



Antitumor and antioxidant activities of *Bryonia laciniosa* against Ehrlich's Ascites Carcinoma bearing Swiss albino mice

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SUMMARY

The plant *Bryonia laciniosa* (Family: Cucurbitaceae) has been indicated for the treatment of various diseases one among which is cancer. The purpose of this study was investigating experimentally the possible anti-tumor effect and antioxidant role of *Bryonia laciniosa* leaves in animal model. The methanol extract of *Bryonia laciniosa* (MEBL) administered at the doses of 62.5, 125 and 250 mg/kg in mice for 14 days after 24 h of tumor inoculation. The effect of MEBL on the growth of transplantable murine tumor, life span of EAC bearing mice, hematological profile and liver biochemical parameters (lipid peroxidation, antioxidant enzymes) were estimated. Treatment with MEBL decreased the tumor volume and viable cell count thereby increasing the life span of EAC bearing mice and brought back the hematological parameter more or less normal level. The effect of MEBL also decreases the levels of lipid peroxidation and increased the levels of glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). The present work indicates that the methanol extract of *Bryonia laciniosa* exhibited significant antitumor and antioxidant activity *in vivo*.

Key words: *Bryonia laciniosa*; Antitumor activity; Lipid peroxidation; Antioxidants

INTRODUCTION

There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. The plant *Bryonia laciniosa* Linn (Family: Cucurbitaceae) is a shrub found wildly in India, Philippines and some parts of Africa. *Bryonia laciniosa* is a well-known medicinal plant currently used mainly as anti-inflammatory drug in homeopathy (Gabrielian and Alexander

Gevorgovich, 1997). It has considerable reputation as a potent adjunct in the treatment of various ailments such as inflammation, tumor and fever (Kritikar and Basu, 1975). The leaf extract of the plant is being used as a cathartic and hot aqueous extract of the roots and seeds have an effect on conception in barren women. Goniothalamine, punicic acid and lipids were previously isolated from the whole plant of *Bryonia laciniosa* (Haque *et al.*, 2000; Mosaddik *et al.*, 2003). Recently we have reported the anti-inflammatory and analgesic and toxicity studies of *Bryonia laciniosa* (Gupta *et al.*, 2003; Sivakumar *et al.*, 2004) in standard animal models. In folklore remedy the plant was used in

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the treatment of cancer among the tribal population in Kolli Hills, South India. However, a fewer reports are available with respect to the pharmacological properties of the plant.

Reactive oxygen species such as superoxide, hydroxyl radical, iron-oxygen complexes, hydrogen peroxide and lipid peroxides are generated by several reactions. These are metabolism of triplet oxygen molecule; one electron reduction of oxygen; catalytic decomposition of hydrogen peroxide and lipid peroxides by metal ions; attack of metal and/or metal-oxygen complex; irradiation of visible light and x-ray, and intake of exogenous radicals (Fridovich, 1976). These radicals react with biological molecules such as DNA, proteins and phospholipids and eventually destroy the structure of other membranes and tissues (Vuillaume, 1987; Meneghini, 1988).

At present, the scientific community is interested in elucidating the role of several therapeutic modalities, currently considered as elements of complementary and alternative medicine, on the control of certain diseases. Plant derived natural products such as terpenoids and steroids etc. have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity (DeFeudis *et al.*, 2003; Takeoka *et al.*, 2003). Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against infection and degenerative diseases. *Bryonia laciniosa* have been indicated for the treatment of several diseases, one among which is cancer (Belkin *et al.*, 1952). From this viewpoint the present study was carried out to evaluate the antitumor activity and antioxidant status of methanol extract of *Bryonia laciniosa* (MEBL) against EAC bearing mice.

