



# Journal of Medical Sciences

ISSN 1682-4474

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# Research Paper

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**JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publish original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued six times per year on paper and in electronic format.**

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J. Med. Sci., 2 (4): 202-205  
July-August, 2002

## ***Aristolochia indica* Whole Plant Extract as an Antineoplastic Agent**

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The research work was conducted to determine the antitumor effect of *Aristolochia indica* (whole plant extract) against Ehrlich ascites carcinoma (EAC) in mice. Significant ( $P < 0.001$ ) increases of survival times ( $45 \pm 2$  days) of the *A. indica* extract ( $50 \text{ mg kg}^{-1}$ ) treated tumor bearing mice were confirmed with respect to the control group ( $23 \pm 1.45$  days). *A. indica* extract ( $50 \text{ mg kg}^{-1}$ ) inhibited the tumor cell growth (94.12 %) and reversed the changes of hematological parameters consequent to tumor inoculation i.e. hemoglobin,  $9.2 \pm 0.26$  vs  $7.5 \pm 0.3 \text{ gm dl}^{-1}$ ; red blood cells,  $7.1 \pm 0.21$  vs  $2.44 \pm 0.2$  ( $\text{cells ml}^{-1}) \times 10^9$ ; white blood cells,  $11 \pm 0.17$  vs  $30 \pm 0.49$  ( $\text{cells ml}^{-1}) \times 10^6$ ; lymphocyte,  $61.6 \pm 1.3$  vs  $38.5 \pm 2.1$  %; neutrophil,  $30 \pm 3.08$  vs  $55.2 \pm 1.75$  %; monocyte,  $4.5 \pm 0.5$  vs  $3.56 \pm 0.47$  %, for treated and control (EAC bearing) mice respectively.

**Key words:** *A. indica*, Ehrlich ascites carcinoma, antitumor activity

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## Introduction

*Aristolochia indica* is a native of India and Eastward. This plant is a remedy for intermittent fever, dropsy and loss of appetite (Lily, 1960). Root extract of *A. indica* possesses antitumor activity. It inhibits the growth of human CA-755 and Hela cells. It is effective against adenocarcinoma (Klein, 1962). Root extract of *A. indica* contains phenanthrene derivatives aristolochine alkaline, isoaristolochic acid, allatoin etc. (Pakrashi *et al.*, 1977), which possess therapeutic values.

All of these findings prompted to look for the medicinal uses of *A. indica* plant as a whole. In this research paper, report for the first time, the antitumor effect of *A. indica* against Ehrlich ascites carcinoma. In addition effect of *A. indica* on hematological and immunomodulatory parameters (normal peritoneal cells) have been observed and cytotoxic effect of *A. indica* has also been evaluated.

## Materials and Methods

**Plant materials:** The research work was conducted in the Laboratory of Biochemistry Department, Rajshahi University, Bangladesh during July 1998 – April 2000. *A. indica* plant was collected from Natore District, Bangladesh and whole plants were sun dried, pulverized, then stored as a powder in a polythene bag.

**Extraction and preparation of the test sample:** *A. indica* (powdered, 500 gm) was extracted in a soxhlet apparatus consequently with petroleum ether (C<sub>2</sub>H<sub>5</sub>-O-C<sub>2</sub>H<sub>5</sub>) methanol (CH<sub>3</sub>OH) and finally chloroform (CHCl<sub>3</sub>) (using rotatory evaporator) a blackish brown material was obtained (2.5% w/w), which was kept in a desiccator. For experiment 0.2 gm of test sample was dissolved in 100 ml distilled water containing 0.2 % dimethyl sulfoxide (DMSO) to make a stock solution. Suitable dilutions were made from the stock solution before animal experimentation.

**Animals:** Swiss Albino male mice of 6-8 weeks of age, weighing 20-25 gm were collected from International Center for Diarrhoeal Disease Research Bangladesh (ICDDR'B). Animals were fed with standard mouse pellet collected from ICDDR'B and water was given ad libitum.

**Experimental tumor model:** Ehrlich ascites carcinoma cells were obtained from Indian Institute of Chemical Biology (IICB), Calcutta 700032, West Bengal, India and were maintained in our laboratory by weekly intraperitoneal (i.p.) transplantation.

