Evaluation of antitumor activity and antioxidant status of *Alternanthera brasiliana* against Ehrlich ascites carcinoma in Swiss albino mice

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ABSTRACT

Objective: The main objective of the present study was to explore the antitumor activity of the ethyl acetate extract of the *Alternanthera brasiliana* (EAAB) and its antioxidant status against Ehrlich ascites carcinoma (EAC) in Swiss albino mice. **Materials and Methods:** Based on the preliminary *in vitro* cytotoxicity studies, EAAB was selected for anti-tumor and antioxidant effects. Anticancer activity of EAAB was evaluated against EAC in Swiss albino mice at the doses of 200 and 400 mg/kg. EAAB was administered for 14 consecutive days after induction of cancer. After 24 h of the last dose and 18 h of fasting, half of the mice were sacrificed and rest were kept alive for assessing any increase in life span. The antitumor effect of EAAB was assessed by evaluating tumor volume, viable and nonviable tumor cell count, tumor weight, hematological and biochemical parameters of EAC bearing host. Furthermore, the antioxidant and histopathological parameters were evaluated. **Results:** EAAB treatment has shown significant decrease in tumor volume, viable cell count, tumor weight and elevated the life span of EAC tumor bearing mice in a dose dependent manner. In hematological profile count of RBC, hemoglobin, and WBC were found reverted to normal. EAAB also significantly decreased the levels of lipid peroxidation and significantly increased the levels of GSH, SOD and Catalase. **Conclusion:** From the above results it may be concluded that EAAB has potent dose dependent antitumor activity and is comparable to that of 5-flourouracil.

Key words: 5-Flourouracil, alternanthera brasiliana, antioxidant, antitumor, ehrlich ascites carcinoma

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INTRODUCTION

Cancer, otherwise termed as malignant neoplasm, is a class of diseases where in a group of cells show abnormal proliferation, invasion and sometimes metastasis.[1] Cancer is the second most common cause of death in humans standing next to cardiovascular disorders.[2] Currently it accounts for 8.2 million deaths across the globe, 14.1 million new cases have been registered in 2012 and is projected for a continuous rise, with an estimated 19.3 million new cases per year by 2025.[3] Despite all great strides that have been made in the treatment of cancer over the past 50 years, it still continues to be a major health concern, therefore, extensive efforts have been devoted to search for new therapeutic approaches. One such approach is exploration of natural plant products for their anticancer effects. Plants used in traditional medicines have been accepted currently as one of the main sources of anticancer agents.[4] There is a mounting interest in exploring diverse species of plants to identify their possible therapeutic applications, and this is attributed to their presumed safety, cost effectiveness, and easy availability.[5] Natural products have historically provided new drugs against a wide variety of diseases, and cancer is certainly no exception. In the recent past, scientific research on plants used in ethnomedicine has led to the discovery of many valuable drugs such as vincristine, camptothecin, podophyllotoxin, taxol, combretastatin, *etc.*[6]

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Alternanthera brasiliana belonging to the family Amaranthaceae is an herbaceous plant, native to different countries like Brazil, Australia and India.[7] Though almost all of its parts are used in traditional system of medicine, leaves are the most important parts which are using medicinally. In traditional system of medicine the plant used as a galactagogue, cholagogue, abortifacient, diuretic and treatment of indigestion.[8] Previous phytochemical constituents reported in this plant are flavonol glycosides (3-*O*-robinobioside derivatives of kaempferol and quercetin), vitamins (riboflavin and niacin), betacianin, and steroids such as β-sitosterol.[8] Various bioactivities, including wound healing, anti inflammatory and analgesic, lymphocyte proliferation inhibition, antioxidant, antimicrobial and antiviral properties of the plant were reported.[8] However, anti-tumor activity on the *A. brasiliana* has not been defined so far. Hence, the present study based on the ethno medical claims was sought to evaluate the scientific validity for the antitumor activity of the *Alternanthera brasiliana* leaves against EAC tumor model.