MATERIALS AND METHODS

Plant material and extraction

The leaves of *Bryonia laciniosa* (Family: Cucurbitaceae) were collected in the month of April 2003 from the

Kolli Hills, Tamilnadu, India. The plant material was taxonomically identified by the Botanical survey of India, Shibpur, Howrah and the voucher specimen GMS-25 was retained in our laboratory for future reference. The dried powdered leaves were extracted by methanol in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure and a semi solid mass was obtained and vacuum dried to yield a solid residue (14.25%). The extract showed positive test for steroids, triterpenoids and lipids. The extract at the doses of 62.5, 125 and 250 mg/kg and 5-fluorouracil (20 mg/kg) in saline were used for the present study.

Chemicals and reagents

Chloro-2, 4-dinitrobenzene [CDNB] was purchased from Sigma chemicals, USA, Thiobarbituric acid (Loba Chemie, Bombay, India), 5, 5'- Dithio-bis-2-nitrobenzoic acid [DTNB] (Sisco research laboratory, Bombay), Nitroblue tetrazolium chloride [NBT] (Sigma chemicals, USA) and other solvent and / or reagent obtained was used as received. EAC cells were obtained from Chittaranjan National Cancer Institute (CNCI), Kolkata, India. The EAC cells were maintained by intraperitoneal inoculation of 2×10^6 cells/mouse. Studies were carried out using male Swiss albino mice weighing 20 ± 2 g. were obtained from Indian Institute of Chemical Biology (IICB), Kolkata, India. All procedures described were reviewed and approved by the University Animal Ethical Committee.

Animals

Male Swiss albino mice weighing between 18 - 22 g were used for the present study. They were maintained under standard environmental conditions and were fed with standard pellet diet and water *ad libitum*.

Toxicity study

A short-term toxicity study was also carried out for a period of 14 days which is period of the study of antitumor activity. Healthy Swiss albino mice were divided into 4 groups of 8 animals in each. Group 1,

(vehicle control) was received propylene glycol 5 ml/kg intraperitoneally once daily for 14 days. Group 2, 3, and 4 received MEBL at the doses of 62.5, 125 and 250 mg/kg intraperitoneally once daily for 14 days. After 24 h of the last dose the mice were sacrificed. Blood collected and hematological parameters were determined as described in hematological studies. Liver and other important internal organs were removed, weighed and observed for pathological changes. The blood was centrifuged at 3,000 rpm at 4°C for 10 minutes to separate serum. The activities of serum glutamate oxaloacetate transaminase level (SGOT) and serum glutamate pyruvate transaminase (SGPT) were assayed (Bergmeyer and Bernt, 1974). The alkaline phosphatase activity in the serum was measured according to the method of King (1965). Further, liver biochemical parameters were estimated by methods described in estimation of biochemical parameters.

Treatment schedule

Tumor was induced by injecting 0.2 ml of 2×10^6 cell/ml of Ehrlich's Ascitic Carcinoma (EAC) in to peritoneal cavity of mice. Prior to the administration of EAC cells to mice, the animals were divided into six groups (n = 12) and given food and water ad libitum. All the groups were injected with EAC cells (2×10^6 cells/mouse) intraperitoneally except normal group. This was taken as day 0. On the first day normal saline (0.9%, w/v, NaCl, 5 ml/kg/mouse/day) administered into normal group 1. EAC control mice were received only vehicle (propylene glycol 5 ml/kg/day/mouse) as groups 2. The different doses of *Bryonia laciniosa* (62.5, 125 and 250 mg/kg/mouse/day) and standard drug 5-Fluorouracil (20 mg/kg) were subsequently administered in groups 3, 4, 5 and 6 respectively for 14 days intraperitoneally. On 15th day, after the last dose and 18 h fasting six mice from each group were sacrificed for the study of antitumor activity, hematological, and antioxidant enzymes estimation and rest of the animal of each group were kept to check the mean survival time (MST) and increase

in the lifespan of the tumor bearing mice.

