Research Article

THE EFFECTS OF ALCOHOLIC *HEDERA HELIX* PLANT EXTRACT ON EHRLICH ASCITIC TUMOUR CELLS *IN VITRO* AND ITS ANTITUMOUR ACTIVITIES *IN VIVO*.

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ABSTRACT

The ethanol extract of *Hedera helix* has been seen to be cytotoxic to splenic lymphocytes due to the presence of saponins whose effect is reduced by addition of serum to the medium. The serum proteins bind to the saponins and reduces its cytotoxic effect. The alcoholic *Hedera* extract shows cytotoxic effect against Ehrlich ascitic cells. When the extract was injected in tumour bearing mice, tumour disappeared in 83.3% animals. Further treatment by alcoholic *Hedera* extract on the 16.7% cases of tumour bearing animals where the tumour did not disappear, the plant extract curbed the growth of the tumours and increased the survivality of the animals compared to control animals.

Keywords: *Hedera helix*; goat serum; disappearance of tumour; curb in growth of tumour.

INTRODUCTION

Hedera helix Linn. is a common ivy of family Araliaceae found growing in the Darjeeling hills. It was identified by the Professors of the Botany Department of St. Joseph's College, Darjeeling. It is an evergreen woody climber scaling the walls and covering the walls with a thick canopy of leaves. It is also grown as an ornamental plant. In folklore medicine of the Darjeeling Hills, 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. Some workers have reported the anti-inflammatory and antioxidant properties of *Hedera*[1-4], its antispasmodic properties[5] and its allergic effect after contact[6]. The effect of dry extracts on respiratory functions of children with chronic bronchial asthma has been carried out[7,8]. Some antitumour activities of the *Hedera* plant extract has also been reported[9-13]. The plant is reported to contain saponins in its leaves and stems[14-16]. The saponins have been reported to be cytotoxic to cells[17]. This paper also reports findings of the alcoholic extract of the *Hedera* plant on splenic lymphocytes where it is seen that saponins of the plant is cytotoxic to splenic cells which decreases in the presence of serum. The *in vitro* effects of alcoholic extract of *Hedera* plant on Ehrlich ascitic tumour cells have been carried out and its efficacy against growth of tumour in Swiss mice has been reported.

MATERIALS AND METHODS

Experimental animals

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 tumour induction

For solid tumor induction, $2x10^6$ 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[18].

Hedera helix plant extract preparation

For the preparation of *Hedera* extract, leaves of the plant were collected and washed with water to wash away dirt. After soaking away excess water, 10gms of the leaves were taken and crushed to a paste in a mortar and pestle. 15ml of ethanol were added and kept in a refrigerator at 4°C 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°C for further use[19].

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). The cells were then suspended in RPMI 1640 supplemented with 25mM HEPES, penicillin, streptomycin and 10% heat inactivated sterile goat serum[20].

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 *Hedera* extract was added to the culture plates

and incubated at 37°C in 5% CO_2 for various hours. Cell survival studies were performed by Trypan blue dye exclusion test. As *Hedera* extract was made in ethyl alcohol, an equivalent amount of ethanol were added to ascitic and normal spleen cells and incubated for same hours as control. The total number of viable cells were counted in a Haemocytometer.

***In vivo* experiments:**

Ten mice bearing palpable tumors, 25µl of *Hedera* extract was injected intravenously 5 times after every 5 days. The disappearance or non disappearance of tumour was noted. In the mice where tumour failed to disappear, the growth of the tumour at different weeks was noted on the basis of mean diameter (cm) of tumour. 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 t test[21,22].

RESULTS AND DISCUSSION

For *in vitro* studies of effect of alcoholic *Hedera* extract on splenic lymphocytes, the lymphocytes were incubated in 5µl, 10µl, 20µl and 25µl of alcoholic *Hedera* extract in presence of 10% goat serum. In 5µl *Hedera* extract, 66.7% of the spleen lymphocytes were living after 24hrs incubation. However in high concentration of 20µl and 25µl alcoholic *Hedera* extract, all the lymphocytes became inviable (Fig 1). Control experiments with spleen cells incubated in alcohol only (Fig 2) showed that 61% of lymphocytes living at 24hrs incubation. This shows that alcohol in *Hedera* extract is not the factor that led to cell death in the experiment where 10% goat serum was taken.

