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Bacopa monniera Linn. extract modulates antioxidant and marker enzyme status in fibrosarcoma bearing rats

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Antioxidative property and tumor inhibitive property of *B. monniera* (20mg/kg body wt, sc) was examined in 3-methylcholanthrene induced fibrosarcoma rats. Antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and the levels of glutathione (GSH) and the rate of lipid peroxidation (LPO) in the liver and kidney tissues were assessed. A significant increase was noted for the rate of LPO with a corresponding decrease in the antioxidant enzyme status in fibrosarcoma bearing rats. In fibrosarcoma bearing rats, the tumor markers like lactate dehydrogenase (LDH), creatine kinase (CK), alanine transaminase (ALT), aspartate transaminase (AST) and sialic acid (SA) were increased in the serum. Treatment with *B. monniera* extract significantly increased the antioxidant enzyme status, inhibited lipid peroxidation and reduced the tumor markers. It can be concluded that *B.monniera* extract promotes the antioxidant status, reduces the rate of lipid peroxidation and the markers of tumor progression in the fibrosarcoma bearing rats.

Keywords: 3-Methylcholanthrene, Fibrosarcoma, Antioxidants, Marker enzymes, *Bacopa monniera*

Cancer chemopreventive agents, many of which are natural products, are capable of preventing and inhibiting the process of carcinogenesis[1]. *Bacopa monniera* Linn (Syn. *Herpestis monniera* Linn H.B & vernacular Brahmi), has been recommended in the treatment of tumors, ascites, inflammations and CNS related disturbances in Ayurvedic transcripts[2,3]. The active constituents of *B. monniera* are saponins, alkaloids, betulic acid and phytosterols[4,5]. The ethanolic extract of *B.monniera* is recognized to exhibit antioxidative property[6]. Anticancer activity of *B.monniera* has been reported on S-180 cells *in vitro*[7] and walker carcinoma, *in vivo*[8]. Among the phytoconstituents of the ethanolic extract of *B. monniera*, Bacoside-A a principal saponin, exhibits maximum cytotoxicity in Brine shrimp[9].

Fibrosarcoma are solid tumors, characterized by the malignant proliferation of fibroblasts[10], arising in the external soft tissues as a gray white, firm, lobulate mass with a good circumscription[11]. They have a low metastatic rate, but are characteristically recurrent and radioresistant with a poor prognostic index[12]. 3-Methylcholantherne induced experimental fibrosarcoma serves as a convenient tool to study the tumor inhibitory properties of potentially exploitable drugs on solid tumors. Based on the previous reports, an attempt has been made to evaluate the tumor inhibitory efficacy of *B.monniera* extract by assessing the antioxidant and marker enzyme status in the liver and kidney tissues of the fibrosarcoma bearing animals as they have been shown to exhibit profound modifications[13,14].

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Materials and Methods

Drug—The whole plant of *B. monniera* was collected in March 2000 and authenticated by Dr. P. Brinda, Pharmacognist, Captain Srinivasa Murthi Drug Research Institute, Chennai. Shade dried and finely powdered plant material (1 kg) was mixed with 90% ethanol (5 l) by stirring continuously and extracted in the cold after soaking for 48 hrs. The extract was filtered and distilled on to a water bath to a syrupy mass and the last traces of the solvent were removed in vacuum (yield 50 g). The extract showed the presence of Bacoside-A, by comparing with the authentic specimen by CO-TLC, over silica gel using (*n*-butanol : acetic acid : water, 4:1:5 upper layer) as developing system. Dilute sulphuric acid (1:1) was used as the spray reagent. After spraying, the plates were dried at 110°C for 5 min. The compound

developed pink spots. The lyophilized extract was the used for the experimental study.

Chemicals—3-methylcholanthrene was obtained from Sigma (St. Louis & Co, USA). All other chemicals used were of analytical grade.