Tumor growth response

Antitumor effect of MEBL was assessed by observation of changes with respect to body weight, ascetics tumor volume, packed cell volume, viable and nonviable tumor cell count, MST and percentage increase in life span (% ILS). Transplantable murain tumor was carefully collected with the help of a sterile 3 ml syringe and measured the tumor volume and the ascitic fluid was with draw in a graduated centrifuge tube and packed cell volume was determined by centrifuging at 1,000 g for 5 min. Viable and nonviable cell cont of ascitic cell were stained by the trypan blue (0.4% in normal saline) dye exclusion test and count was determined in a Neubauer counting chamber. The effect of MEBL on tumor growth was monitored daily by recording the mortality and percentage increase in life span (% ILS) was calculated using following formula $ILS (\%) = [(Mean\ survival\ of\ treated\ group / Mean\ survival\ of\ control\ group) - 1] \times 100$.

Hematological studies

Blood was obtained from the tail vein, 24 h after last dose. For the total count blood was drawn into RBC or WBC pipettes, diluted and counted in a Neubauer counting chamber. Sahli's Hemoglobinometer determined the hemoglobin concentration. Differential count of leukocytes was done on a freshly drawn blood film using Leishman's stain. Hemoglobin content (D'Amour *et al.*, 1965), RBC and WBC count (Wintrobe *et al.*, 1961) and differential leukocyte count (Dacie and Lewis, 1958) was estimated from the peripheral blood of normal, EAC control and treated animal groups.

Biochemical assays

The liver was excised, rinsed in ice-cold normal saline followed by cold 0.15 M Tris-HCl (pH 7.4), blotted and weighed. The homogenate was processed for estimation of lipid peroxidation, GSH, SOD and CAT. Assay for microsomal lipid peroxidation was

carried out by the measurement of thiobarbituric acid reactive substances (TBARS) in the tissues reported by Ohkawa *et al.* (1979). The pink chromogen produced by the reaction of malondialdehyde, which is a secondary product of lipid peroxidation reaction with thiobarbituric acid was estimated at 532 nm. Reduced glutathione (GSH) in the tissues was assayed by the method of Ellman (1979). GSH estimation is based on the development of yellow color when 5, 5'-dithiobis (2-nitro benzoic acid) di-nitrobenzoic acid was added to compounds containing sulphhydryl group. SOD was assayed by the method of Kakkar *et al.* (1984). The assay was based on the 50% inhibition of formation of NADH-Phenazinemethosulphate-Nitroblue tetrazolium formazan at 520 nm. The activity of CAT was assayed by the method of Abei (1983). Proteins were estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Statistical analysis

Total variation present in a set of data was performed by using one way analysis of variance (ANOVA) and the results are expressed as mean \pm SEM.

RESULTS

The present investigation indicates that the MEBL showed significant anti-tumor and antioxidant

activity in EAC bearing mice. The effects of MEBL (62.5, 125 and 250 mg/kg) at different doses on tumor volume, viable and non-viable cell count, survival time and ILS, were shown in Table 1 and 2. Administration of MEBL reduces the tumor volume, packed cell volume and viable tumor cell count in a dose dependant manner when compared to EAC control mice. In EAC control mice the median survival time was 21.0 ± 0.77 days. Whereas, it was significantly increased median survival time (25 ± 0.30 , 28 ± 0.25 , 34 ± 0.32 and 31 ± 0.21 days) with different doses (62.5, 125 and 250 mg/kg) of MEBL and standard drug respectively.

As shown in Table 3, the hemoglobin content in the EAC control mice (11.3 g %) was significantly decreased when compared with normal mice (13.8 g %). MEBL at the dose of 125 and 250 mg/kg the hemoglobin content in EAC treated mice were increased to 10.6 ± 1.04 g % to 11.7 ± 1.03 and 12.4 ± 1.62 (g %). Moderate changes in the RBC count were also observed in the extract treated mice. The total WBC counts were significantly higher in the EAC treated mice when compared with normal mice. Whereas, MEBL treated mice significantly reduced the WBC counts as compared to that of control mice. As shown in table 4, the differential leukocyte count, the percentage of neutrophils was increased while the lymphocyte count was decreased in the extracts treated mice when compared with EAC control mice.