MATERIALS AND METHODS

Drugs and chemicals

5-FU (5-Fluoro uracil), MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide), Trypan blue, RPMI-1640 medium, FBS (Fetal bovine serum), DTNB (5, 5'-dithio-bis-(2-nitrobenzoic acid), Thiobarbituric acid, DMSO, Catalase and SOD assay kits were purchased from Sigma Aldrich. All other chemicals used in the study were of analytical grade (AR).

Plant material

Leaves of *A. brasiliana* were collected from the medicinal garden of the Department of Pharmacology and Toxicology, College of Veterinary Sciences (CVSc), Khanapara, Assam, India. The plant was authenticated by taxonomist of NEIST, Jorhat, Assam.

Preparation of extract

The shade dried coarsely powdered (250 g) *A. brasiliana* was extracted with solvents like petroleum ether, chloroform, ethyl acetate and methanol (70%) by using hot continuous percolation process (Soxhlet) and the different extracts were concentrated by using rotary vacuum evaporator (Buchi) at 50°C, dried in a vacuum dessicator and stored at − 20°C till further use. The yields obtained were 12.1%, 11.2%, 12.5% and 11.6% in respective solvents.

To screen the anticancer activity of the above extracts preliminary *in vitro* cytotoxic assays (Trypan blue and MTT assays) were performed in EAC cells. Ethyl acetate extract of the *A. brasiliana* (EAAB) found to be the most effective in inducing cytotoxicity. Hence, EAAB was chosen for further study.

Phytochemical screening

The phytochemical screening was carried out by standard methods.[9]

Animals

Swiss albino mice of about 8 weeks of age with an average body weight of 20-25 g were used for the experiment. The mice were grouped, housed in poly acrylic cages and maintained under standard laboratory conditions (temperature 25 ± 2°C and relative humidity 55-60% with dark/light cycle (12/12 h) and were allowed free access to standard dry pellet diet and water *ad libitum*. The animals were acclimatized to laboratory conditions for 1 week before initiation of the experimental studies. All the described experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee, Gauhati Medical College, Assam. (MC/32/2012/35).

Acute toxicity study

The acute toxicity study of EAAB was performed in Swiss albino mice, no gross behavioral changes and mortality were observed at dose level of 2000 mg/kg. Therefore, the LD_{50} value of EAAB should be more than 2000 mg/kg. Generally 1/5 to $1/10^{th}$ of the lethal dose was taken for effective dose calculation. Hence, 200 and 400 mg/kg were selected as doses of EAAB for the current study.[10]

Transplantation of tumor cells

The EAC cells were obtained from North-East Hill University (NEHU), Shillong, India. The cells were maintained in Swiss albino mice. Ascitis fluid containing EAC cells aspirated from the peritoneal cavity of mice were washed with saline and given intraperitoneally (2×10^6 cells/mouse) to fresh mice to develop ascitic tumor for every 10 days and it was used for both *in vitro* and *in vivo* study.[11]

In vitro cytotoxicity studies

Trypan blue dye exclusion method

Trypan blue dye exclusion method was used to assess the *in vitro* cytotoxicity of the EAAB in EAC cells.[12] Briefly, stock cell suspension of EAC was adjusted to 1×10^6 cells by phosphate buffer saline (PBS) using countess cell counter. The cells were incubated with desired test drug concentrations of EAAB (4, 8, 16, 32 and 64 μg/ml) for 3 h in co_2 co_2 incubator by maintaining 5% co_2 and 37°C. 5-FU was used as standard control. After the completion of incubation, 10 μl of trypan blue was added to equal amount of incubation mixture and mixed well. The total number of live cells and dead cells were counted using an automated countess cell counter. The percentage cell death and IC_{50} values were determined.

MTT assay

In vitro cytotoxicity was determined using a standard MTT assay with small modification for the individual test system.[13] In brief, EAC cells aspirated from the peritoneum of EAC inoculated mice was adjusted to 1×10^5 cells/ml using RPMI medium suspended with 10% FBS. 100 µl of EAC cells were plated in 96 well plate and incubated with different concentrations of EAAB and 5-FU for 48 h at 37°C in CO_2 incubator. After 48 h media was removed and cell cultures were incubated with 100 µl MTT reagent (1 mg/ml) for 4 h at 37°C. The formazan produced by the viable cells was solubilized by addition of 100 µl DMSO. The 96 well plate contain cell suspension was placed in an incubation shaker for 5 min and absorbance was measured at 570 nm by the micro plate reader, percentage cytotoxicity and IC_{50} values were calculated.

