ORIGINAL ARTICLE

Evaluation of anticancer activity of methanol extract of *Commelina benghalensis* Linn. against Ehrlich ascites carcinoma in albino mice

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Abstract

Aim: Evaluation of anticancer activity of methanol extract of *Commelina benghalensis* L. (MECB) by the experimental parameters such as tumor volume, tumor cell count, viable and non-viable cell count, mean survival time, increase lifespan, red blood cells (RBC) and white blood cells count and hemoglobin level (Hb), and histological architecture of liver section of three treatment group of mice compared to Ehrlich Ascites Carcinoma (EAC) control group of mice. **Materials and Methods:** The anticancer activity of methanol extract of the aerial part of CB, family Commelinaceae has been evaluated against EAC cells (EAC 10^7 cells/mouse) in Swiss Albino mice at the dose of 200 and 400 mg/kg body weight. The MECB was administered intraperitoneal (i.p.) route for 9 consecutive days to tumor-bearing mice. 5-Flurouracil (5-FU) 20 mg/kg body weight was also administered to i.p. for 9 days as a standard anticancer drug. **Result:** The MECB decreased the EAC cell count, viable cell count, and percentage inhibition of total cell count. At the same time, there was an increase in the percentage of lifespan (% ILS), non-viable cell count, RBC count, and Hb level. These parameters were compared with the EAC bearing mice in a dose-dependent manner. The histological architecture of liver section in EAC bearing mice was observed steatosis, lymphocyte accumulation and normal architecture of nucleus, parenchyma, and regeneration of hepatic cells in three treated group of mice. **Conclusion:** This study reveals that the MECB has significant anticancer activity against EAC cell in mice.

Key words: *Commelina benghalensis* L, Ehrlich ascites carcinoma, histology activity index, methanol extract of *Commelina benghalensis,* tumor volume

INTRODUCTION

Phytochemical compounds and extract obtained from medicinal plants have an important role in the treatment of various diseases.[1-3] According to the WHO, 2004 approximately 12.5% population was affected by cancer, and the mortality rate was high. Large numbers of drugs are available from natural sources, either by structural modifications or by semi-synthetic chemical compounds for the treatment of cancer. Modern anticancer drug has cytotoxicity. Hence, compounds obtained from plants have major importance in the field of oncological therapy due to their low toxicity and comparatively low cost[4] than the modern anticancer drug. Herbal compounds vinca alkaloids - vincristine, vinblastine, vinorelbine; taxanes-paclitaxel, docetaxel; camptothecin derivatives-topotecan, irinotecan; epipodophyllotoxins - etoposide, etoposide phosphate, teniposide, etc. are used for cancer treatment.[5]

Commelina benghalensis (CB) is a succulent, creeping perineal plant, height of the creeping stem approximately

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Received: 25-01-2018
Revised: 24-02-2018
Accepted: 08-03-2018

0.4 m, stem-branched, leaves-opposite, flowers - 5–6 blue flowers present in a short stalk, roots appear at the node of creeping stems, about 1600 seeds are present in a matured plant.[6]

It has been reported that CB contains flavonoids, alkaloids, steroids, triterpenes, lactones, coumarins, resins, phenols, reducing agents, tannins, quinones, amino acids, and saponins,[7,8] β-carotene, and lutein are present in the higher level.[9,10]

CB has pharmacological importance in leprosy, headache, fever, constipation, jaundice, snake bite,[6,11,12] candida infection, inflammation in the conjunctiva, psychosis,[13] epilepsy,[14] diuretic,[15] laxative,[16] antioxidant, and anticancer.[15,17]

CB L. is grown in India, tropical and subtropical countries such as Pakistan, Africa (Northern and Southern Africa), China, and Bangladesh.

MATERIALS AND METHODS

The aerial parts of CB were collected from a village of East Midnapur, WB. A herbarium of the flowering plant of CB was prepared and identified as CB of family-Commelinaceae by Central National Herbarium, Shibpur, Howrah, India (BSI, Howrah) vide certificate number CNH/Tech II/2015/I/229. All the collected plants were cleaned, dried, sliced, and further dried under shade at room temperature. Then, properly dried CB was grinded, and 1.6 kg coarse powder was produced. This powder was extracted with methanol as menstruum followed by multiple macerations. The extracted liquid content was evaporated under reduced pressure in rotary evaporator. Then, concentrated extract was lyophilized and 100 g solid extract was obtained. This methanol extract of CB was named as MECB and stored in well-closed container in a refrigerator.