Table 1. Effect of methanol extract of *Bryonia laciniosa* on tumor volume, packed cell volume, viable and non-viable tumor cell count of EAC bearing mice

Parameters	EAC Control (2×10^6 cells/ mouse/ml)	MEBL (62.5 mg/kg) +EAC	MEBL (125 mg/kg) + EAC	MEBL (250 mg/kg) + EAC	Standard 5-flourouracil (20 mg/kg)+ EAC
Body weight (g)	26.22 ± 0.12	23.34 ± 0.17	22.52 ± 0.13	21.55 ± 0.13	20.23 ± 0.19
Tumor volume (ml)	4.41 ± 0.07	3.73 ± 0.03	2.72 ± 0.03	1.44 ± 0.01	-
Packed cell volume (ml)	2.31 ± 0.06	1.22 ± 0.05	0.96 ± 0.02	0.27 ± 0.01	-
Viable tumor cell count $\times 10^7$ cells/ml	11.22 ± 0.07	9.33 ± 0.06	5.51 ± 0.04	1.71 ± 0.06	-
Nonviable tumor cell count $\times 10^7$ cells/ml	0.34 ± 0.02	0.67 ± 0.07	0.82 ± 0.06	1.34 ± 0.09	-

Values are mean \pm SEM. Number of mice in each group (n = 6). $P < 0.01$, Experimental groups was compared with EAC control.

Table 2. Effect of the methanol extract of *Bryonia laciniosa* on survival time on EAC bearing mice

Groups	Experiment	Median survival (days)	Life span (%)	Increase of life span
1	Normal control (Normal saline 5 ml/kg b.w.)	-	-	-
2	EAC control (2×10^6 cells) + Propylene glycol (5 ml/kg b.w.)	21 \pm 0.77	100	-
3	MEBL (67.5 mg/kg) + EAC (2×10^6 cells)	25 \pm 0.30	125.0	25.0
4	MEBL (125 mg/kg) + EAC (2×10^6 cells)	28 \pm 0.25	133.3	33.3
5	MEBL (250 mg/kg) + EAC (2×10^6 cells)	34 \pm 0.32	161.9	61.9
6	5-Flurouracil (20 mg/kg) + EAC (2×10^6 cells)	31 \pm 0.21	147.6	47.6

Values are mean \pm SEM. Number of mice in each group (n = 6). $P < 0.01$, Experimental groups was compared with control.

Table 3. Effects of methanol extract of *Bryonia laciniosa* on hematological parameters of EAC treated mice

Parameters	Normal Saline (0.5 ml/kg)	EAC (2×10^6 cells) Control + Vehicles	EAC (2×10^6 cells) + MEBL 62.5 mg/kg	EAC (2×10^6 cells) + MEBL 125 mg/kg	EAC (2×10^6 cells) + MEBL 250 mg/kg	EAC (2×10^6 cells) + MEBL Standard
Hemoglobin (g %)	13.8 \pm 1.10	11.3 \pm 0.39 ^b	10.6 \pm 1.04	11.7 \pm 1.03	12.4 \pm 1.62	11.6 \pm 1.62
Total RBC (cells/ml $\times 10^9$)	6.4 \pm 0.54	4.5 \pm 0.45	4.4 \pm 0.32 ^b	5.6 \pm 0.53 ^b	6.1 \pm 0.68	5.7 \pm 0.54
Total WBC (cells/ml $\times 10^6$)	6.7 \pm 0.58	18.9 \pm 1.67 ^b	15.4 \pm 1.34	11.6 \pm 0.77	7.1 \pm 0.70	8.4 \pm 0.53
Cells/femur 1×10^6 /ml	18.9 \pm 1.68	14.9 \pm 1.47 ^b	15.8 \pm 1.45 ^a	16.5 \pm 1.45 ^a	17.4 \pm 1.48	16.7 \pm 1.22
Cells/spleen 2×10^6 /ml	16.7 \pm 1.88	28.4 \pm 1.47 ^b	24.95 \pm 2.27 ^b	20.5 \pm 1.70 ^b	14.4 \pm 1.42	19.7 \pm 1.27

Values are mean \pm SEM (n = 6). EAC control group compared with normal group ^b $P < 0.05$. Experimental groups were compared with EAC control. ^a $P < 0.01$, ^b $P < 0.05$.

