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Anti-inflammatory and Antitumour effects of Ethanolic Extract of Himalayan Cobra Lily- *Arisaema speciosum.*

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Abstract
Arisaema speciosum is a wild lily of family Araceae. This paper reports anti-inflammatory properties of the ethanolic extract of the corm of *Arisaema speciosum* .The ethanolic extract of corm of *Arisaema speciosum* showed *in vitro* cytotoxic effect against Ehrlich ascitic cells. When the extract was injected in tumour bearing mice, tumour disappeared in 40% animals. Injection of ethanolic extract also curbed the growth of the tumours and increased the survivality of the animals compared to control animals.

Key words: *Arisaema speciosum,* anti-inflammatory, antitumour, disappearance of tumour, curb in growth of tumour.

INTRODUCTION

In modern medicine the major modes of treatment of malignancy is by surgery followed by chemotherapy or radiotherapy[1]. Use of chemopreventive agents in the early stage of carcinogenesis is theoretically more rational than attempting to eradicate fully developed tumours with chemotherapeutic drugs[2]. These agents have a narrow margin of safety, and the therapy may fail due to drug resistance and dose-limiting toxicities which may severely affect the host normal cells[3]. Hence the search and use of natural products especially from plants for the control of cancer has widened the quest for the search for treatment of malignancy[4].

The Himalayas have always been a paradise of wild flowers and plants which have been used for centuries as folklore medicine for the treatment of human ailments[5,6] *Arisaema speciosum*(Wall.) commoly known as showy cobra-lily(Plate1), inhabits cool mountain ranges of the Himalayas and is found growing at 2000-3000m. It is also found growing in and around Darjeeling hills. The main distinguishing feature of the plant is its corm which is rhizomatous, annulated, and cylindrical with length over 15cm and diameter 4-10cm (Plate 2). The single leaf is three-segmented. The spathe is chocolate-purple, striped and drawn into a tail-like apex[7,8,9]. It was identified by the Professors of the Botany Department of St. Joseph's College, Darjeeling. In folklore medicine it is used for the cure of benign warts; the effect of the plant extract on restriction of the growth and multiplication of cells in warts suggests that the plant may have some potential against malignancy.

Plate 1
Arisaema speciosum in bloom.

Plate 2
Corm of *Arisaema speciosum.*

Inflammation is a fundamental protective response which can be harmful in conditions such as life-threatening reactions to insect bites, toxins and in chronic diseases such as rheumatic arthritis ,atherosclerosis, lung fibrosis and cancer [(10)].Inflammation can also accelerate cancer and chronic inflammation is regarded as an essential factor for the progression of neoplastic process[(11)].

The anti-inflammatory effect of ethanolic extract of the corm of *Arisaema speciosum* was carried out using 2% formalin for induction of inflammation. Inflammatory experiments were carried out using Diclofenac as the reference drug. The *in vitro* effects of alcoholic extract of *Arisaema* corm on Ehrlich ascitic tumour cells was carried out and its efficacy against growth of tumour in Swiss mice has been reported.

MATERIALS AND METHODS:

Mice: Six to eight week old Swiss (albino) mice were used.

Ascitic cell line:

Ehrlich's lymphoma cell line obtained from Centre or Life Sciences, North Bengal University was maintained by serial passage in mice by intra peritoneal injections of $1x10^6$ fibrosarcoma cells per animal every 15 days.

All animal experiments were carried out according to the guidelines of the Animal Ethics Committee.

Solid tumor induction:

For solid tumor induction of $2x10^6$ ascitic fibrosarcoma cells were injected subcutaneously per animal at the left hand side of the abdomen of the left thigh. Mice with 14 days of tumor growth after induction having palpable tumor growth were taken as tumour-bearing mice[(12)] .

***Arisaema* plant corm extract preparation:**

For the preparation of *Arisaema* extract, corms of the plant were collected and washed with water to wash away dirt. After soaking away excess water, 10gms of the cut corm was taken and crushed to a paste in a mortar and pestel. 15ml of ethanol were added and kept in a refrigerator at 4^0C for 12 hrs. The extract was then filtered through Whatman filter paper no. 1; the filtrate was then filtered through Millipore filter paper and the final solution obtained was stored at 4^0C for further use [(13)] .