Animals

The male Swiss albino mice having body weight 20 ± 2 were selected for *in vivo* anticancer study after obtaining the permission from Animal Ethics Committee of Jadavpur University vide their letter number AEC/PHARM/1502/12/2015. This *in vivo* animal study was carried out under the guideline of CPCEA (Government of India). The mice were kept under standard environment condition, fed with standard laboratory diet and water *ad libitum*.

Acute Toxicity Study

The acute toxicity study of MECB on male Swiss Albino mice was done under the guideline of OECD. No mortality was found up to the dose 2000 mg/kg body weight, and therefore, considered as safe.

Study of Antitumor Activity

To perform the experiment, the mice were divided into five groups, and each group consists of six mice.

- Group-I: Normal control (propylene glycol 5 mL/Kg b.w.)
- Group II: Ehrlich ascites carcinoma (EAC) control (10^7 cells/mouse)
- Group III: Test drug (MECB 200 mg/kg b.w.)
- Group IV: Test drug (MECB 400 mg/kg b.w.)
- Group V: Standard drug (5 fluorouracil/5-FU, 20 mg/kg b.w.)

To carry out this experiment, EAC cells were collected from Chittaranjan National Cancer Research Centre, Kolkata, India, and subcultured in our laboratory weekly. 10^7 EAC cells were inoculated intraperitoneal (i.p.) in the day "o," to all mice of Group II-V. MECB was suspended with propylene glycol and 200 mg/kg and 400 mg/kg b.w. doses were injected into i.p. of the mice of Groups III and IV in the day "Ist." 20 mg/kg b.w. of 5-FU (standard drug) was administered i.p. to the mice of Group V. 5 mL/kg b.w. propylene glycol was injected i.p. to the mice of Group I. The treatment of the drug was continued for 9 consecutive days. The mice were sacrificed in the day 10th, and EAC cells, tumor volume, percentage inhibition of total count, percentage inhibition of tumor volume, total count, viable count, non-viable cell count, percentage inhibition of viable cells, and increase of life span (ILS) were evaluated.[18,19]

Study of Hematological Parameter

On the 10th day, blood was collected from the heart of the sacrificed mice from each group and white blood cells (WBC), red blood cells (RBC) count, and hemoglobin (Hb) level were determined. The parameters of EAC Group II were compared to the other groups for statistical analysis.[18,19]

Study of Histological Architecture of Liver

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 (H and E) for histological examination.

Statistical Analysis

All data are expressed as mean ±SEM (n = 6 mice per group). The data were analyzed by one-way ANOVA between the treated groups and the EAC control followed by Dunnett's Multiple Comparison test.

RESULTS AND DISCUSSION

The LD_{50} value of the methanol leaf extract of CB. was carried out as per the OECD guideline, but no mortality of the mice was observed even at a dose of 2000 mg/kg body weight and was, therefore, considered as safe.

The anticancer property of MECB was evaluated by their ability to inhibit cancer cell growth in ascitic fluid of Swiss albino mice. The percentage inhibition of total cell count (%TCI), percentage inhibition of viable cell count, percentage increase in lifespan (%ILS), and improved hematological parameters were considered the potency of the anticancer property of MECB.

Intraperitoneal administration of MECB to the mice at the doses of 200 mg/kg b.w. and 400 mg/kg b.w. significantly reduced the total cell count and tumor volume compared to EAC control group (Group II). Percentage TCI, the percentage inhibition of tumor volume was observed 79.92% and 78.81%, respectively, at the dose of 200 mg/kg b.w. of MECB. Similarly, percentage TCI and percentage inhibition of tumor volume were observed 89.51% and 94.77%, respectively, at the dose of 400 mg/kg b.w. of MECB [Table 1 and Figures 1 and 2].

MECB at the doses of 200 mg/kg b.w. and 400 mg/kg b.w. significantly ($p<0.01$) decrease the viable cell count [Table 2 and Figure 3] and significantly ($P < 0.05$) increased the non-viable cell count [Table 2 and Figure 4] in the MECB treated mice (Groups III and IV) compared with EAC control mice.

