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ANTICANCER EFFECT OF *ABUTILON INDICUM* (LINN) SWEET. AGAINST EHRLICH ASCITES CARCINOMA

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ABSTRACT

The effect of ethanolic and aqueous extracts of the leaves of *Abutilon indicum* (Linn.) Sweet. against Ehrlich Ascites Carcinoma (EAC) has been evaluated in Swiss albino mice. A significant increase in the life span and a decrease in the cancer cell number and tumour weight were noted in the tumour-induced mice after treatment with extracts. The hematological parameters were also corrected by the extracts in tumour-induced mice. These observations are suggestive of the protective effect of *Abutilon indicum* in EAC.

KEYWORDS: *Abutilon indicum*; Ehrlich Ascites Carcinoma; Anticancer.

INTRODUCTION

Cancer is one of the ailments which cannot be completely subdued by chemotherapy. The chemotherapeutic agents though effective against various types of tumour are not totally free from side effects. Historically, plants were a folkloric source of medicinal agents, and as modern medicine developed, numerous useful drugs were developed from lead compounds discovered from medicinal plants. Today, this strategy remains an essential route to new pharmaceuticals. Since 1961, nine plant-derived compounds have been approved for use as anticancer drugs in US: vinblastine, vincristine, navelbine, etoposide, teniposide, taxol, taxotere, topotecan and irinotecan.[1] This fostered our attempts to evaluate some plant products against cancer, as they are less likely to cause serious side effects. Many Indian spices[2] and plants[3] are quoted to be useful in different types of cancer.

Abutilon indicum (Linn.) Sweet., belonging to the family Malvaceae, found throughout tropical India and Ceylon. Root, bark, seeds, fruits and leaves are used in many diseases traditionally. Its hepatoprotective[4,5], hypoglycemic[6], analgesic[7] and antiestrogenic[8] activities have also been reported. It is used by practitioners of traditional systems of medicine against colon cancer. In order to evaluate the possible anticancer activity, a study against EAC was planned.

MATERIALS AND METHODS

Plant material

The leaves of *Abutilon indicum* (Linn) Sweet. were collected from the foothill of Yercaud, Salem District, Tamilnadu in the month of April and authenticated by a botanist and a voucher specimen (AIK-1) has been kept in our museum for future reference. The plant parts were shade dried at room temperature for 10 d and coarsely powdered and passed through sieve No. 60.

Preparation of extract

The powder of leaves of *Abutilon indicum* was extracted separately by continuous hot extraction process using soxhlet apparatus with different solvents in increasing order of polarity from petroleum ether, chloroform, acetone, alcohol, to finally chloroform:water.[9] After extraction, the extracts were concentrated under reduced pressure in tared vessel. The marc of crude drug powder was then once again subjected to successive extraction with other solvents and the extractive values were calculated with reference to the air-dried drug. The dried extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

Animals

Swiss albino mice (20-25 g), obtained from Perundurai Medical College, Perundurai, were used for the study. They were housed in polypropylene cages in controlled temperature (27±2^{o} C), humidity (55±10%) and fed with standard chow diet and water *ad libitum*. The animals were exposed to an alternate cycle of 12 h of darkness and light each. They were given a week time to get acclimatized with laboratory condition. Before test, the animals were fasted for atleast 12 h. The experimental protocols were subjected to the scrutinization of the IAEC and were cleared by the same.

Cells

EAC cells were obtained through the courtesy of Amala Cancer Institute, Thrissur, Kerala (originally brought from Prof. G. Klein, Stockholm, Sweden) and given by intraperitoneal transplantation of 10^6 cells/mouse.