Animals—Adult male albino rats of Wistar strain weighing 80-110 g were used for the study. The rats were fed with commercial pellet rat chow and water *ad libitum*. They were maintained under standard lab conditions with 12 hr light and dark cycle. All the animal experiments were carried out according to the guidelines of Institutional Animal Ethics Committee. The animals were divided into following four groups of 6 each: Group I: normal animals (control), Group II: fibrosarcoma induced control animals, Group III: normal animals treated with *B.monniera* (20 mg/kg body weight, sc), Group IV: fibrosarcoma bearing rats treated with *B. monniera* (20 mg/kg body weight, sc). Fibrosarcoma was induced in male albino rats using 3-methylcholanthrene according to the method described by Nagarajan *et al.*[15]. Serially transplanted animals were maintained for the experiments by subcutaneous injections of the tumor tissue suspended in saline (2×10^6 cells) in the flank area of the rats. A palpable tumor mass was detectable from the 7^{th}-10^{th} day of transplantation.

Dose-dependent effects assessed revealed that a minimum effective dosage of 20 mg/kg body weight exhibited positive effects while a dosage beyond 80mg/kg body weight revealed weakness, weight loss and lethargy. However, no mortality was observed. The effects were reversible with the withdrawal of the extract. The minimum effective concentration of *B.monniera* (20 mg/kg body weight) was chosen for further experiments. Treatment with the extract was started at the 10^{th} day of transplantation and comprised a 30 day regime.

At the end of the experimental period, the animals were anaesthetized with ether and blood was drawn from the external jugular vein and serum separated by centrifugation. The animals were sacrificed and the liver and kidney tissues dissected out were washed in ice-cold physiological saline, weighed and homogenized in Tris-HCl buffer, 0.5 *M*, *p*H 7.4 at 4°C. The homogenate was used for the analysis of total protein[16], glutathione (GSH)[17], lipid peroxidation (LPO)[18], superoxide dismutase (SOD)[19], catalase (CAT)[20] and glutathione peroxidase (GPx)[21]. Serum was used for the assay of markers such as lactate dehydrogenase (LDH)[22], creatine kinase (CK)[23], aspartate transaminase (AST)[24], alanine transaminase (ALT)[24] and sialic acid (SA)[25].

Statistical analysis—The data were analyzed using one way ANOVA followed by Duncan's multiple comparison test. The results from the experimental groups were compared with respective controls. Values of $P<0.05$ were considered statistically significant.

Results

A decrease in the status of antioxidants such as SOD, CAT, GSH and GPx with a concomitant increase in the rate of LPO was observed in the fibrosarcoma bearing rats when compared to the control in the liver and kidney tissues (Table 1). On treatment with *B.monniera*,

Table 1—Effect of *B.monniera* on (A) liver and (B) kidney antioxidant enzymes in fibrosarcoma bearing rats [Values are mean ±SD for 6 animals in each group]

Treatment	Control	Fibrosarcoma	*B. monniera* (20 mg/kg: sc)	Fibrosarcoma +*B. monniera* (20 mg/kg: sc)	F Value
SOD[1]	A 4.05 ± 0.42	1.28 ± 0.17[a]	4.35 ± 0.38	3.86 ± 0.37[b]	67.86
	B 2.37 ± 0.20	1.75 ± 0.15[a]	2.32 ± 0.27	2.36 ± 0.12[b]	14.40
CAT[2]	A 14.13 ± 1.2	9.91 ± 0.89[a]	14.94 ± 1.27	11.68 ± 1.22	23.63
	B 42.59 ± 3.77	32.88 ± 2.92[a]	48.1 ± 3.39	40.95 ± 4.8[b]	16.57
GPx[3]	A 2.02 ± 0.35	2.31 ± 0.20	2.29 ± 0.19	2.74 ± 0.22[b]	22.58
	B 2.91 ± 0.28	2.00 ± 0.25[a]	2.54 ± 0.50	2.79 ± 0.20[b]	9.07
GSH[4]	A 3.70 ± 0.41	2.01 ± 0.21[a]	3.89 ± 0.28	3.13 ± 0.88[b]	10.01
	B 2.30 ± 0.28	1.73 ± 0.15[a]	2.91 ± 0.26	2.08 ± 0.19	9.71
LPO[5]	A 140.4 ± 14.21	178.11 ± 18.5[a]	149.83 ± 14.98	140.08 ± 15.58[b]	7.61
	B 70.95 ± 7.01	98.16 ± 9.49[a]	75.83 ± 8.06	76.16 ± 8.72[b]	12.64

[1]: units/mg protein,[2]: nmoles of H_2O_2 decomposed/min/mg protein,[3]: nmoles of GSH oxidised/min/mg protein,[4]: nmoles of CDNB/min/mg protein, [5]: nmoles of MDA/100 mg protein.