One of the most important criteria for judgment of efficacy of an anticancer drug is the increase of the lifespan of the experimental animals. In this study, lifespan of the MECB

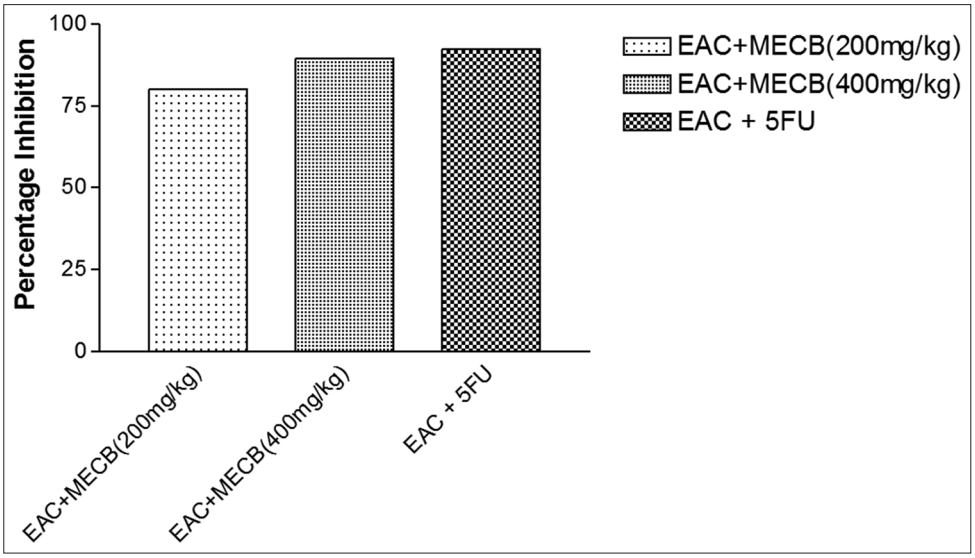

Figure 1: Percentage inhibition of total cell count

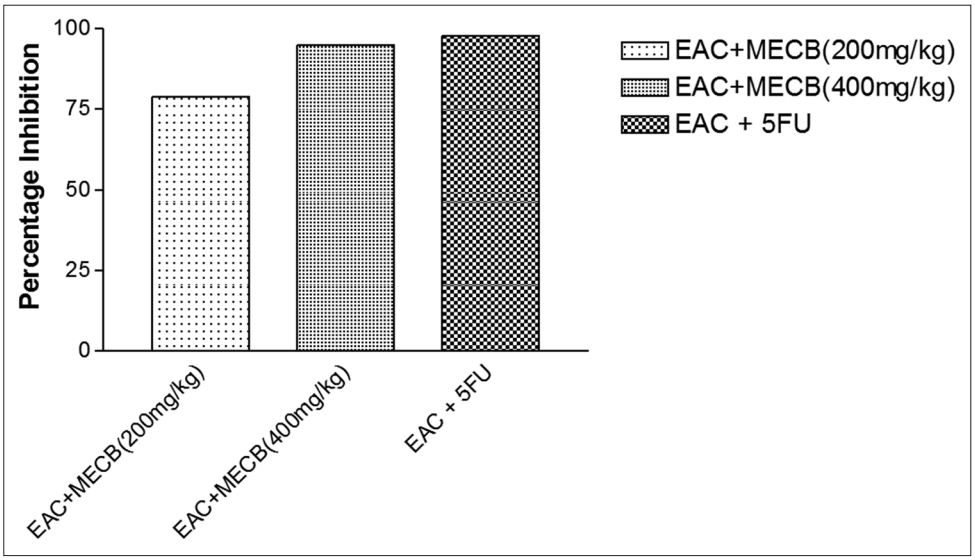

Figure 2: Percentage inhibition of tumor volume

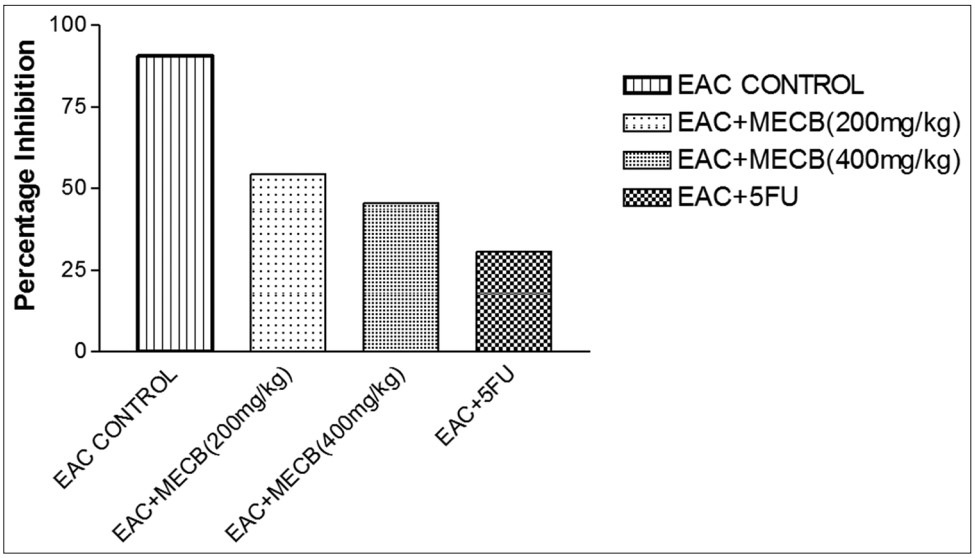

Figure 3: Percentage inhibition of viable cell count

Table 1: Anticancer activity of MECB against EAC bearing mice (total EAC cells count, total tumor volume, percentage TCI, and percentage inhibition tumor volume)

Group	Compound	Total cell count (×10^7)	%TCI	Tumor volume (mL)	%TVI
I	Normal	-	-	-	-
II	EAC+control	7.617±0.078	0.00	6.89±0.16	0.00
III	EAC+MECB (200 mg/kg)	1.532±0.098	79.92	1.46±0.17	78.81
IV	EAC+MECB (400 mg/kg)	0.8022±0.005	89.51	0.36±0.03	94.77
V	EAC+5-FU	0.5992±0.006	92.30	0.15±0.030	97.88

Each value represents the mean±SEM (n=6 mice per group). Experimental groups were compared with EAC control group (P<0.01).
EAC: Ehrlich Ascites Carcinoma, TCI: Inhibition of total cell count, MECB: Methanol extract of *Commelina benghalensis*

Table 2: Anticancer activity of MECB against EAC bearing mice (total count of viable, non-viable and % inhibition of viable, non-viable)