Evaluation of *in vivo* antitumor activity

Treatment schedule

The Swiss albino mice were divided into five groups ($n = 12$ each group) and given food and water *ad libitum*. Except group 1 animals in all other groups received EAC cells (2×10^6cells/mouse i.p.). The day of tumor implantation was considered as a day zero. Group 1 was served as normal control and received same amount of vehicle p.o., and group 2 was served as EAC control. After 24 h, EAC implanted Group 3 and 4 were being treated with EAAB (200 and 400 mg/kg, p.o.), group 5 was treated with standard drug 5-FU (20 mg/kg i.p.) once daily for 14 consecutive days and measure the body weight of all studied animals daily. After administration of last dose, half of the animals ($n = 6$) in each group were kept fast for 18 h and 0.5 ml of blood was withdrawn by retro orbital plexus[14] under ketamine anesthesia for estimation of hematological and serum biochemical parameters. After blood withdrawal, mice were sacrificed by cervical dislocation method for collection of liver and ascitic fluid. Part of the liver was used for the histopathological study and remaining part was used for estimation of antioxidant parameters. Rests of the animals in each group were allowed to natural death for determination of mean survival time (MST) and percentage increased life span (%ILS). [Figure 1]

Antitumor activity and antioxidant status of the EAAB were assessed in EAC animals with respect to the following parameters.

Tumor growth response

The antitumor effect of EAAB was assessed by change in tumor volume, tumor weight, viable and non viable cell count, MST and % ILS.[15]

Tumor volume and weight

Volume of the ascitic fluid was measured by taking it in centrifuge tube and expressed in milliliter (ml). Tumor weight was measured by taking the weight of mice before and after collection of the ascitic fluid from the peritoneal cavity and expressed in grams (gm).[15]

Tumor cell (viable and non-viable) count

The viability and non-viability of the cells were determined by trypan blue assay. The cells were stained with 0.4% trypan blue. The cells which are viable they did not take the dye and those that took the dye were non-viable.[15]

Percentage increase in life span (%ILS)

Mean survival time (MST) for each group was noted and anti tumor activity of the EAAB was compared with that of control group by measuring % ILS. Formulas used are as follows:[16]

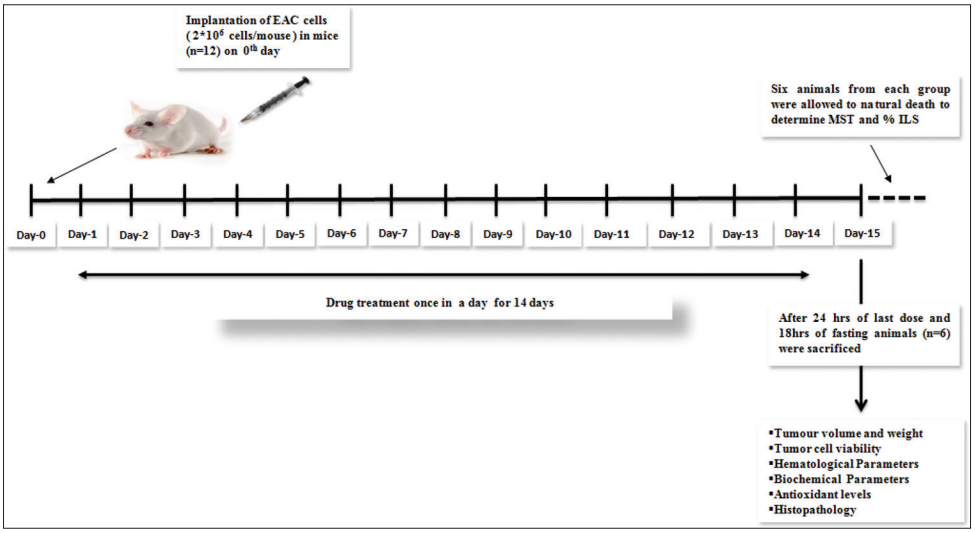

Figure 1: Experimental design of the study

MST= (day of first death + day of last death)/2
%ILS= [(MST of treated group/MST of control group)-1] × 100

Effect of EAAB and 5-FU treatment on survival time in EAC bearing mice was also analyzed by using the Kaplan–Meier method.