**Tumor cell growth inhibition:** *In vivo* tumor cell growth inhibition (Sur *et al.*, 1997) were carried out with chloroform extract and standard drug bleomycin. Animals (6 in each group) were inoculated (i.p.) with 2 × 10<sup>6</sup> cells mouse<sup>-1</sup>. Treatment with chloroform extract (crude) at dose of 50, 20, 10 mg kg<sup>-1</sup> i.p. and bleomycin (Pure, 0.3 mg kg<sup>-1</sup> i.p.) started after 24 hrs of inoculation. The control group was treated with 0.2 % DMSO. All the treatments were continued for 4 consecutive days. Animals were sacrificed on day 5 after transplantation and tumor cells were collected by repeated intraperitoneal wash with 0.9 % saline. Viable tumor cell counts were made with a hemocytometer. Total number of viable cells per animal of the treated group was compared with those of control group.

**Survival time:** Survival time of EAC bearing mice treated with the test sample and bleomycin were carried out under similar experimental conditions as stated in previous experiment for 10 days using the dose for chloroform extract 50, 20 and 10 mg kg<sup>-1</sup> and for bleomycin 0.3 mg kg<sup>-1</sup> i.p. Mean survival time (MST) for each group containing 6 mice was noted. Survival time of treated groups were compared with those of control group (c) with the following calculation (Sur *et al.*, 1984):

$$\text{Percent increase of life span over control} = \frac{\text{MST of treated group}}{\text{MST of control group}} \times 100-100$$

Where

$$\text{MST} = \frac{\sum \text{Survival time (days) of each mouse in a group}}{\text{Total no. of mice}}$$

$$\text{Life span with respect to control (T/C \%)} = \frac{\text{MST of treated group}}{\text{MST of control group}} \times 100$$

**Brine shrimp lethality bioassay:** Twelve vials were taken (two vials for each concentration) for this study. Five ml of sea water was given to each of the vials. 5, 10, 20, 40 and 80 µg ml<sup>-1</sup> solutions of chloroform extract were transferred to 10 vials and 2 vials were used as control. With the help of pasteur pipette 10 living shrimps were inoculated into each of the vials. After 24 hr., vials were observed and number of survival noplui in each vial was counted (Attaur and Thomsen, 1999 and Meye, 1982).

**Effect of *A. indica* on normal peritoneal cells:** Effect of chloroform extract of *A. indica* on normal peritoneal cells was observed by the procedure as described by Sur and Ganguly (1994). Two groups of normal mice (n=4) were used. The first group was treated with chloroform extract (50 mg kg<sup>-1</sup> i.p.) for three consecutive days. The untreated second group was used as control. Total peritoneal exudate cells and number of macrophages (with 0.1% neutral red) were counted 24 hours after treatment and compared with the untreated control.

**Hematological studies:** In order to detect the influence of chloroform extract (50 mg kg<sup>-1</sup>, i.p.) on hematological status of normal and EAC bearing mice, comparison was made among four groups (n= 4) of mice on the 12th day, after tumor transplantation. The four groups comprised:

- normal mice served as control
- normal mice treated with chloroform extract (50 mg kg<sup>-1</sup> i.p.)
- EAC bearing mice
- EAC bearing mice treated with chloroform extract (50 mg kg<sup>-1</sup> i.p.)

Treatment was continued for 10 consecutive days (Khanam *et al.*, 1997).

Blood was collected from individual mice for experiment. Total counts of white blood cells (WBC) and red blood cells (RBC) as well as the hemoglobin (Hb) content were determined by standard methods (Rusia, 1998) using cell diluting fluids and a hemocytometer. The differential count was carried out with Wright stain.

Data were statistically analyzed by using students "t" test and significance was assigned at p<0.05 (Ghosh, 1984).

## Results

**Tumor cell growth inhibition:** Treatment with chloroform extract (crude, 50 mg kg<sup>-1</sup>.i.p.) resulted in a excellent tumor cell growth inhibition (P<0.001), as evident from 94.12 % reduction of tumor cells, which was found to be 94.44 % for bleomycin (pure, 0.3 mg kg<sup>-1</sup> i.p.) treatment (Table 1). Significant (P<0.001) cell growth inhibition was also observed with *A. indica* extract at dose 20 and 10 mg kg<sup>-1</sup> which were 87.3 and 69.5 % respectively.