Then, spleen lymphocytes were incubated with an optimum level of 20% goat serum; 80.3% cells were living at 24hrs incubation in 5 µl *Hedera* extract concentration and 68.2 % lymphocytes were living in 25µl *Hedera* extract concentration at 24hrs incubation (Fig 3). Higher concentrations of alcoholic *Hedera* extract proved to be cytotoxic to the splenic lymphocytes. The percentage of splenic lymphocytes in control experiments when incubated in same concentration of ethanol showed that ethanol was not the factor that was bringing about cytotoxicity of the cells. Saponins of the plant extract must have brought about the cytoxicity of the cells. Saponins in *Hedera* extract has emulsifying properties and decrease surface tension. They tend to alter the permeability of the cell plasma membrane and exert general cytotoxicity in all organized cells [17]. The living cells in 5µl alcoholic *Hedera* extract could be because of the low amount of saponins in the extract. It was also shown by Danloy *et al* in 1994 that when BSA is added to medium containing *Hedera* extract, the saponins get bound and do not cause vacuolization of cytoplasm and do not bring about membrane alterations leading to cell death. Similarly, in the present study, increasing the serum concentration of the medium to an optimum level of 20% in which the splenic lymphocytes were incubated with alcoholic *Hedera* extract, showed a high percentage of splenic lymphocytes living. Thus, to avoid interference of saponins in the experiments where ascitic cells were incubated with alcoholic *Hedera* extract and to neutralize the saponins of the plant, 20% goat serum was used in the medium for all *in vitro* experiments.

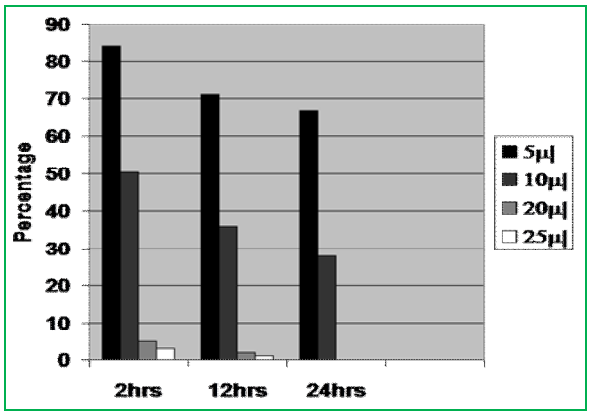

Figure 1: Diagram showing the comparative survival of splenic lymphocytes at different hours of treatment with alcoholic Hedera extract with 10 % goat serum in medium.Amount of alcoholic plant extract used is shown in the box.

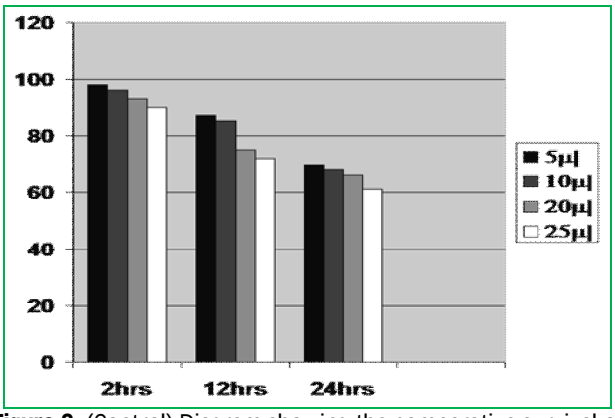

Figure 2: (Control) Diagram showing the comparative survival of splenic lymphocytes in ethanol at various hours of incubation. Amount of ethanol used is shown in the box.