Determination of antitumour activity

The animals acclimatized to our laboratory conditions were divided into six groups viz. G1, G2, G3, G4, G5 and G6 of six each and used for the study[10]. The EAC cells were injected intraperitoneally (10^6 cells/mouse) to all the mice of the six groups. On the second day the animals of G2 and G3 were treated with 500 and 1000 mg/kg, i.p.,[6] of ethanolic extract of *Abutilon indicum* (EAI), respectively. G4 and G5 were treated with 500 and 1000 mg/kg, i.p.,[6] of aqueous extract of *Abutilon indicum* (AAI), respectively, while the mice of G6 were treated with 20 mg/kg of 5-fluorouracil[11] and the treatment was continued for the next 10 days. G1 was not allocated any treatment after inoculation with EAC cells. The mice were observed for the next 11 days for the development of carcinoma. On day 11, the following parameters were estimated.

1. Cancer cell number
2. Packed cell volume (PCV)
3. Decrease in tumour weight of the mice
4. Increase in life span (ILS)

Determination of hematological parameters

Apart from the above mentioned parameters, the effects of EAI and AAI on hematological parameters were also studied in the mice of all the groups. Blood was collected from the mice of all the groups by puncturing retro-orbital plexus and counted for RBC and WBC. For comparison a control group designated as G7 was used which was neither inoculated with cancer cells nor treated with the extracts.

Statistical analysis

The results are expressed as mean ±SEM. The evaluation of the data was done using one way ANOVA followed by Newman–Keul's multiple range test; differences below $P < 0.05$ implied significance.

RESULTS

On preliminary phytochemical screening of EAI and AAI revealed the presence of flavonoids, glycosides and our findings are in agree with the findings of Matlawska and Sikorska[12]. Effects of EAI and AAI on the EAC induced mice are shown in Table 1. Both the extracts were reduced the cancer cell number to a significant level at both the doses. But protection against EAC was more pronounced at higher dose level of extract, i.e. at 1000 mg/kg and indicates the dose dependent activity of the extracts. The ethanolic extract at a dose of 1000 mg/kg, i.p., showed a maximum activity, which reduced the cancer cell number to $0.90\pm0.08x10^6$ in the treated mice. Following inoculation with EAC cells, there was profound proliferation of tumour cells in the peritoneal cavity of the mice. As a result the PCV in the tumour control mice was found to be high (38%). Intraperitoneal administration of the EAI had reduced the PCV to 25 and 21% for the dose of 500 and 1000 mg/kg, respectively. The AAI had reduced the PCV to 28 and 23 % for the dose of 500 and 1000 mg/kg, respectively. Also a decrease in tumour weight was noted in the EAI and AAI treated mice. The EAI treated mice survived up to 36 days and AAI treated mice survived up to 34 days, whereas the tumour control mice survived up to 21 days only. Hence the percentage increases in lifespan (ILS) of the EAI-treated mice increased up to 71.42%.

Regarding the effect of EAI and AAI on the hematological parameters (Table 2), it was found that the tumour bearing mice showed reduced number of RBC but an increase in WBC compared to normal control mice. Following treatment with EAI and AAI, the RBC count was significantly increased and WBC count was significantly reduced to normal. The hemoglobin, lymphocytes and monocytes were found to be increased with a significant reduction of protein and neutrophil count. The EAI and AAI treatment could change these altered parameters to near normal and the hematological parameters were almost restored to its normal in the animals treated with the standard drug 5-Fu.

DISCUSSION

Cancer is a group of more than 100 different diseases characterized by uncontrolled cellular growth, local tissue invasion and distant metastases[13] and the free radicals have been implicated in carcinogenesis[14]. Supportive to this, many plant extracts containing antioxidant principles have been reported to possess antitumour activity[15]. Hence plants containing flavonoids, glycosides, etc., are constantly being screened for antitumour activity. Some of the active principles present in this plant are reported to be antioxidant. Hence this plant was chosen to study the antitumour activity against EAC.