Group	Compound	Viable cell count (×10^7)	Non-viable cell count (×10^7)	% of viable cells	% of non-viable cells
I	Normal	-	-	-	-
II	EAC+control	6.903±0.049	0.711±0.097	90.62	9.33
III	EAC+MECB (200 mg/kg)	0.8167±0.062*	0.715±0.052***	54.44	46.67
IV	EAC+MECB (400 mg/kg)	0.3652±0.059*	0.437±0.061**	45.52	54.47
V	EAC+5-FU	0.1835±0.022*	0.415±0.023**	30.62	69.28

Each value represents the mean±SEM (n=6 mice per group). *Experimental groups were compared with EAC control group (P<0.01). **Experimental groups were compared with EAC control group (P<0.05). ***Experimental groups were compared with EAC control group (P>0.05). EAC: Ehrlich Ascites Carcinoma

treated mice (Groups III and IV) compared with EAC control mice [Table 3 and Figure 5].

MECB treated mice (Groups III and IV) at the doses 200 mg/kg b.w. and 400 mg/kg b.w. in EAC bearing mice significantly (P < 0.01) increased both the level of RBC and Hb while significantly (P < 0.001) reduced the level of WBC compared with EAC control group [Table 4 and Figures 6-8].

Table 3: Percentage increase in lifespan (%ILS)

Group	Compound	MST (in days)	% ILS
I	Normal	-	-
II	EAC+control	19.35	0.00
VII	EAC+MECB (200 mg/kg)	25.41	57.15
VII	EAC+MECB (400 mg/kg)	38.02	96.48
IX	EAC+5-FU	43.33	123.92