Anti-inflammatory experiment

For evaluation of antiinflammatory property animals were divided into 3 groups of eight mice each (Group A,B and C). Group A mice were used as control and each animal of Group A received distilled water (3ml/Kg ip) only. Twenty minutes before induction of inflammation Group B test mice received ethanolic *Arisaema* extract 2.5-10ml/Kg wt i.p.. Group C animals received Diclofenac as reference drug 100mg/Kg i.p.

Induction of inflammation

20µl freshly prepared 2% formalin was used as the oedemataogenic agent. It was injected on left hind paw of mice[(14)].

Calculation of % inflammation (oedema)

Percent inhibition were calculated as follows;

Increase in paw thickness in control and experimental animals $Pc=Pt-Po$,$P_T=P_t-P_o$ respectively.

$$\% \text{ inhibition} = \frac{P_c - P_T}{P_c} \times 100$$

Where P_t is paw thickness at time t

P_o is initial paw thickness

P_c is increase in paw thickness of control animals

P_T is the increase in paw thickness of the treatment animals [(15)]

Pedal inflammation was evident 5-8 minutes after formalin injection. The paw thickness was measured using vernier calipers before and after formalin treatment. The thickness of paw was recorded at 30,60,90,120,150 and180 minutes after formalin injection.

Lymphocyte preparation:

The spleen of normal Swiss albino mice was aseptically removed and the cells were dissociated in Phosphate Buffered Saline (PBS) (pH 7.0-7.2) with the help of a stainless steel wire mesh. Erythrocytes were lysed by treatment with Tris buffered NH_4Cl (0.84 % pH 7.2)[(16)]. The cells were then suspended in RPMI 1640 supplemented with 25mM HEPES, penicillin, streptomycin and 10% heat inactivated sterile goat serum [(17)] .

***In vitro* viability assay:**

Splenic lymphocytes and ascitic fibrosarcoma cells were transferred to culture plates at a density of $1x10^6$ cells in 0.2 ml medium. 5µl, 10µl, 20µl and 25µl doses of ethanolic *Arisaema* corm extract was added to the culture plates and incubated at 37^0C in 5% CO_2 for various hours. Cell survival studies were performed by Trypan blue dye exclusion test. As *Arisaema* extract was made in ethyl alcohol, an equivalent amount of ethanol were added to ascitic cells and incubated for same hours as control. The total number of viable cells were counted in a Haemocytometer.

***In vivo* experiments:**

To ten mice bearing palpable tumors, 25µl of

Arisaema extract was injected intravenously 5 times after every 5 days. The disappearance or non disappearance of tumour was noted. The significance of the datas were calculated. Separately, for control experiments, 10 animals with palpable tumour were taken and injected intravenously with PBS for 5 times after every five days. The survival of these animals were separately noted.

All the tests used to generate the significant P values mentioned in the paper were calculated following the Student's t test [18,19]

RESULTS AND DISCUSSION

After induction of inflammation, paw thickness increased steadily in all experimental and control animals. Paw thickness increased upto 90 mins of induction and then decreased steadily in treatment animals. Within 180 mins, paw thickness came back to normal size in diclofenac treated animals. In animals treated with 50µl *Arisaema* extract showed better anti-inflammatory property than 25 µl *Arisaema* extract (Table I)

Percent inhibition was highest with 70% inhibition with 50 µl *Arisaema* extract at 180 minutes after induction of inflammation as compared to reference drug Diclofenac which was 94.44% inhibition at 180 mins after induction (TableII) Lower dose of 25µl *Arisaema* extract showed 42% inhibition at 180 mins after induction.

The anti-inflammatory property of *Arisaema* is evident but not as strong as Diclofenac. However, some mice could not tolerate higher dose of intraperitoneal injections of *Arisaema* extract thus further works using concentrated strengths of *Arisaema* extract in ethanol tolerable to animals needs to be carried out.

Diclofenac is a non-steriodal anti-inflammatory drug(NSAID). Diclofenac has been reported to suppress inflammation induced by various phlogistic agents in experimental animal models. diclofenac is commonly employed in the treatment or management of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis and for its anti-inflammatory and analgesic effects. Diclofenac reduces inflammation, swelling and arthritic pain by inhibiting prostaglandin synthesis and/or production. The drug also affects polymorphonuclear leukocyte functions *in vitro*, thereby reducing chemotaxis, superoxide toxic radical formation, oxygen-derived free radical generation and neutral protease production[20].

TABLE I: Effect of ethanolic *Arisaema speciosum* plant extract and Diclofenac on left paw oedema induced by formalin.