Table 4. Effect of methanol extract of *Bryonia laciniosa* on differential counts of white blood cells in EAC bearing mice

Design of Experiment	Neutrophil (%)	Eosinophil (%)	Lymphocyte (%)	Monocyte (%)
Normal saline (5 ml/kg)	17.5 \pm 1.25	0.6 \pm 0.01	80.1 \pm 2.31	1.8 \pm 0.15
EAC (2×10^6 cells) + Propylene glycol (5 ml/kg)	66.6 \pm 0.01	1.5 \pm 2.48 ^b	32.2 \pm 0.07 ^b	0.9 \pm 0.03 ^b
EAC (2×10^6 cells) + MEBL 62.5 mg/kg	54.2 \pm 3.44 ^b	1.1 \pm 0.03 ^a	43.7 \pm 2.48 ^b	1.0 \pm 0.05 ^a
EAC (2×10^6 cells) + MEBL 125 mg/kg	43.9 \pm 2.57 ^b	0.6 \pm 0.02 ^a	54.3 \pm 2.22 ^b	1.2 \pm 0.03 ^a
EAC (2×10^6 cells) + MEBL 250 mg/kg	37.4 \pm 2.34 ^b	0.6 \pm 0.03 ^a	60.8 \pm 2.81 ^b	1.2 \pm 0.09 ^a
EAC (2×10^6 cells) + Standard drug (5-Flurouracil 20 mg/kg)	45.3 \pm 4.33 ^b	0.7 \pm 0.02 ^a	52.7 \pm 2.33 ^b	1.3 \pm 0.05

Values are mean \pm SEM (n = 6). EAC control group was compared with normal group ^b $P < 0.05$. Experimental groups were compared with EAC control. ^a $P < 0.01$, ^b $P < 0.05$

The levels of LOP, GSH, SOD and catalase were summarized in Table 5, the levels of lipid peroxidation in liver tissue were significantly increased in EAC control mice (1.45 n moles MDA/g of tissue) as compared to the normal mice (0.97 n moles MDA/g of tissue). Treatment with MEBL (62.5, 125 and 250 mg/kg) were significantly decrease the LOP levels (1.37, 1.29 and 1.19 n moles MDA/g of tissue) in a dose dependent manner. The GSH content in

liver tissues of normal mice was found to be 2.36 mg/g wet tissue. Inoculation of EAC drastically decreased the GSH content to 1.69 mg/g wet tissue. Whereas, treatment with different doses of MEBL the GSH levels were reverts to normal level (2.86, 2.14 and 2.29 mg/g wet tissue) respectively.

As shown in table 5, SOD level in the liver of EAC bearing mice was significantly decreased (3.29 Unit/mg protein) when compared with normal mice

Table 5. Effect of different doses of methanol extract of the *Bryonia laciniosa* on different biochemical parameters in liver in EAC bearing mice

Parameters	Normal Saline (0.5 ml/kg)	EAC (2 × 10 ⁶ cells) Control + MEBL (Vehicles)	EAC (2 × 10 ⁶ cells) + MEBL 62.5 mg/kg	EAC (2 × 10 ⁶ cells) + MEBL 125 mg/kg	EAC (2 × 10 ⁶ cells) + MEBL 250 mg/kg
Lipid peroxidation (n moles MDA/g of tissue)	0.97 ± 0.03	1.45 ± 0.03 ^b	1.37 ± 0.02 ^a	1.29 ± 0.01	1.19 ± 0.01 ^a
GSH (mg/g of tissue)	2.36 ± 0.03	1.69 ± 0.12 ^b	2.86 ± 0.17 ^a	2.14 ± 0.21 ^a	2.29 ± 0.03 ^b
SOD (Units/mg Protein)	4.38 ± 0.43	3.29 ± 0.27 ^b	3.59 ± 0.22 ^b	3.96 ± 0.33 ^a	4.22 ± 0.01 ^a
Catalase (Units/mg tissues)	2.59 ± 1.91	1.63 ± 0.11 ^b	1.78 ± 0.11 ^b	1.97 ± 1.17 ^a	2.14 ± 0.01 ^b