EAC: Ehrlich ascites carcinoma

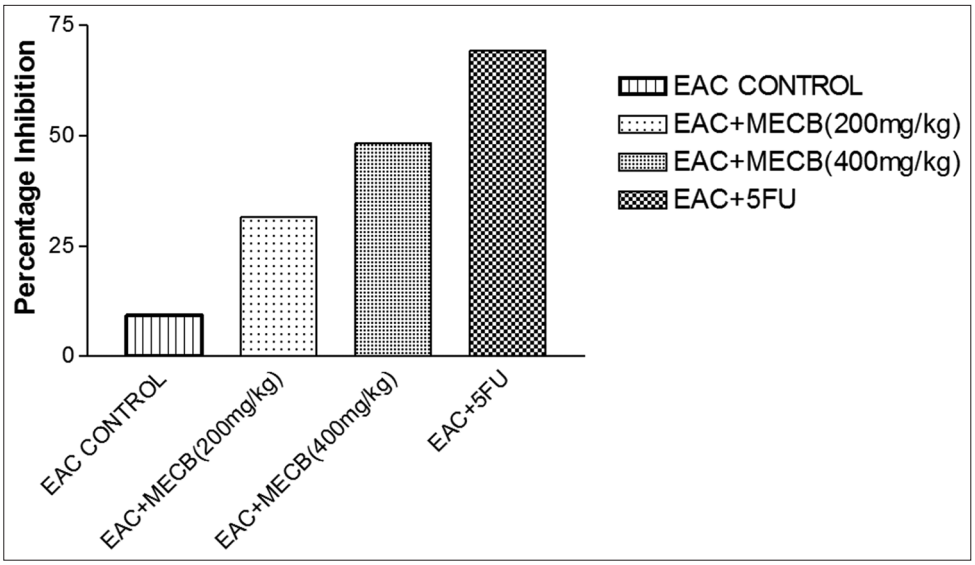

Figure 4: Percentage inhibition of non-viable cell count

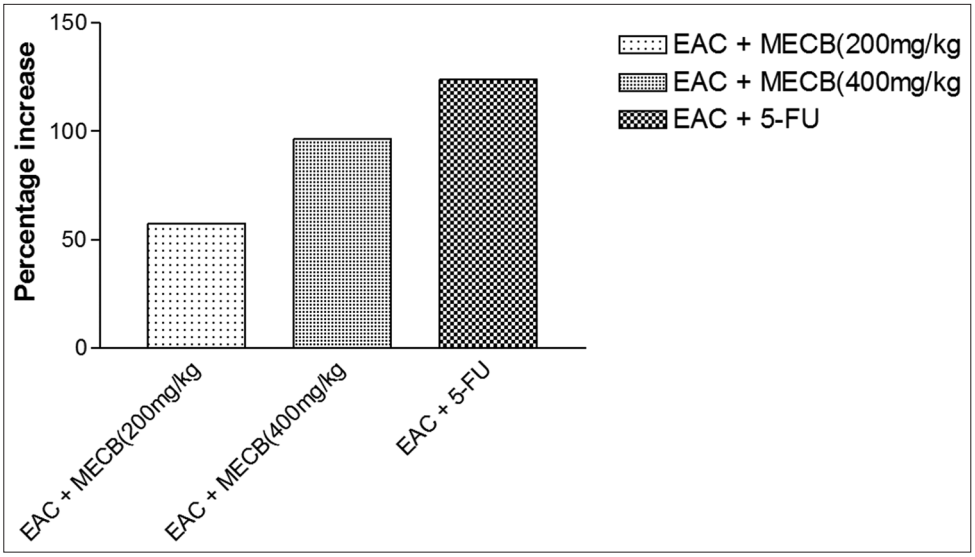

Figure 5: Percentage increase in life span

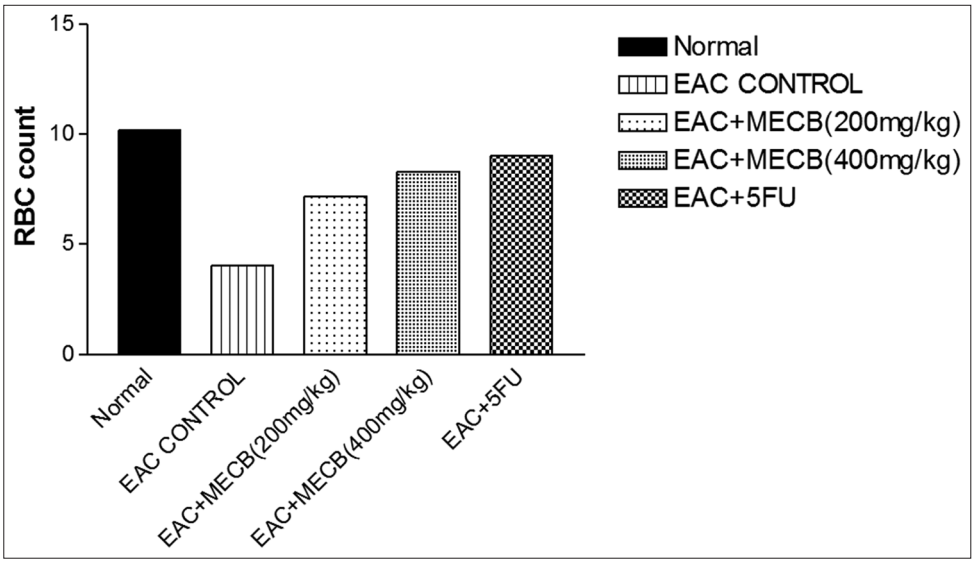

Figure 6: Red blood cell

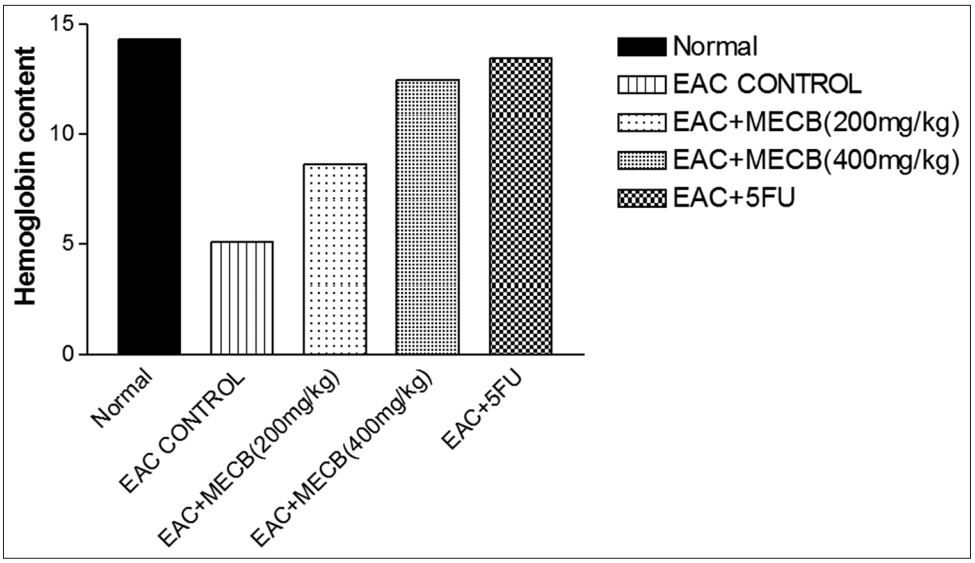

Figure 7: Hemoglobin

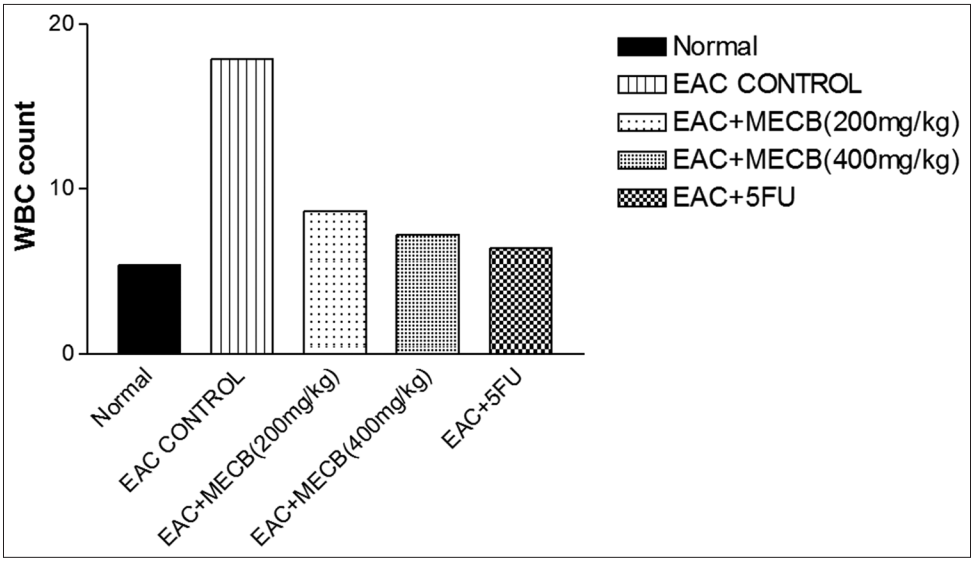

Figure 8: White blood cell

Table 4: Hematological parameters - WBC, RBC, and Hb content

Group	Compound	WBC count (×10^9/L)	RBC count (10^{12}/L)	Hb (g/dL)
I	Normal	5.415±0.189	10.19±0.285	14.29±0.168
II	EAC+control	17.88±0.378	4.045±0.222	5.117±0.2065
III	EAC+MECB (200 mg/kg)	8.67±0.363	7.17±0.137	8.616±0.1699
IV	EAC+MECB (400 mg/kg)	7.242±0.1248	8.308±0.1079	12.46±0.113
V	EAC+5-FU	6.423±0.1601	9.020±0.095	13.44±0.120

Each value represents the mean±SEM (n=6 mice per group). Experimental groups were compared with EAC control group (P<0.01)
EAC: Ehrlich ascites carcinoma, Hb: Hemoglobin, WBC: White blood cell, RBC: Red blood cell

Histological findings such as steatosis and lymphocyte accumulation were observed in the liver of EAC bearing mice by histological examination.

