ml/mouse)	EAC + MEEC (50 mg/kg)	EAC + MEEC (100 mg/kg)	EAC + MEEC (200 mg/kg)	EAC + 5-FU (20 mg/kg)
Body weight (g)	28.6±0.25	24.78±0.19**	24.75±0.19	22.54±±1.17**	21.57±0.03**
Mean Survival Time (days)	21.0±0.74	24.2±0.22*	27.4±0.15*	30.2±0.26**	35.6±0.23**
Increase Life Span (%)	-	15.24	30.48	43.81	69.52
Tumor volume (ml)	3.9±0.02	3.42±0.1**	3.11±0.03**	2.8±0.02**	-
Packed cell volume (ml)	2.34±0.04	1.49±0.02	0.96±0.06	0.35±0.01**	-
Viable tumor cell count ($x10^7$ cells/ml)	8.9±0.87	8.96±0.02	4.42±0.13	3.6±0.12	0.92±0.13**
Non-viable tumor cell count ($x10^7$ cells/ml)	0.4±0.02	1.35±0.04**	1.62±0.02**	0.7±0.02	-

Data are expressed as the mean of results in 8 mice ± S.E.M; **P<0.01 Experimental groups compared with the EAC control group; Body weight of normal mice is 21.8±0.19

Table 2: Effect of methanol extract of *Ervatamia coronaria* (MEEC) on hematological parameters of EAC bearing mice

Parameters	Normal (0.9 % NaCl, 5 ml/kg)	EAC control ($2x10^6$ cells/ml/mouse)	EAC+MEEC (50 mg/kg)	EAC+MEEC (100 mg/kg)	EAC+MEEC (200 mg/kg)	EAC+5-FU (20 mg/kg)
Hemoglobin (g %)	12.2±1.1	9.3±0.39***	9.91±1.2**	10.76±0.46**	10.3±1.03**	12.6±0.69
RBC (cells/ml $x10^6$)	6.4±0.54	4.5±0.45***	4.38±1.5	4.88±0.33**	5.91±0.23**	6.1±0.38**
WBC (cells/ml $x10^6$)	6.7±0.58	18.9±1.67***	16.54±1.2**	14.2±0.80**	12.4±0.77	6.9±0.71**
Monocytes (%)	1.8±0.15	1.0±0.07***	1.11±0.04**	1.24±0.12	1.35±0.77	1.4±0.04
Lymphocytes (%)	80.1±2.31	32.2±2.47***	41.29±0.08**	48.1±0.40**	50.7±2.48	46.7±2.48**
Neutrophils (%)	17.6±1.26	64.5±1.76***	58.1±0.22	51.5±0.12**	47.3±3.31**	53.3±4.23

Data are expressed as the mean of results in 8 mice ± S.E.M.
**P<0.01 Experimental groups compared with the EAC control group
***P<0.001 Experimental groups compared with the normal group

Table 3. Effect of methanol extract of *Ervatamia coronaria* (MEEC) on hematological, biochemical parameters and body weight of normal mice

Parameters	Normal (0.9 % NaCl, 5 ml/kg)	MEEC (100 mg/kg)	MEEC (250 mg/kg)	MEEC (500 mg/kg)
Hemoglobin (g %)	12.5±0.2	9.2±0.4	9.8±0.9	10.4±0.3
RBC (10^6/mm^3)	6.7±0.1	6.6±0.52*	6.3±0.1	6.5±0.4
Total WBC (10^6/mm^3)	5.8±0.1	5.8±0.5	6.4±0.2	6.9±0.8
SGPT (U/L)	64.1±0.5	65.1±0.43**	71.8±0.08	72.1±0.5**
SGOT (U/L)	40.5±0.3	41.6±0.2	42.1±0.28	43.4±0.4
Serum urea (mg/dl)	23.6±1.8	21.2±0.4	22.3±0.11**	23.4±3.1
Serum calcium (mg/dl)	10.1±0.9	10.5±0.8	10.4±0.12	10.7±1.5
Serum phosphate (mg/ml)	4.8±0.7	4.6±0.7	4.5±0.4	5.2±0.1**
LPO (nmol MDA/mg protein)	0.96±0.4	0.92±0.7	0.96±0.4	0.94±0.9
GSH (mg/g wet tissue)	2.45±0.6	2.46±0.9	2.48±0.2***	2.52±0.7
SOD (U/mg protein)	4.53±0.1	4.55±0.2	4.68±1.9	4.82±0.1
CAT (U/mg protein)	26.6±0.4	26.98±0.4**	27.97±2.3	28.18±0.03**
Body weight (g)	22.5±0.5	22.4±0.2	22.8±2.1	22.7±2.2

Data are expressed as the mean of results in 8 mice ± S.E.M.

