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Anti-tumor activity of aqueous extract of *Biophytum sensitivum* Linn

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ABSTRACT

The present study was investigating experimentally the possible antitumor effects of aqueous extract of Biophytum sensitivum Linn (AEBS) leaves against Dalton's Ascitic Lymphoma (DAL) bearing Swiss albino mice. The AEBS administered orally at the doses of 100 and 200 mg/kg b. wt, in mice for 28 days after 24 h of tumor inoculation. The effects AEBS on the growth of murine tumor, life span of DAL bearing mice were studied. Treatment with AEBS decreased the tumor volume and viable cell count there by increasing the life span of DAL bearing mice. The present work indicates that the aqueous extract of B. sensitivum exhibited significant antitumor activity.

Key words: *B. sensitivum* Linn, Dalton's Ascitic Lymphoma, Aqueous extract.

INTRODUCTION

Since medieval times, plants have been the source of medicines for the treatment of diseases. Regardless of the availability of a wealth of synthetic drugs, plants remain even in the 21st century an integral part of the health care in different countries, especially the developing ones. In the late 90's the WHO stated that a big percentage of the world's population depends on plant based therapies to cover the needs of the primary health care [1]. Moreover, towards the end of the 20th century, plant based OTC products, nutroceuticals and food supplements comprising the complementary and alternative therapies have gained a big share in the drug market in the developed countries. Medicinal plants either through systematic screening programs or by serendipity - possess an important position in the drug discovery and many modern drugs have their origin in traditional medicine of different cultures. Hence, despite the advantages of the synthetic and combinatorial chemistry as well as molecular modeling, medicinal plants remain an important source of new drugs, new drug leads and new chemical entities [2]. The latter study reported that of the 877 small molecule new chemical entities (NCEs) introduced between 1981

and 2002 nearly the half (49%) were natural products, semi-synthetic natural products, semi-synthetic natural products analogues or synthetic compounds based on natural products.

The areas of cancer and infectious diseases have a leading position in utilization of medicinal plants as a source of drug discovery. Among FDA approved anticancer and anti-infectious preparations drugs of natural origin have a share of 60% and 75% respectively [3]. It is worthy to mention the vivid current interest in discovery of natural drugs for cancer treatment and chemoprevention [4, 5]. Huge number of plant species is screened and bioassayed for this purpose worldwide [6], 10 million; half of these are in developed countries [7]. Among the cancer patients in the USA the use of complementary and alternative medicine represented mainly by plants ranges between 30-75% [6]. This in turn justifies the interest in search of possible anticancer agents from the flora of different countries. In accordance with this worldwide trend, the current study was undertaken to screen the ethanolic extracts of 67 plant species found in the Jordanian flora or sold by the local herbalist shops. Among the screened plants there are only few plants recommended by the traditional healers for the treatment of cancer (i.e *Arum palestinum*) while some tested plants are belonging to the genera with reported anticancer activities (i.e. *Salvia dominica*). In many countries, cancer is the second leading cause of death after heart diseases [8]. The estimated worldwide incidence of different carcinomas is about 10 million; half of these are in developed countries. Among the cancer patients in the USA the use of complementary and alternative medicine represented mainly, by plants ranges between 30-75%. This in turn justifies the interest in search of possible anticancer agents from the flora of different countries.

The plant *B. sensitivum* belongs to family oxalidaceae and the stems are erect, from 2.5-25 cm long, short or slender or glabrous or hairy. Leaves are sensitive, crowded into a rosette on the top of the stem 3.8-7.5 cm long. Flowers dimorphic, 8 mm across, yellow; peduncles many of various lengths up to 10 cm long. Sepals are lanceolate, acute with parallel nerves. Corolla much exceeding the sepals, lobes rounded. Style nearly glabrous [9].

The plant *B. sensitivum* have been reported various pharmacological activities including protective effect on radiation-induced damage in mice [10], immunomodulatory activity [11], inhibition of tumor specific angiogenesis [12], effect on cell mediated immune response in mice [13], chemoprotective effect [14], anti-angiogenic effect [15], antioxidant potential [16], anti-inflammatory activity [17] and alters the cytokine profile and inhibits iNOS and COX-2 expression in LPS/Con A stimulated macrophages [18]. The present study was carried out to evaluate the antitumor activity of aqueous extract of *B. sensitivum* L against DAL bearing mice.

MATERIAL AND METHODS

Plant material

Leaves of *B. sensitivum* was collected from Maruthamalai, Coimbatore, Tamil Nadu, India and authenticated by Dr P. Jayaraman, Plant anatomy Research centre, Chennai, Tamil Nadu, India. Voucher specimens (BSL/074/08) were deposited at our college Museum for future reference.

Preparation of the extract

The powdered material (1000g) of leaves of *B. sensitivum* was extracted separately using aqueous by cold maceration. The extract was dried under reduced pressure and it was stored in desiccators for further studies.

Preliminary phytochemical screening
The aqueous extract was subjected to preliminary screening for various active phytochemical constituents [19].

Animals
Wistar Albino mice of either sex, weighing 20-22g, were purchased from M/S Venkateshwara Enterprises (P) Ltd, Bangalore, India and housed under standard environmental conditions (temperature: 24 ± 1^0 C, light/ dark cycle: 12 h). The mice were fed with standard Pellet diet (Amrut (P) Ltd, Bangalore) and water *ad libitum*.