The liver section of EAC treated control group was compared with the tested formulations in different treated groups (200 mg/kg, 400 mg/kg, and 20 mg/kg). The histological observations of the test groups suggested that normal architecture of liver nucleus, parenchyma, and hepatic cells were regenerated, which was damaged in EAC disease (tumor) control group. All three tested formulations (with 200, and 400 mg/kg body weight of MECB, and

Table 5: HAI using the knodell score-CB plant extract

Organ	Findings	Gr-I	Gr-II	Gr-III	Gr-IV	Gr-V
	Sinusoidal dilatation around CV	0	2	2	1	0
	Eosinophilic hepatocytes	0	0	0	0.5	0
Liver	MNC infiltration	0	3	1	1	1
	Degeneration (binucleated hepatocytes) near central vein	0	2	1	1	0.5
	Inflammatory cells foci	0	1	1	0	0
	MEAN	0.00	1.60	1.00	0.70	0.30
	SEM	0.00	0.51	0.32	0.20	0.20

CV: Central vein, HAI: Histology activity index, *C. benghalensis: Commelina benghalensis*

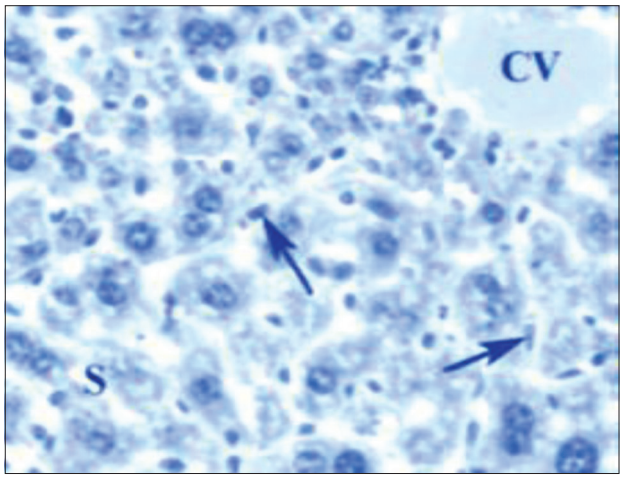

Figure 9: Photomicrograph of liver section of a normal control mice showing branching and anastomosing cords of hepatocytes radiating from the central vein. The hepatocytes have vesicular nuclei, and some are binucleated. Cells are separated by sinusoids (S) lined by flat endothelial cells and Von Kupffer cells (arrows) (H and E, ×100)

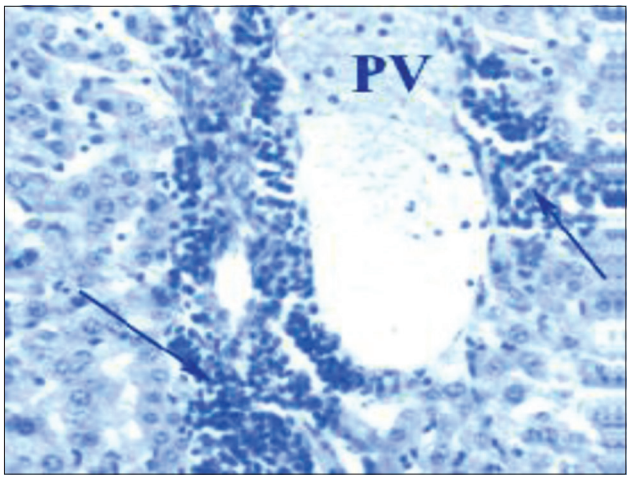

Figure 10: Photomicrographs of liver of mice bearing Ehrlich ascites carcinoma showing vacuolated hepatocyte nucleus (arrowhead), widen portal tract with the dilated congested portal vein, and mononuclear cellular infiltration (arrow) (H and E, ×100)

20 mg/kg of 5-Fluorouracil) showed significant improvement of the damaged central vein (CV), decreased degenerated hepatocytes and deformed necrosis of tissue, which was damaged in tumor disease control Group II (EAC control) [Figures 9-12].

Carcinogenesis is associated with cirrhosis and cirrhosis correlates with primary liver disease. Liver is easily affected by various types of diseases, cancer being one of them. EAC easily affects the liver of mice. From the experiment, the liver section of EAC disease control group was compared with normal, 5-FU and plant extracts treated groups.