Values are mean ± SEM (n = 6). EAC control group was compared with normal group ^bP < 0.05. Experimental groups were compared with EAC control. ^aP < 0.01, ^bP < 0.05

Table 6. Short term toxicity effect of methanol extract of the *Bryonia laciniosa* on different biochemical parameters

Parameters	Normal Saline (0.9% NaCl 0.5 ml/kg)	MEBL 62.5 mg/kg	MEBL 125 mg/kg	MEBL 250 mg/kg
Hb (g %)	12.4 ± 1.51	12.5 ± 1.3	12.3 ± 0.92	12.6 ± 1.35
RBC (10 ⁶)	6.5 ± 0.54	6.2 ± 0.52	6.6 ± 0.62	6.8 ± 0.35
WBC (10 ³)	5.5 ± 0.34	5.9 ± 0.33	7.4 ± 0.54	8.7 ± 0.78
SGPT (U/l)	65.6 ± 4.73	68.7 ± 4.48	74.3 ± 5.22	87.4 ± 4.04 ^a
SGOT (U/l)	38.9 ± 2.64	43.97 ± 0.37	46.7 ± 1.76	48.3 ± 3.45 ^a
Serum Urea (mg/dl)	21.7 ± 1.03	22.4 ± 1.57	22.4 ± 1.45	22.6 ± 1.02
Lipid peroxidation (n moles MDA/g of tissue)	0.97 ± 0.03	0.98 ± 0.04	0.97 ± 0.02	0.99 ± 0.02
GSH (mg/g of tissue)	2.36 ± 0.03	2.39 ± 0.12	2.46 ± 0.17	2.54 ± 0.21
SOD (Units /mg of Protein)	4.38 ± 0.43	4.42 ± 0.27	4.49 ± 0.22	4.56 ± 0.33 ^a
Catalase (Units /mg tissues)	2.59 ± 1.91	2.63 ± 0.11	2.68 ± 0.11	2.74 ± 1.17

Values are mean ± SEM (n = 8). The experimental groups compared with normal groups ^aP < 0.01.

(4.38 Unit/mg protein). Administration of the MEBL significantly increased the SOD level (3.59, 3.96 and 4.22 unit/mg of protein in tissues) at the doses of 62.5, 125 and 250 mg/kg respectively. The CAT level were decreased in EAC control mice (1.63 Unit/mg protein) when compared with normal mice (2.59 unit/mg of protein in tissues) treatment with MEBL at the doses of 62.5, 125 and 250 mg/kg it brought back to normal levels (1.78, 1.97 and 2.14 unit/mg of protein in tissues).

Short term toxicity studies

The MEBL was also evaluated for its short-term toxicity in mice. The hematological profile and biochemical parameter were shown in Table 6. No harmful effect was noticed in either liver or kidney function of the extract treated animals. However,

the mice which is received 250 mg/kg or above showed slight toxic symptoms. These include inactiveness, loss of appetite, slow movement, and dizziness, erection of hairs and hypothermia. Administration of repeated daily doses of 62.5, 125 and 250 mg/kg for 14 days did not influence the body weight of the mice. The weights of liver, kidney, brain and spleen were also not altered by the treatment. But at the higher dose of MEBL (250 mg/kg) were significantly altered the enzyme levels such as SGPT (87.4 U/l) and SGOT (48.3 U/l) when compared with normal mice.