Hemotological parameters

At the end of the experiment blood was collected from retro orbital plexus and used for the estimation of hemotological parameters like Hemoglobin (Hb), Red blood cell (RBC) and White blood cell (WBC) count by using standard procedures.[17]

Biochemical parameters

The blood samples were allowed to clot and the serum was separated by centrifugation at 8000 rpm for 5 min. Serum was used for the estimation of serum glutamate pyruvic transaminase (SGPT), Serum glutamic oxaloacetate transaminase (SGOT), Triglycerides (TGL) and alkaline phosphatase (ALP). These parameters were analyzed by using automated vet test analyzer (IDEXX) using the respective strips, manufactured by the IDEXX laboratories.

Tissue antioxidant parameters

The antioxidant assays were performed with the 10% w/v liver homogenate. It was prepared in ice cold PBS solution and centrifuged at 1500 rpm for 15 min at 4°C. The MDA estimation was done according to the method of Ohkawa *et al.*,[18] with some modifications. Briefly, 0.2 ml of homogenate was added to 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid solution adjusted to pH 3.4 with sodium hydroxide and 1.5 ml of 0.8% of thiobarbituric acid. The mixture was made up to 4 ml with distilled water. The mixture was heated on water bath at 95°C for 60 min. After that the mixture was cooled under tap water, centrifuged at 10000 rpm and collected the supernatant. Absorbance of supernatant was measured at 532 nm.

Reduced glutathione (GSH) was estimated by using Ellman method.[19] Homogenate (60 µl) and 1% trichloroacetic acid (60 µl) mixed and kept at 4°C for 30 min. Centrifuge the homogenate at 10,000 rpm for 5 min at 4°C collected the Supernatant and added three times volume of DTNB. Incubated the mixture for 10 min at 37°C and absorbance was taken at 412 nm.

The total protein assay was done according to the method of Lowry.[20] Briefly 0.2 ml of homogenate makes up to 1 ml with 0.85% sodium chloride, Add 1.0 ml of the Lowry Reagent Solution, Mix well. Allow solutions to stand at room temperature for 20 min. With rapid and immediate mixing, add 0.5 ml of the Folin and Ciocalteu's Phenol Reagent Working Solution to each tube. Allow color to develop for 30 min and absorbance was measured at wavelength between 500-800 nm. Super oxide dismutase (SOD) and Catalase (CAT) levels were estimated by using commercially available kits supplied by Sigma Aldrich.

Histopathology study

The dissected liver initially kept in 10% buffered formalin, then dehydrated in alcohol and embedded in paraffin. The paraffin blocks were sectioned at 5 µm intervals and stained with hematoxylin and eosin for histological examination.

Statistical analysis

All the values were expressed as mean ± SEM (n = 6). Statistical analysis was carried out by using one way ANOVA followed by Dunnets *post hoc* test with GraphPad Prism 5.0 (San Diego, CA, USA) and values of $P < 0.05$ were considered to be statistically significant.

RESULTS

Preliminary phyto chemical screening

The EAAB revealed the presence of various phytoconstituents like alkaloids, glycosides, saponins, triterpenes, Terpenoids and flavonoids.

In vitro cytotoxic study

The EAAB has shown concentration dependant cytotoxicity on EAC cells in both Trypan blue [Figure 2] and MTT assays [Table 1], the IC_{50} values of EAAB were 33.54 µg and 33.69 µg respectively in both the assays.