Toxicity studies
Healthy mice of either sex, starved over night, were divided into 3 groups (in each group 6 animals) and were orally fed with aqueous extract of *B. sensitivum* in escalating dose levels 100, 200 and 300 mg/kg body weight, respectively. The rats were observed continuously for 2 h for behavioral, neurological and autonomic changes and after a period of 24 and 72 h for any death.

Treatment
Tumor was induced by injecting 0.2 ml of $2X10^6$ cell ml^{-6} of Delton's Ascitic Lymphoma (DAL) in to peritoneal cavity of mice. The animals were divided in to 5 groups (n= 12). All the groups were injected with DAL cells ($2X10^6$ cells/mouse) intraperitonealy except normal group. This was taken a s day 0. On the first day normal saline (0.9 % w/v^{-1}, Nacl, 5ml/kg/day/mouse) administered into normal (group 1). DAL mice were received only vehicle (propylene glycol 5 ml/kg/day/mouse) as group 2. The different doses of the aqueous extract of *B. sensitivum* (100 and 200 mg/kg/day/mouse) and standard drug Vincristin (0.8 mg/kg^{-1}) were subsequently administered in group 3, 4 and 5, respectively for 14 days intraperitonealy. On the 15^{th} day, after the last dose and 18 h fasting 6 mice from each group were sacrificed for the study of antitumor activity and hematological estimation and rest of the animal of each group were kept to check the Mean Survival Time (MST) and percentage increase in the life span (%ILS) of the tumor bearing mice [20].

Tumor growth response
Antitumor effect of aqueous extract of *B. sensitivum* was assessed by observation of changes with respect to body weight, Ascetic's tumor volume, packed cell count. MST and %ILS were also calculated. Transplantable murrain tumor was carefully collected with the help of a sterile 3 ml syringe and measured the tumor volume and the ascetic fluid was with draw in a graduated centrifuge tube and packed cell volume was determined by centrifuged tube at 1000 rpm for 5 min, viable and nonviable cell count of ascetic cell were stained by the trypan blue (0.4% in normal saline) dye exclusion test and count was determined in Neubarer counting chamber. The effect of aqueous extract of *B. sensitivum* on tumor growth was monitored daily by recording the mortality and % ILS was calculated using following formula

$$\text{ILS (\%)} = \frac{\text{Mean survival of treated group}}{\text{Mean survival of control group}} \times 100$$

Statistic analysis
Total variation present in set of data was performed by using one way Analysis of Variance (ANOVA) and the results are expressed as mean ± SEM.

Table 1. Effect of aqueous extract of *B. sensitivum* on tumor volume, packed cell volume and viable and non-viable tumor cell of DAL bearing mice

Parameters	Body weight (g)	Tumor volume (ml)	Packed cell volume	Viable tumor count 10^7cells ml^{-1}	Non-viable tumor cells count X 10^7cells ml^{-1}
DAL control ($2X10^6$ cell/mouse/ml)	30.26±2.28	5.48±0.10	2.32±0.08	14.28±2.28	0.48±0.18
AEBS 100 (mg/kg)+ DAL	24.66±2.46	1.26±0.08	1.92±0.064	7.16±0.68	0.86±0.22
AEBS 200 (mg/kg)+ DAL	26.22±2.84	2.42±0.06	0.86±0.08	3.24±0.24	1.26±0.44
Vincristin (0.08mg/kg^{-1}) + DAL	28.24±4.24	1.14±0.10	0.72±0.04	2.22±0.26	1.68±0.66

Values are means ±SEM Number of mice in each group (n=6). Experimental group was compared with DAL control, (Weight of normal mice 20±0.22)

Table 2: Effect of aqueous extract of *B. sensitivum* on survival time on DAL bearing mice

Experiment	Mean survival (days)	% Increase in life span (%ILS)
Normal control Saline 5 ml/kg b.wt	-	-
DAL Control ($2X10^6$ cells+ propylene glycol (5ml/kg b.wt)	24.42±0.36	-
AEBS 100 mg/kg)+ DAL Control ($2X10^6$ cells)	28.84±0.26	29.23
AEBS 200 mg/kg)+ DAL Control ($2X10^6$ cells)	34.10±0.50	60.92
Vincristin (0.08mg/kg^{-1}+ DAL Control ($2X10^6$ cells)	36.26±2.26	62.94

Values are means ±SEM Number of mice in each group (n=6). Experimental group compared with control.

RESULTS AND DISCUSSION

The AEBS showed significant anti-tumor activity in DAL bearing mice. The effect of AEBS (100 and 200 mg/kg) at different doses on tumor volume, viable and nonviable cell count, survival time and ILS were shown in table 1 and 2.

The AEBS were showed significant antitumor activity against the transplantable murine tumor. The reliable criteria for judging the value of any anticancer drugs are the prolongation of life span of animals. A reduction in the number of ascetic tumor cells may indicate either an effect of AEBS on peritoneal macrophages or other components of the immune system [21] therefore increase their capacity of killing the tumor cells, or a direct effect on the tumor cell growth. AEBS inhibited significantly the tumor volume, viable cell count and enhancement in survival time of DAL bearing mice and thereby acts as anti-neoplasticagents [22].

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