**Survival time:** The mean survival time (MST) for the control group was found to be 23 days, while it was 45 days for the group treated with *A. indica* extract (50 mg kg<sup>-1</sup> 10 days), T/C % was

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Table 1: Effect of chloroform extract of *A. indica* on EAC cell growth in mice (*in vivo*)

Treatments	Doses mg kg <sup>-1</sup>	No. of tumor cell 10 <sup>7</sup> / mouse on day 5 after tumor inoculation	% of cell growth inhibition
Control (vehicle)	-	5.33 ± 1.33	-
<i>Aristolochia indica</i> (6)	50	0.34 ± 0.24**	94.12
<i>Aristolochia indica</i> (6)	20	0.74 ± 0.13**	87.3
<i>Aristolochia indica</i> (6)	10	1.779 ± 0.40**	69.5
Bleomycin	0.3	0.312 ± 0.037**	94.44

Values represent mean ± SEM No. of mice per group given in parenthesis \*\*; P < 0.001

Table 2: Survival of EAC bearing mice following *Aristolochia indica* treatment

Treatment (n = 6)	Dose (mg kg <sup>-1</sup> day <sup>-1</sup> i.p.)	MST(days)	T/C%	Increase in life span over control (%)
Control (vehicle)	-	23 ± 1.45	100	-
<i>Aristolochia indica</i>	50	45 ± 2**	195	95
<i>Aristolochia indica</i>	20	34 ± 1.76*	147	47
<i>Aristolochia indica</i>	10	26 ± 2	113	13
Bleomycin	0.3	45 ± 1.76**	195	95

\*\*; P < 0.001, \*; P < 0.05 EAC: Ehrlich ascites carcinoma

Table 3: Effect of *A. indica* treatment on normal peritoneal cells in mice

Treatments	Dose/day (mg kg <sup>-1</sup> )	Macrophage × 10 <sup>6</sup> Mean ± SEM	Total peritoneal cells × 10 <sup>6</sup>
Control (Normal)	-	2.53 ± 0.29	20.2 ± 3.16
Normal + <i>A. indica</i>	20	2.7 ± 0.28	20.5 ± 3.21
	50	3.2 ± 0.31	20.75 ± 3.49

Table 4: Effect of *A. indica* (20 and 50 mg kg<sup>-1</sup>.p. 10 days) treatment on hematological parameters in normal and tumor bearing mice

Treatments (mg kg <sup>-1</sup> i.p)	Hb (gm dl <sup>-1</sup> )	RBC × 10 <sup>9</sup> ml <sup>-1</sup>	WBC × 10 <sup>9</sup> ml <sup>-1</sup>	Lymphocyte (%)	Neutrophil (%)	Monocyte (%)
Normal mice (vehicle) control	12.0 ± 0.3	8.3 ± 0.57	5.6 ± 0.12	68.0 ± 1.0	24.75 ± 1.54	5.5 ± 0.5
EAC bearing mice	7.5 ± 0.3**	2.44 ± 0.2**	30.0 ± 0.49**	38.5 ± 2.1**	55.2 ± 1.75**	3.56 ± 0.47
EAC + <i>A. indica</i> 50	9.2 ± 0.26**	7.1 ± 0.21*	11.0 ± 0.17**	61.6 ± 1.3*	30.0 ± 3.08	4.5 ± 0.50
EAC + <i>A. indica</i> 20	8.3 ± 0.2*	3.5 ± 0.3**	25.0 ± 0.34**	48.3 ± 2.3**	44.6 ± 1.8**	3.66 ± 0.40
Normal + <i>A. indica</i> 50	9.4 ± 0.5*	5.96 ± 0.08*	5.0 ± 1.15	74.0 ± 2.4	17.12 ± 1.66*	3.5 ± 0.5
Normal + <i>A. indica</i> 20	10.6 ± 0.3*	6.2 ± 0.17*	5.3 ± 0.8	70.2 ± 1.72	20.56 ± 2	4 ± 0.51

Hematological parameters were studied on day 12 after tumor transplantation \*\*; P < 0.001, \*; P < 0.05-0.01