[a]$P<0.05$ when compared with the control

[b]$P<0.05$ when compared with the Fibrosarcoma bearing rats (FB)

(Group IV) there was a significant increase in the activities of antioxidant enzymes and glutathione with a corresponding decrease in LPO when compared to the fibrosarcoma bearing rats (Group II). *B. monniera* treated group alone (Group III) did not show any significant change.

An elevation in the levels of SA (Fig. 1) and the activities of ALT, AST, CK, and LDH were observed in the fibrosarcoma bearing rats (Group II) when compared to control (Table 2). Treatment with *B.monniera* (Group IV) significantly reduced these tumor markers in the serum when compared with the fibrosarcoma bearing rats (Group II). *B. monniera* alone did not show any significant change (Group III).

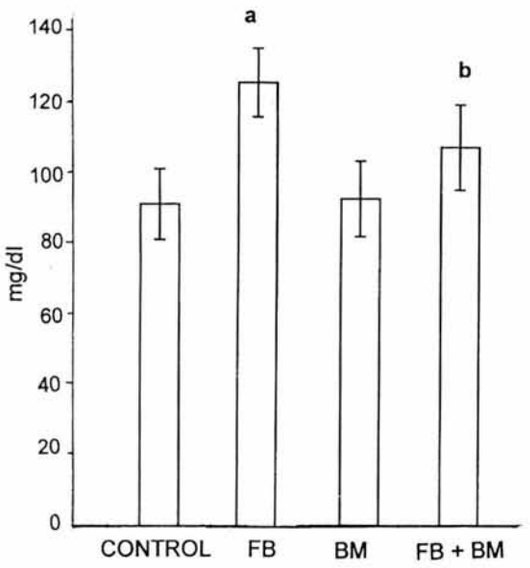

Fig. 1—Effect of *B. Monniera* on levels of sialic acid in fibrosarcoma bearing rats [$^{a}P<0.05$ when compared with the control; $^{b}P<0.05$ when compared with the fibrosarcoma bearing rats (FB). BM-*B.monniera* treated group; FB + BM-fibrosarcoma rats treated with *B. monniera*]

Discussion

Need for assessing the body weight arises while evaluating drugs for their therapeutic efficiency against cancer. *B.monniera* treated group III animals did not show any significant change in their body weight when compared to the control Group I animals, which showed a normal growth pattern indicating the nontoxic nature of *B.monniera*[26].

Malondialdehyde (MDA) a major end product and index of LPO, cross-links DNA and protein and nucleotides on the same and opposite strands thereby promoting carcinogenesis. Therefore, it is observed to be increased in tumor conditions[27]. The results of the present study also indicate that Group II fibrosarcoma bearing rats showed an increased rate of lipid peroxidation, which is found to be decreased in *B. monniera* treated group IV fibrosarcoma bearing rats. Malignant conditions are characterized by reduced activity of antioxidant enzymes and require an enhanced and effective antioxidant scavenging system. Natural products are source of antioxidants, which can antagonize the initiation and promotion phases of carcinogenesis. Deleterious actions of oxidants progressive to neoplastic conditions, are affronted by first line defense antioxidants such as SOD, CAT and GPx[28]. A decreased status of such antioxidant enzymes were observed in group II fibrosarcoma bearing rats in the present study. *B. monniera* treated group IV fibrosarcoma bearing animals had a significant rise in these enzyme activities. *B. monniera* has been identified to exhibit significant antioxidative and antilipid peroxidative property [6]. This may be the reason for the changes observed in *B. monniera* treated fibrosarcoma bearing rats (Group IV). Superoxide inhibitory activity in polymorpho nuclear cells is reported to be promoted by the extract of *B. monniera*[29]. Antioxidant and protective effect of various saponins have been reported earlier [30,31]. *In vitro* studies on the mode of cytotoxicity