*P<0.05; **P<0.01 and ***P<0.001 Experimental groups compared with the normal control group

REFERENCES

1. Abdullaev, F.I., R.R. Luna, B.V. Roitenburd and A.J. Espinosa, Pattern of chiledhood cancer mortility in mexico. Arch Med Res; 2000; 31: 526-531.
2. Abdullaev, F.I., In Plant-drived agents against cancer. Gupta SK, editor. Pharmacology and therapeutics in the new millennium. New Delhi: Narosa Puplising House; 2001; 345-354.
3. Osawa, T., S. Kawakishi, M. Namiki, In Antitumutagenesis and Anticarcinogenesis Mechanism II Kuroda, Shankel DM, Waters MD, editors.. New York, Plenum; 1990; 139-153.
4. Di Carlo, G., N. Mascolo, A.A. Izzo and F. Capassao, Flavonoids: old and new aspects of a class of natural therapeutic drugs. Life Sci; 1999; 65: 337–353.
5. Keith, M.W., A.L. Sally, W.S. Michael, J.G. Thomas and M.M. Garry, Taxus Spp. Needles contain amounts of taxol comparable to the stem bark of taxus brevifolia: analysis and isolation. J Nat Prod; 1990; 53: 1249–1255.
6. Roja, G., M.R. Heble MR, The quinoline alkaloid Camptothecin and 9-methoxy camptothecin from tissue cultures and mature trees of Nathapodytes foetida. Phytochemistry; 1994; 36: 65–66.
7. DeFeudis, F.V., V. Papadopoulos, K. Drieu, *Ginkgo biloba* extracts and cancer: a research area in its infancy. Fundam Clin Pharmacol; 2003; 17: 405-417.
8. Van Beek, T.A., R. Verpoorte, A. Baerheim Svendsen, A.J.M. Leeuwenberg, N.G. Bisset NG, *Tabernaemontana* L. (Apocynaceae): a review of its taxonomy, phytochemistry, ethnobotany and pharmacology. J Ethnopharmacol; 1984; 10: 1-56.
9. Pawelka, K.H., J. Stoeckigt, Indole alkaloids from cell suspension cultures of *Tabernaemontana divaricata* and *Tabernaemontana iboga*. Plant Cell Rep; 1983; 22: 105–107.
10. Atta-Ur-Rahman., A. Muzaffar, N. Daulatabadi, Ervatinine, an indole alkaloid from *Ervatamia coronaria*. Phytochem; 1985; 24 (10): 2473–2474.
11. Yu, Y., J.K. Liu, The constituents of *Ervatamia divaricata*. Yunnan Zhiwu Yanjiu; 1999; 21 (2): 260–264.
12. Rastogi, K., R.S. Kapil, S.P. Popli, New alkaloids from *Tabernaemontana divaricata*. Phytochem; 1980; 19: 1209–1212.
13. Sharma, P., G.A. Cordell, Heyneanine hydroxyindolenine, A new indole alkaloid from *Ervatamia coronaria* Var. Plena. J Nat Prod; 1988; 51 (3): 528-531.
14. Van Der Heijden, R., Indole alkaloids in cell and tissue cultures of *Tabernaemontana* species. Pharm Weekbl (Sci. Ed); 1989; 11 (6): 239–241.
15. Kirtikar, K.R., B.D. Basu, Indian Medicinal Plants, v 2, Bishen mahendra pal singh Dehradun, India; 1975; 842-844.
16. Hsu, Y.T., Study on the Chinese drugs used as cancer remedy. J Southeast Asian Res; 1967; 3: 63–66.
17. Taesotikul, T., A. Panthong, D. Kanjanapothi, R. Verpoorte, J.J.C. Scheffer, Hippocratic screening of ethanolic extracts from two *Tabernaemontana* species. J Ethnopharmacol; 1989; 27 ½: 99–106.
18. Dhar, M.L., M.M. Dhar, B.N. Dhawan, B.N. Mehrotra, C. Ray, Screening of Indian plants for biological activity: Part – I. Ind J Exp Biol; 1968; 6: 232–247.
19. Da Sil Va, N.H., A.A. De Melo, M.M.C.V. Casado, A.T. Hendriques, D.C. Wandscheer, O.E. Da Sil Va, Indole alkaloids with potential endocrine activity. Rev Inst Antibiot Univ Fed Pernambuco Recife; 1984; 22 ½: 27-32.
20. Yamamoto, T., H. Takahashi, K. Sakai, T. Kowithayakorn, T. Koyano, Screening of Thai plants for anti-HIV-1 activity. Natural Med; 1997; 51 (6): 541-546.
21. Thompson, W.R., C.S. Weil, On the construction of tables for moving average interpolations. Biometric; 1952; 8: 51-54.
22. Kavimani, S., K.T. Manisenthil Kumar, Effect of methanol extract of *Enicostemma littorale* on Dalton's lymphoma. J Ethnopharmacol; 2000; 71: 349-352.
23. D'Armour, F.E., F.R. Blood, D.A. Belden, The manual for laboratory work in mammalian physiology, 3rd ed, Chicago, The University of Chicago Press; 1965; 4-6.
24. Wintrobe, M.M., G.R. Lee, D.R. Boggs, T.C. Bithel, J.W. Athens, J. Foerester,1961. Clinical Hematology, 5th ed, Philadelphia, 326.