In vivo antitumor model

Tumor growth response

The development of tumor was observed on the day 5, from that day a steady increase in body weight was observed up

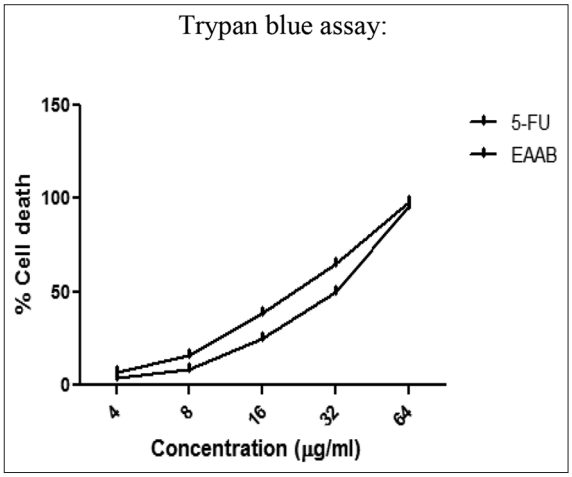

Figure 2: *In vitro* cytotoxic effect of EAAB on EAC cells by trypan blue exclusion assay. Values are represented as mean ± SEM (n = 3). IC_{50} for EAAB = 33.54 µg and 5-FU = 31.39 µg

to end of the study (15th day). The maximum gain of body weight was observed in the EAC control group. In case of EAAB and 5-FU treated groups the body weight was significantly ($P < 0.001$) reduced at all the doses [Figure 3].

The tumor volume, tumor weight and viable cell count were found to be significantly ($P < 0.001$) decreased and non viable cell count was significantly ($P < 0.001$) increased in EAAB treated animals at the doses 200 and 400 mg/kg and 5-FU (20 mg/kg) when compared with EAC control animals [Table 2].

The EAC bearing mice administered with EAAB and 5-FU for 14 days and the days of survival were recorded. With EAAB treatment, the survival of EAC bearing mice significantly ($P < 0.001$) increased as compared to EAC bearing control group. [Figure 4].

In treated group mean survival time was significantly increased to 31.05 ± 2.04 (%ILS = 53.33), 36.12 ± 2.45 (%ILS = 78.37), and 39.78 ± 2.86 (%ILS = 96.44), respectively when compared to EAC control group [Table 2].

Hematological and biochemical parameters

There was two-fold increase in the WBC count, drastic fall in the RBC count and hemoglobin content in the EAC control group as compared to normal control group. Administration of both doses of EAAB and 5-FU in EAC bearing mice significantly ($P < 0.001$) reverted the above changes to normal [Table 2]. The amounts of SGPT, SGOT, TGL and ALP were elevated in the EAC control group. Treatment with EAAB and 5-FU significantly reduced ($P < 0.001$) all the elevated biochemical parameters to normal when compared with EAC control group [Figure 5].

Antioxidant parameters

The levels of liver antioxidant markers like GSH, SOD and Catalase were decreased while the levels of malondialdehyde (MDA) increased in EAC control group when compared to normal control group. Significant reversal of these changes toward normal levels were observed when treated with EAAB at the both doses [200 mg/kg ($P < 0.05$) and 400 mg/kg ($P < 0.001$)] and 5-FU ($P < 0.001$). [Figure 6].

Histopathological study

Pathological findings such as steatosis and lymphocyte accumulation were observed in the liver of EAC bearing mice by histopathological examination. Treated animals showed almost normal liver histology [Figure 7].

DISCUSSION

Historically, ethno medicine has played an important role in antitumor drug discovery. It constitutes a common

Table 1: *In vitro* cytotoxicity of EAAB in EAC cells after 48h exposure by MTT assay

Concentration (µg/ml)	% Cytotoxicity	
	EAAB	5-FU
4	3.67±0.18	4.56±0.14
8	7.25±0.25	8.76±0.52
16	20.58±1.12	24.21±1.08
32	48.59±2.03	55.49±1.41
64	96.41±2.56	98.45±2.98

Values are represented as mean±SEM (n=3). IC_{50} values for EAAB=33.69 µg, 5-FU=31.82 µg. EAAB=Extract of the *Alternanthera brasiliana*

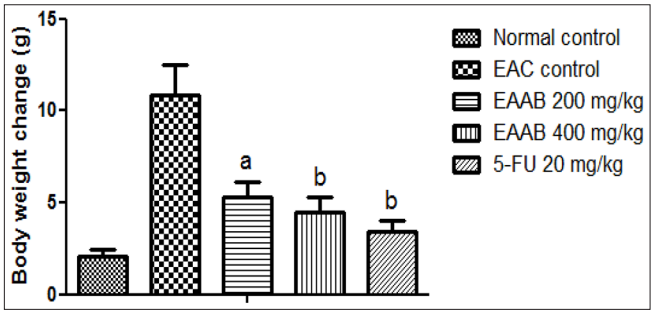

Figure 3: Effect of EAAB and 5-FU treatment on body weight change in EAC bearing mice. Values are represented as mean ± SEM ($n = 6$). [a]$P < 0.01$, [b]$P < 0.001$ as compared to EAC control group