The liver of control mice [Figure 9] appeared to be formed of the classical hepatic lobules. Each lobule showed radially arranged hepatocytes forming cords around the CV. Hepatocytes appeared polygonal in shape with rounded central vesicular nuclei. Blood sinusoids were seen separating the cords of the liver cells and lined by flattened endothelial cells and Von Kupffer cells. Minimal amount of thin, fine collagen fibers were seen around the CV and portal areas [Figure 9]. In Figure 10, mice liver sections showed hypertrophy of hepatic cells, vacuolated and necrosis in the cytoplasm with deeply stained pyknotic nuclei. A widening of the portal tract and mononuclear cellular infiltration around the congested portal vein were seen. The cellular architecture of the hepatic lobules seemed to have degenerated after inoculation of EAC cells in mice. The cells were positively stained with H and E, and the cytoplasmic material was less vacuolated [Table 5].

The best way to use histologic data such as histology activity index (HAI) has been already reported using Knodell score.[20] The original Knodell score was calculated as the sum or scores of periportal necrosis, intralobular injury, portal inflammation, and fibrosis. These scores are a very good way to show differences in histologic response between control and receiving different doses of test items, and these have been used successfully for better interpretation of the experimental results.

CONCLUSION

In this present study, it was noted that MECB significantly reduced tumor volume, viable cell count and normalized the Hb level and increased lifespan in comparison with

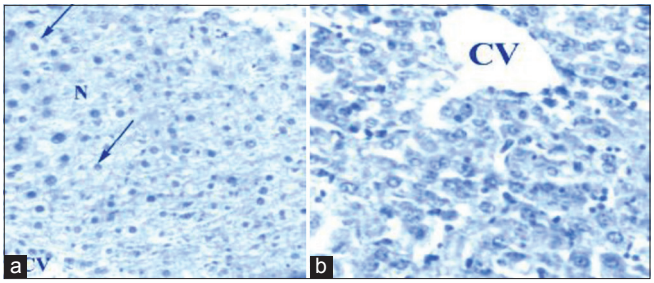

Figure 11: (a and b) Photomicrographs of liver of mice bearing Ehrlich ascites carcinoma, treated with 200, and 400 mg/kg body weight of methanolic extract of CB, showing dose-dependent improvement of the structural damages of the liver tissue, also showing less damage of central vein, less degenerated hepatocytes, and deformed necrosis of tissue (N) (H and E, ×100)

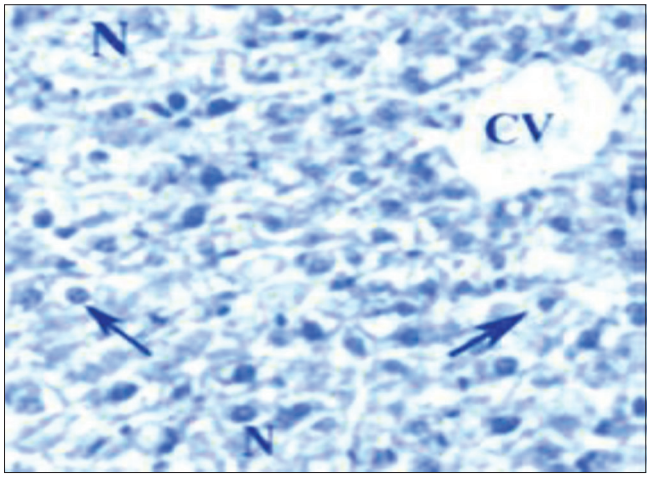

Figure 12: Photomicrograph of mice liver in Ehrlich Ascites Carcinoma treated with 5-Fluorouracil (20 mg/kg, i.p.) showing significant improvement and more or less normal structure of the liver tissue, showing thin wall central vein, hepatic portal vein, and large neoplastic hepatocytes (N) are regenerated look like to normal liver section (H and E, ×100)

EAC control group. Furthermore, the Study of Histological observation of test groups suggested normal architecture of liver tissue, less thick wall CV, deformed necrosis of tissue (N) and nucleus, parenchyma, and hepatic cells were regenerated, which was damaged in EAC treated control Groups (I-V). These parameters and observations indicate that MECB has significant anticancer activity.

ACKNOWLEDGMENTS

The authors are thankful and expressed their gratitude to the officers and staff of the Department of Health and F.W., Govt. of W.B. for approval the NOC. Authors are also grateful to the Professors and Staff of Jadavpur University, Kolkata, BSI Howrah. Authors are also grateful to the Professor G.C. Maity, Former HOD (Botany), University of Kalyani, for his valuable suggestions and guidance.

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Source of Support: Nil. **Conflict of Interest:** None declared.