Table 2—Effect of *B.monniera* treatment on serum tumor markers in fibrosarcoma bearing rats
[Values are expressed as mean ±SD for 6 animals in each group]

Treatment	LDH[1]	CK[2]	ALT[3]	AST[3]
Control	279.56 ± 31.60	74.10 ± 5.99	35.31 ± 2.59	13.09 ± 1.09
Fibrosarcoma	380.81 ± 32.6 [a]	71.08 ± 14.88 [a]	46.69 ± 2.03[a]	31.79 ± 3.1 [a]
B.monniera (20 mg/kg: sc)	287.09 ± 31.89	81.80 ± 5.61	37.89 ± 4.21	14.08 ± 1.75
Fibrosarcoma *B.monniera* (20 mg/kg: sc)	315.31 ± 25.90 [b]	61.80 ± 5.61[b]	39.93 ± 4.20[b]	17.31 ± 2.03 [b]
F-value	13.57	41	5.32	17.41

[1]: IU/L; [2]: μ mole of phosphorus liberated/ sec/ mg protein ; [3]: n mole of phosphorous liberated /sec /mg protein.
[a]$P<0.05$ when compared with the control
[b]$P<0.05$ when compared with the fibrosarcoma bearing rats (FB)

explain that saponins do not cause oxidation-mediated cytotoxicity[32]. Hence in the present study, the enhanced antioxidant status observed in the *B. monniera* treated fibrosarcoma bearing rats could be attributed to the antioxidant nature of *B. monniera* and its components.

LDH, a cytosolic enzyme is involved in biochemical regulation reactions of the body tissues and fluids. An elevation of LDH in the serum has been observed in ovarian cancer[33] and other malignant conditions[22] and is due to the changes in the membrane permeability and leakage of soluble enzymes. In the present study, *B. monniera* treated fibrosarcoma bearing rats were found to have significantly reduced activity of LDH when compared to the untreated fibrosarcoma bearing group II animals. Serum sialic acid is elevated in accordance with the tumor size and stage[34]. The increased level of sialic acid in turn facilitates uncontrolled cellular proliferation and loss of contact inhibition[35]. Sialic acid levels in the present study was significantly increased in the fibrosarcoma bearing animals, whereas the *B. monniera* treated fibrosarcoma bearing animals of group IV exhibited a profound decrease. Steroidal saponins and triterpenoid saponins have been recognized for their anticarcinogenic property[36,37]. Saponins are also renowned for various activities like membrane permeabilisation and immunostimulation[38]. Bacoside-A, a steroidal saponin[33], and other dammarene triterpenoid and triterpenoid saponins[39,40,41], the phytoconstituents of *B. monniera*, may exhibit such membrane permeabilising and immuno stimulating activities and possibly altered the status of LDH and SA as observed in the *B. monniera* treated fibrosarcoma rats. Creatine kinase activity was reported to be increased 10 fold in lung carcinoma cell lines and in progressive states of cancer[42]. Increased breakdown of ATP which in turn is due to the increased energy requirements of the tumor bearing animals is the contributive cause. Saponins can bind specifically to tumor cells and can initiate non specific cell aggregation[43] and hence can exhibit potent cytotoxic action[44] on tumor cells. This may reduce the tumor burden by direct cytotoxicity and inhibit tumor progression by enhancing contact inhibition, attributing for the changes observed in the levels of SA and the activities of other tumor markers like CK, ALT and AST. Increased serum ALT and AST has been observed in fibrosarcoma bearing rats[45]. These levels were decreased in the *B. monniera* treated fibrosarcoma bearing rats of Group IV. The presence of alkaloids and saponins and their combined anticarcinogenic effects could also be a possible factor that can explain the tumor inhibitory activity of *B. monniera* as observed in the present study.

From the data presented, it can be concluded that *B. monniera* extract promoted the antioxidant status thereby reducing the rate of lipid peroxidation and lowering the markers of tumor progression in the *B. monniera* treated fibrosarcoma bearing rats.

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