Table 2: Effect of EAAB on tumour volume (ml), tumour weight (gm), viable and nonviable cell count (cells×10^7cell/ml), mean survival time, percentage increase life span and hematological parameters like RBC (cells×10^6/µl), WBC (cells×10^3/µl) and hemoglobin content (g/dl) in EAC bearing mice

Parameters	Normal control	EAC control	EAC+EAAB 200 mg/kg	EAC+EAAB 400 mg/kg	EAC+5-FU 20 mg/kg
Tumor volume	-	6.45±0.47	3.25±0.55[b]	1.22±0.14[b]	1.05±0.11[b]
Tumor weight	-	4.85±0.35	2.30±0.21[b]	1.05±0.08[b]	0.95±0.11[b]
Viable cell	-	9.85±0.85	3.25±0.26[b]	1.85±0.12[b]	1.42±0.09[b]
Nonviable cell	-	0.45±0.06	1.65±0.21[a]	2.65±0.32[b]	3.05±0.26[b]
MST (days)	-	20.25±1.57	31.05±2.04[a]	36.12±2.45[b]	39.78±2.86[b]
%ILS	-	0	53.33	78.37	96.44
RBC	5.62±0.21	2.25±0.08	3.86±0.12[b]	4.63±0.16[b]	4.91±0.23[b]
WBC	5.14±0.62	10.28±0.81	7.86±0.65[b]	6.24±0.43[b]	5.89±0.56[b]
Hemoglobin	12.45±1.04	6.12±0.82	10.29±0.96	11.32±1.15[a]	12.06±1.24[a]

Values are represented as mean±SEM (n=6). [a]P<0.01, [b]P<0.001 as compared to EAC control group. EAAB=Extract of the *Alternanthera brasiliana*, MST=Mean survival time, %ILS=Percentage increase life span, RBC=Red blood cell, WBC=White blood cell, EAC=Ehrlich ascites carcinoma

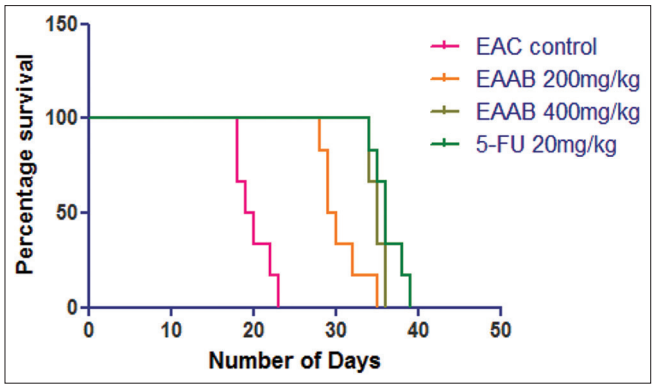

Figure 4: Effect of EAAB and 5-FU treatment on median survival time in EAC bearing mice. Data were analyzed using Kaplan–Meier method. Survival time plotted as days post tumor transplant and ***$P < 0.001$ when compared to EAC control group

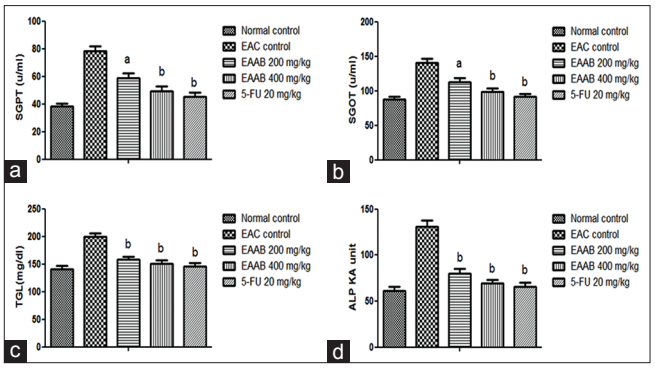

Figure 5: Effect of EAAB on serum biochemical parameters such as SGPT (3a), SGOT (3b), TGL (3c) and ALP (3d) in EAC bearing mice. Values are represented as mean ± SEM ($n = 6$). [a] $P < 0.01$, [b] $P < 0.001$ as compared to EAC control group