Treatment	***Time (mins) and paw thickness (mm)***					
	30 mins	***60 mins***	***90 mins***	***120 mins***	***150 mins***	***180 mins***
Control (PBS)	0.36±0.08	0.41±0.02	0.48±0.07	0.47±0.03	0.46±0.01	0.45±0.05
25µl *Arisaema Extract*	0.35±0.06	0.40±0.05	0.42±0.02	0.41±0.08	0.39±0.01	0.37±0.02
50µl *Arisaema* Extract	0.33±0.1	0.35±0.01	0.38±0.03	0.37±0.01	0.35±0.15*	0.32±0.45*
Diclofenac	0.30±0.07	0.31±0.056	0.31±0.067*	0.30±0.02*	0.29±0.14*	0.28±0.10*

All values are mean ± SD (n=8); * p<0.001 with respect to control

Note: Original thickness of paw of 1and 2 treatment animals were .25mm and of 3and 4 treatment animals were 0 .26 mm

TABLE II .Percent inhibition of formalin- induced inflammation by ethanolic *Arisaema speciosum* plant extract.

Treatment	***Percentage Inhibition***					
	30 mins	***60 mins***	***90 mins***	***120 mins***	***150 mins***	***180 mins***
Control (PBS)	0	0	0	0	0	0
25µl *Arisaema Extract*	10.00	24.00	27.00	28.60	39.00	42.00
50µl *Arisaema* Extract	36.40	43.80	47.80	50.00	57.00	70.00
Diclofenac	66.67	71.43	80.95	85.00	89.47	94.44

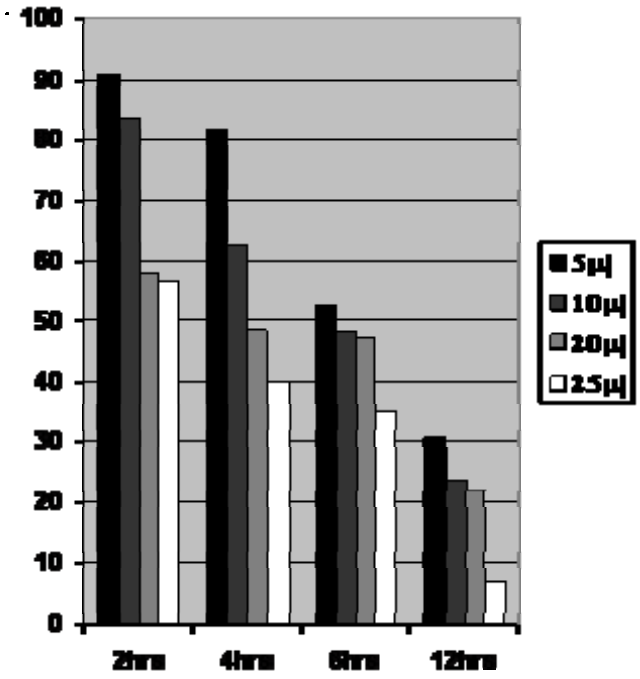

Figure 1-Diagram showing the comparative survival of ascitic cells at different hours of ethanolic *Arisaema* extract treatment . Amount of ethanolic plant extract used is shown in the box.

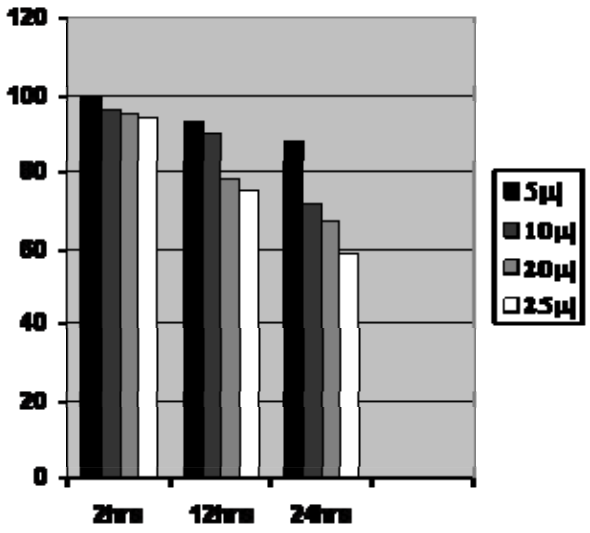

Figure 2.- Diagram showing the comparative survival of ascitic cells at different hours in ethanol *in vitro.* Amount of ethanol used is shown in the box.