Intraperitoneal inoculation of EAC cells in the mice produced an enormous increase in the cancer cell count which indicated that there is progression of cancer in the animals. The decrease in the cancer cell number observed in the EAI and AAI treated group of mice (G2-G5) indicates that the test drug is having significant inhibitory effect on the tumour cell proliferation. The increase in tumour weight of test groups may be due to accumulation of peritoneal fluid as an abnormal enlargement of peritoneal cavity was observed in tumour-induced mice. Treatment with EAI and AAI reduced the tumour weight and hence increased the life span. From the hematological studies it was understood that the significant rise in WBC in G1, might be a defensive mechanism against cancer cells. As the progression of cancer was brought under control by EAI and AAI, the WBC count got reduced in test groups.

These observations on the effect of EAI and AAI on various parameters studied to evaluate the antitumour activity enabled us to conclude that it possesses antitumour activity. The extract treated mice restored the hematological parameters to almost its normal and proved its dose dependent activity. However further investigations are essential for the isolation of the active principle of EAI, AAI and it's mechanism of action.

TABLE 1: Effects of EAI and AAI on EAC induced mice

Groups	Treatment	Dose (mg/kg, i.p.,)	Cancer cell number ($\times 10^6$)	Packed cell volume (%)	Increase in tumour weight (g)	Number of days survived	Increase in life span (%)
G1	Positive control	-	1.35±0.10	38±0.50	7.54±0.35	21±0.58	-
G2	EAI	500	0.82±0.06[b]	25±0.31[a]	5.25±0.18 [b]	32±0.26[a]	52.38
G3	EAI	1000	0.90±0.08[a]	21±0.40[a]	4.01±0.19[b]	36.16±0.31[a]	71.42
G4	AAI	500	0.94±0.08[a]	28±0.40[a]	5.65±0.16 [b]	30±0.30[a]	42.86
G5	AAI	1000	1.05±0.10[a]	23±0.43[a]	4.55±0.16 [b]	34±0.26[a]	61.90
G6	5-Fu	20	0.70±0.06[b]	18±0.31[a]	2.98±0.02	41.5±0.76[a]	97.62

Newman–Keul's multiple range test is used.

a and b Significantly different from G1 at $P < 0.05$ and $P < 0.01$, $n = 6$.

TABLE 2: Effects of EAI and AAI on hematological parameters

Groups	Treatment/ Dose (mg/kg)	Hb (g/dl)	RBC (10^6/mm^3)	WBC (10^3/mm^3)	Protein (g %)	Neutrophils %	Lymphocytes %	Monocytes %
G1	Positive control	8.3±0.08[a]	2.6±0.09[a]	22.4±0.1[a]	14.1±0.04[a]	68±0.58[a]	29±0.87[a]	1±0.0[b]
G2	EAI- 500	10.9±0.06[a]	3.0±0.05[b]	11.3±0.04[a]	10.2±0.04[a]	44±0.10[a]	43±0.26[a]	1±0.1[a]
G3	EAI-1000	12.8±0.04[a]	3.9±0.04[a]	8.8±0.06[a]	9.0±0.05[a]	38±0.60[a]	59±0.31[a]	2±0.16[a]
G4	AAI-500	9.8±0.05[a]	3.2±0.04[b]	12.5±0.09[a]	10.8±0.06[a]	49±0.10[a]	40±0.45[a]	1±0.4[b]
G5	AAI-1000	11.3±0.08[a]	3.6±0.06[a]	9.5±0.09[a]	9.4±0.04[a]	41±0.58[a]	57±0.60[a]	1±0.81[b]
G6	5-Fu-20	13.9±0.06[a]	4.2±0.06[a]	8.3±0.09[a]	8.56±0.06[a]	33±0.76[a]	63±0.43[a]	3±0.21[a]
G7	Normal	14.7±0.08	4.8±0.05	7.5±0.1	8.4±0.03	30±0.58	68±0.43	2±0.0

Newman–Keul's multiple range test is used.

a and b Significantly different from G1 at $P < 0.001$ and $P < 0.01$, $n = 6$.

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