DISCUSSION

The present study was carried out to evaluate the effect of MEBL on EAC bearing mice. The MEBL

were showed significant antitumor activity against the transplantable murine tumor. The reliable criteria for judging the value of any anticancer drug are the prolongation of life span of animals (Prasad and Giri, 1994). The Ascitic fluid is the direct nutritional source to tumors cells and the rapid increase in ascitic fluid with tumor growth could possibly by a means to meet more nutritional requirements of tumor cells (Clarkson and Burchenal, 1965). A reduction in the number of ascitic tumor cells may indicate either an effect of MEBL on peritoneal macrophages or other components of the immune system (Kleeb *et al.*, 1999) therefore increasing their capacity of killing the tumor cells, or a direct effect on tumor cell growth. MEBL inhibited significantly the tumor volume, viable cell count and enhancement in survival time of EAC bearing mice and thereby acts as antineoplastic agent.

Myelosuppression is a frequent and major complication of cancer chemotherapy. Compared to the EAC control animals, MEBL treatment and subsequent tumor inhibition resulted in appreciable improvements in hemoglobin content, RBC and WBC counts (Table 3). These observations assume great significance as anemia is a common complication in cancer and the situation aggravates further during chemotherapy since a majority of antineoplastic agents exert suppressive effects on erythropoiesis (Price and Greenfield, 1958; Holand, 1982) and thereby limiting the use of these drugs. The improvement in hematological profile of the tumor bearing mice following the treatment with extract could be due to the action of the different phytoconstituents present in the extract.

Lipid peroxidation mediated by free radicals considered being a primary mechanism of cell membrane destruction and cell damage (Plaa and Wistshi, 1976). The oxidation of unsaturated fatty acids in biological membranes leads to a reduction in membrane fluidity and disruption of membrane structure and function (Campo *et al.*, 2001). MDA, the end product of lipid peroxidation was also reported to be higher in carcinomatous tissue than

in non-diseased organs (Yagi, 1987). Increase in the level of TBARS indicate enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defence mechanisms to prevent the formation of excess free radicals (Campori, 1985). With reference to this, the active role of GSH against cellular lipid peroxidation has been well recognized and thereby reduces the reduced glutathione (GSH) activity. GSH can act either to detoxify activated oxygen species such as H_2O_2 or reduce lipid peroxides themselves. In the present study indicated that MEBL significantly reduced the elevated levels of lipid peroxidation and increased the levels of glutathione content and thereby it may act as an antitumor agent.

On the other hand, SOD is a ubiquitous chain breaking antioxidant and is found in all aerobic organisms. It is a metalloprotein widely distributed in all cells and plays an important protective role against ROS-induced oxidative damage. The free radical scavenging system catalase, which are present in all major organs in the body of animals and human beings and is especially concentrated in liver and erythrocytes. Both enzymes play an important role in the elimination of ROS derived from the redox process of xenobiotic in liver tissues (Curtis *et al.*, 1972; Korsrud *et al.*, 1973). It was suggested that catalase and SOD are easily inactivated by lipid peroxides or ROS (Chance *et al.*, 1952). In correlation, it has been reported that EAC bearing mice showed decreased levels of SOD activity and this may be due to loss of Mn^{++} SOD activity in liver (Sun *et al.*, 1989). Inhibition of catalase activity in tumor cell lines was also reported (Marklund *et al.*, 1961). In this study, catalase and SOD were appreciably elevated by administration of MEBL, suggesting that it can restore the levels of SOD and catalase enzymes.

In short term toxicity study the MEBL at the higher dose (250 mg/kg) significantly increase the transaminase activities indicating that it causes hepatorenal dysfunctions and metabolism.

The present study demonstrated that MEBL

increased the life span of EAC tumor bearing mice and decreased the lipid peroxidation and thereby augmented the endogenous antioxidant enzymes in the liver. The above parameters are responsible for the antitumor and antioxidant activities of *Bryonia laciniosa*.

Further investigations are in progress in our laboratory to identify the active principles involved in this antitumor and antioxidant activity and investigate their mechanism.

ACKNOWLEDGEMENTS

The author (T. Sivakumar) thankful to the AICTE, New Delhi for provided the financial assistance of this work. The authors are also thankful to the secretary Mrs. N. Sendamaraai, J.K.K. Rangammal Charitable Trust, Komarapalayam, Tamilnadu, India for the help rendered in all academic aspects.

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