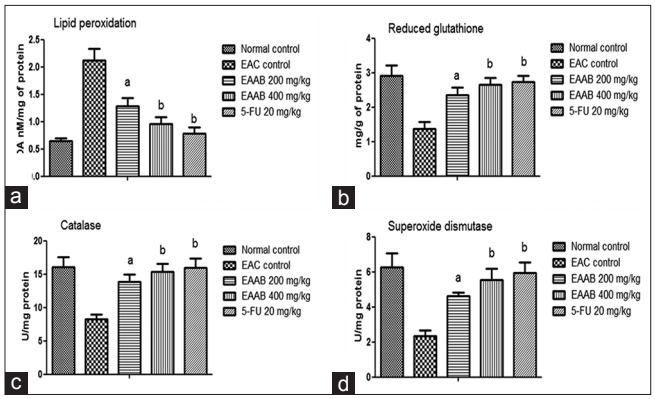

Figure 6: Effect of EAAB on tissue antioxidant parameters like lipid peroxidation (4a), reduced glutathione (4b), Catalase (4c), superoxide dismutase (4d) in EAC bearing mice. Values are represented as mean ± SEM ($n = 6$). [a]$P < 0.01$, [b]$P < 0.001$ as compared to EAC control group

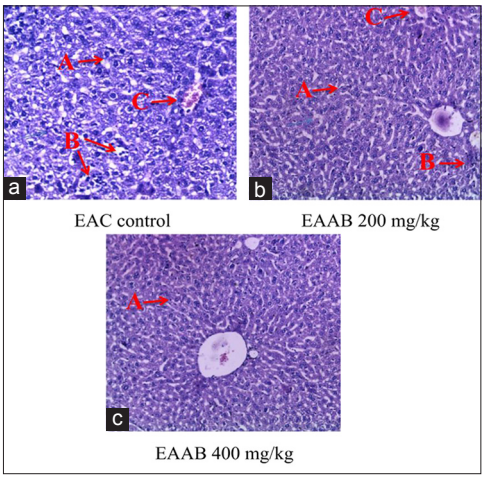

Figure 7: Histology of liver stained with hematoxylin and eosin (×100) of mice from the different groups, on day 15 of the experiment (n=6). a: Hepatocytes, b: Lymphocytes, c: Portal area

substitute for cancer prevention and treatment in distinct countries around the globe and such studies investigating medicinal herbs have been steadily held with interests. Currently, large number of plants possessing anticancer properties have been documented.[21] EAC is a transplantable, poorly differentiated, malignant tumor which appeared initially as a spontaneous murine mammary adenocarcinoma.[22] It can induce both solid and ascitic forms of tumor in almost all strains of mice, due to these concerns it has been widely used from several years to study the antitumor properties of several natural and synthetic compounds.[11,12,15]

Cytotoxic potential of various extracts of *Alternanthera brasiliana* were evaluated using trypan blue assay and it was found that EAAB showed promising anti-cancer activity. The results of trypan blue assay were further confirmed in MTT assay. The IC_{50} values of the EAAB in both the assays was comparable to standard drug 5-FU.

Based on the encouraging data obtained in *in vitro* studies, we decided to proceed further with ethyl acetate extract for screening its antitumor and antioxidant activity in EAC bearing mice. Findings in this study supported the *in vivo* anticancer efficacy of EAAB in ascites tumors.

For the growth of EAC cells ascitic fluid is essential nutritional requirement and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells.[11] The body weight of tumor bearing mice was increased due to increase in ascitic fluid volume. In the present experiment increase in body weight, ascitic tumor volume, tumor weight and tumor cell count were observed in EAC control. Treatment of EAC tumor bearing mice with plant extracts at the doses 200 and 400 mg/kg significantly decreased the body weight, tumor volume, tumor weight, and viable tumor cell count and increased the non viable tumor cell count. These results indicate either a direct cytotoxic effect of plant extracts on tumor cells or an indirect local effect,

which may involve macrophage activation and vascular permeability inhibition.[15] In the present study EAAB at the both doses significantly increases the life span of the EAC treated mice.