WBR: White blood cells, RBC: Red blood cells, HB: Hemoglobin

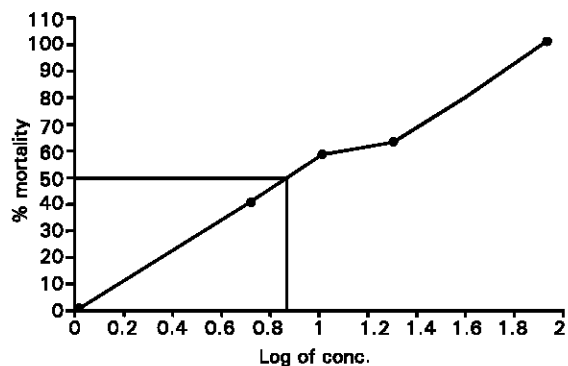


Fig. 1: Effect of *Aristolochia indica* extract on brine shrimp mortality. Conc. = Concentration of chloroform extract *Aristolochia indica*

195 (Table 2). At doses 20 and 10 mg kg<sup>-1</sup> 10 days, the *A. indica* extract showed the MST 34 and 26 days and T/C % was 147 and 113, respectively, bleomycin at dose 0.3 mg kg<sup>-1</sup> 10 days treatment resulted in MST 45 and T/C % was 195.

### Effect of *A. indica* on normal peritoneal cells and macrophages:

The average number of peritoneal exudate cells per normal mouse was found to be 20.2 ± 3.16 × 10<sup>6</sup> of which the macrophage count was 2.53 ± 0.29 × 10<sup>6</sup>. Treatments with *A. indica* extract (50 mg kg<sup>-1</sup> i.p.) for three consecutive days did not alter the peritoneal cells but increased the number of macrophages to some extent (Table 3).

**Hematological parameters:** Along with the growth of tumor, the hematological parameters were found to be altered from normal

values (Table 4). Hemoglobin content of RBC was decreased (7.5 ± 0.3 gm dl<sup>-1</sup>) from normal values (12.0 ± 0.3 gm dl<sup>-1</sup>) and WBC count was increased (30 ± 0.49 cells ml<sup>-1</sup> × 10<sup>6</sup>) from normal values (5.6 ± 0.12 cells ml<sup>-1</sup> × 10<sup>6</sup>). Lymphocyte and neutrophil counts were also significantly changed. All of these depleted parameters were altered towards normal values after treatment with *A. indica* extract at dose of 50 and 25 mg kg<sup>-1</sup> (Table 4). In case of normal mice, *A. indica* at dose 50 mg kg<sup>-1</sup> decreased Hb (9.2 ± 0.26 gm dl<sup>-1</sup>), RBC (7.1 ± 0.21 cells ml<sup>-1</sup> × 10<sup>9</sup>) and WBC (11 ± 0.17 cells ml<sup>-1</sup> × 10<sup>6</sup>) counts, but no effect was observed on blood parameters at dose 20 mg kg<sup>-1</sup> (Table 4).

**Brine shrimp lethality:** To find out the effect of *A. indica* extract on the mortality of brine shrimp noplil median lethal concentration of brine shrimp (LC<sub>50</sub>) was measured and it was found to be 7.24 µg ml<sup>-1</sup>. The plot of percent mortality versus log of chloroform extract concentration showed an approximate linear correlation between them (Fig. 1).

### Discussion

The results showed the antitumor effect of *A. indica* extract against Ehrlich ascites carcinoma in Swiss Albino mice. A significant enhancement of MST was found which was dose depended (Table 2). The antitumor effect of chloroform extract (50 mg kg<sup>-1</sup>) was found to be effective like bleomycin (0.3 mg kg<sup>-1</sup>) (Table 1). Observations of inhibition of *in vivo* tumor cell growth after *A. indica* extract treatment appeared to be correlated with the findings of enhancement of survival time by *A. indica* extract with respect to control.

To evaluate whether *A. indica* extract indirectly inhibited tumor cell growth, the effect of *A. indica* extract was examined on the peritoneal exudate cells of normal mice. But no enhancement in number of macrophages was observed in the total peritoneal cell (Table 3). But *A. indica* possess cytotoxic effect revealed from the linear correlation between percent mortality of brine shrimp noplil

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and the concentration of *A. indica* extract (Fig. 1). Cytotoxic property of *A. indica* may be the causes of killing of tumor cells. At low dose *A. indica* showed no toxic effect on hematological parameters in normal mice (Table 4).

All of these results suggest to carry out further extensive work on antitumor activity of *A. indica* which may bring important information in the treatment of cancer.

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MS received 29th May, 2002; accepted 20th July, 2002