The high degree of cytotoxicity of ascitic cells(Plate 3) by higher dose of *Arisaema* ethanolic extract indicates that the plant has some antitumour properties.

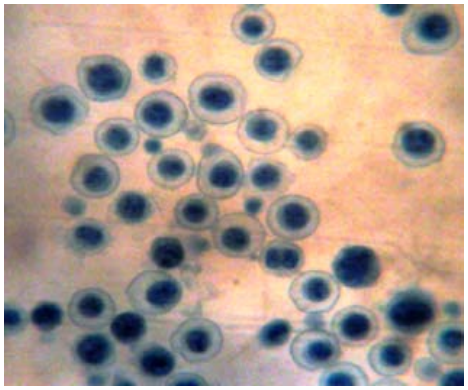

Plate 3. Plate showing Trypan blue dye exclusion test for the ascitic fibrosarcoma cells at 12hrs of *in vitro* treatment with 25µl *Arisaema speciosum* plant extract .Dead ascitic cells have taken up blue colour. (magnification x1600)

Though the activity of *Arisaema* extract *in vitro* is very hopeful, it became necessary to find the effect of *Arisaema* extract *in vivo*.

When mice with palpable tumours were injected with 25µl injection of ethanolic *Arisaema* extract intravenously, tumour disappeared in 40% tumour bearing animals (Fig 3). In 60% cases where the tumour did not disappear, the rate of tumour growth was recorded and it was seen that there was a curb in growth of rate of tumour in animals injected with ethanolic *Arisaema* extract than in control animals (Fig4) .The mice in control animals all died within 5 weeks whereas mice which had been treated with 25µl ethanolic *Arisaema* extract survived 9-10 weeks.

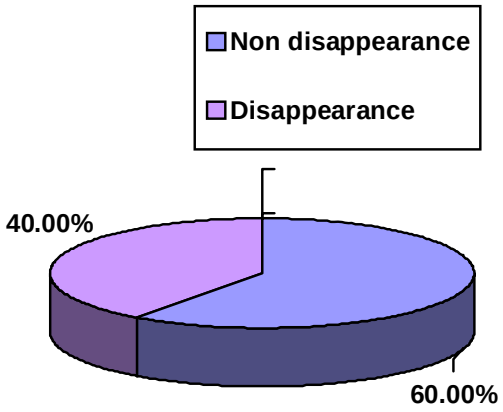

Figure 3. **Percentage of tumour disappearance**

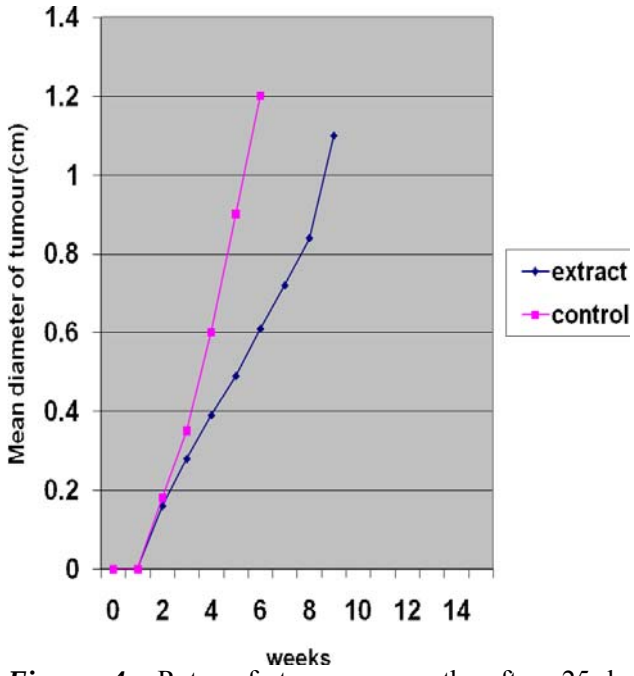

Figure 4 -Rate of tumour growth after 25µl injections of ethanolic *Arisaema* plant extract intravenously in mice for 5 times after each week.

Though some work in plant extraction and identification and synthesis of the various components of the plant is being carried out [(21)], the exact component that brings about this antitumour activity could be isolated and its effect against tumours could be established. This would be a cost effective and a potent herbal medicine for the treatment and cure for malignancy.

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