25. Dacie, J.V., S.M. Lewis, Practical hematology, 2nd ed, London, J and A Churchill; 1958; 38-48.
26. Ohkawa, H., N. Onishi, K. Yagi, Assay for lipid peroxidation in animal tissue by thibarbituric acid reaction. Anal Biochem; 1979; 95: 351-358.
27. Beulter, E., B.M. Kelly, The effect of sodium nitrate on rd cell glutathione. Experientia; 1963; 18: 96-97.
28. Kakkar, P., B. Das, P.N. Vishwanathan, A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophys; 1984; 21: 130-132.
29. Maehly, A.C., B. Chance, In: Methods of Biochemical Analysis, Vol I, Glick D, editor, New York; 1954; 357.
30. Lowry, O.H., N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin-phenol reagent. J Biol Chem; 1951; 193: 265-275.
31. Bergmeyer, H.U., P. Scheibe, A.W. Wahlefeld, Optimization of methods for aspartate aminotransferase and alanine aminotransferase. Clin Chem; 1978; 24: 58-61
32. Tietz, N.W., Fundamentals of clinical chemistry 3rd ed, Philadelphia, WB Saunders Company; 1987; 75.
33. Henry, R.S., Clinical chemistry, Principles and techniques, New York; 1974; Harper and Row.
34. Prasad, S.B., A. Giri, Antitumor effect of cisplatin against murine ascites Dalton's lymphoma. Ind J Exp Biol; 1994; 32: 155-162.
35. Clarkson, B.D., J.H. Burchenal, Preliminary screening of antineoplastic drugs. Prog Cli Cancer; 1965; 1: 625-629.
36. Feninger, L.G., D.B. Mider, In: Advances in cancer Research, Vol II. Academic Press, New York; 1954a; 353-354.
37. Price, V.E., R.E. Greenfild, Anemia in cancer. Adv Can Res; 1958; 5: 199-200.
38. Hogland, H.C., Hematological complications of cancer chemotherapy. Semin Oncology; 1982; 9: 95-102.
39. Fenninger, L.D., G.B. Mider, Energy and nitrogen metabolism in cancer. Adv Can Res; 1954; 2: 229-253.
40. Donehower. R.C., Hydroxy urea. In: Cancer Chemotherapy, Principles and Practice, ed by JM Chabner, JM Collins, JB Lippincott co, Philadelphia; 1990; 225-233.
41. Yagi, K., Lipid peroxides and human diseases. Chem Phys Lipids; 1987; 45: 337-351.
42. Sinclair, A.J., A.H. Barnet, J. Lunie, Free radical and auto-oxidant systems in health and disease. Brit J Hospit Med; 1990; 43: 334-344.
43. Sun, Y., L.W. Oberley, J.H. Elwell, R.E. Sierra RE, Antioxidant enzyme activities in normal and transformed mice liver cells. Intern J Can; 1989; 44: 1028-1033.
44. Marklund, S.L., N.G. Westman, E. Lundgren, G. Roos, Copper and zinc containing superoxide dismutase, catalase and glutathione peroxidase in normal and neoplastic humal cell lines and normal human tissues. Can Res; 1982; 42: 1955-1961.
45. Jiau-Jian, L., W.O. Larry, Over expression of manganese containing superoxide dismutase confers resistance to the cytotoxicity of tumor necrosis factor α and/ or hypothermia. Can Res; 1977; 57: 1991-1998.
46. Ruby, A.J., G. Kuttan, K.D. Babu, K.N. Rajasekaran, R. Kuttan, Antitumor and antioxidant activity of natural curcuminoids. Can Lett; 1995; 94: 783-789.
47. Ming, L., C.P. Jill, J.N. Jingfang, C. Edward, E. Brash, Antioxidant action via p53 mediated apoptosis. Can Res; 1998; 58: 1723-1729.
48. Putul, M., C. Sunit, B. Pritha, Neovascularisation offers a new perspective to glutamine –related therapy. Ind J Exp Biol; 2000; 38: 88-90.