Literature reports reveal that prolongation of life span of tumor bearing animals, reduction in tumor volume and viable cell count, and increased non viable cell count is a reliable criterion for the depiction of potential of any anticancer agent[11,15,23,24] and EAAB meet's this criterion. Thus, a similar inference could be drawn here.

The major problems encountered in cancer chemotherapy are myelosuppression and anemia,[25] which are mainly because of iron deficiency, either due to haemolytic or myelopathic conditions finally leads to reduction in RBC count or hemoglobin content. Similarly, in the present study reduced hemoglobin, RBC count and elevated WBC count was observed in EAC control group, whereas EAAB treatment replenishes the hemoglobin content and maintained the normal values of RBC and WBC, which supports its reduced myelotoxicity and increased hematopoietic protecting activity. Previous studies have shown that most of the plant extracts reduce EAC induced myelotoxicity due to their immune boosting, antioxidant and free radical scavenging activity.[15] EAAB is known for its antioxidant and anti-inflammatory activity,[8] which may be reason for the hematopoietic activity of EAAB.

From past several years, serum enzymes play an important role as diagnostic markers in neoplasia as well as to know the disease condition.[24] Several reports revealing that tumor cell causes liver damage and disturbances in hepatic cell metabolism, which lead to changes in serum enzymes activity.[15,23] Similar results were found in the present study that is elevated levels of SGPT, SGOT, ALP and TGL in EAC control group. The increased levels of above mentioned biochemical parameters may be interpreted as a result of hepato cellular damage by EAC. EAAB treatment at both the doses restored the elevated biochemical parameters to normal range, indicating that protection against tumor cell induced hepatotoxicity.

A decrease in endogenous antioxidant enzymes with enhanced free radical generation and MDA is well documented in carcinogenesis.[26] Several reports documented that Malondialdehyde (MDA) the end product of lipid peroxidation, are seen to be higher in cancer tissues than in normal tissues.[27] GSH plays an important role in endogenous antioxidant system and potent inhibitor of the neoplastic process, found low concentration in cancer tissues and higher in normal tissues.[28] The two enzymes present in free radical scavenging system are super oxide dismutase (SOD) and catalase (CAT). The main function of these two enzymes is to provide a defence against the superoxide anions and hydrogen peroxide. The inhibition of SOD and CAT activities as a result of tumor growth was also reported.[29]

Similarly, in the present study, increase in MDA and decrease in the levels of SOD, CAT and GSH were observed in EAC bearing mice. EAAB administration inhibited the lowering of antioxidant levels and rise in MDA levels in EAC bearing mice demonstrating the potential of EAAB in attenuation of oxidative stress via modulation of antioxidants levels in EAC bearing mice.

Therefore the significant reduction in MDA levels and significant elevation of GSH, SOD and CAT by the extract treatment confirms the potent antioxidant and free radical scavenger activity of EAAB. The histopathological examination of the liver of EAC control mice has shown marked alterations, indicating the toxic effects of the tumor. The normalization of these effects observed in EAAB treated animals further supported the potent hepatoprotective and antioxidant effects of the extract.

It has been reported plant extract having antioxidant potential can inhibit proliferation of cancer cells.[30] Similarly the observed antitumor activity of EAAB in this model may be due to its antioxidant potential.

CONCLUSION

In conclusion, results from the *in vitro* and *in vivo* systems confirm the antitumor and antioxidant activity of EAAB. The strong antitumor activity observed in this model may be due to the hemoprotective and antioxidant nature of the extract. Preliminary phytochemical tests indicate the presence of glycosides, saponins, flavonoids, tannins, and phenols in EAAB. Several such compounds are known to possess strong antitumor, antioxidant and hepatoprotective properties. Further studies aiming to isolate, characterize the active principle and elucidate it's mechanism of action using different cell lines are in progress.

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