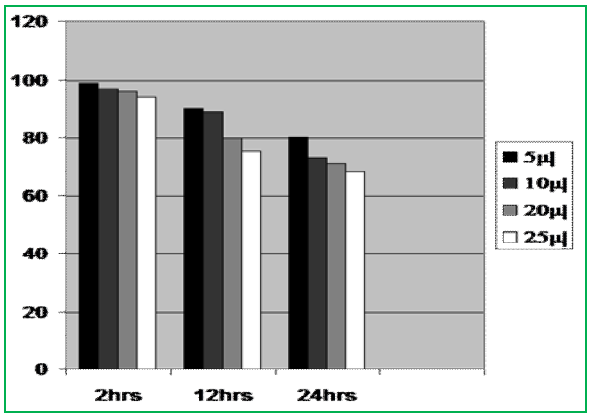

Figure 3: Diagram showing the comparative survival of splenic lymphocytes at different hours of treatment with alcoholic Hedera extract *in vitro* with double amount of serum in

medium. Amount of alcoholic plant extract used is shown in the box.

Further, when ethanolic *Hedera helix* plant extract were used in various concentrations and incubated with ascitic tumour cells, within two hours nearly all the ascitic tumour cells became inviable at 20µl and 25µl concentrations of the plant extract. (Fig. 4) as compared to the Control experiment where ascitic cells were incubated in ethanol only (Fig. 5).

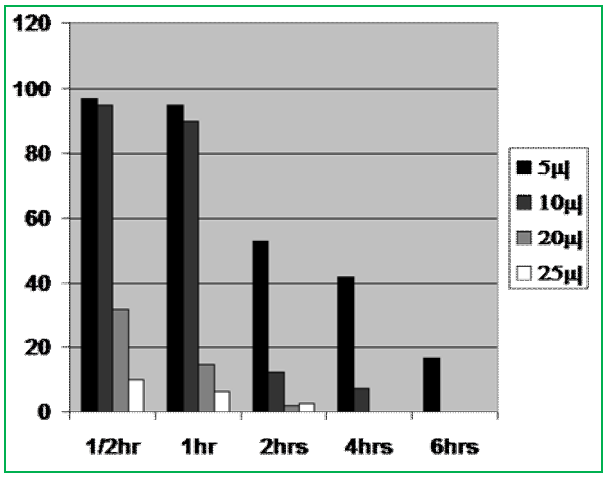

Figure 4: Diagram showing the comparative survival of ascitic cells at different hours of alcoholic Hedera extract treatment *in vitro* with double amount of serum in medium. Amount of alcoholic plant extract used is shown in the box.

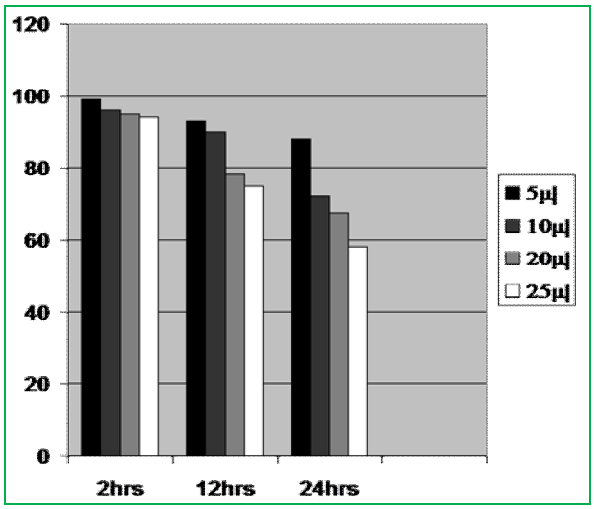

Figure 5: Diagram showing the comparative survival of ascitic cells at different hours in ethanol *in vitro*. Amount of ethanol used is shown in the box.

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

When mice with palpable tumours were injected with 25µl injection of alcoholic *Hedera* extract intravenously, tumour disappeared in 83.3% tumour bearing animals (Fig 6). In 16.7% 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 alcoholic *Hedera* extract than in control animals. (Fig7). The mice in control animals all died within 5 weeks whereas mice which had been treated with 25µl alcoholic *Hedera* extract survived till 13 weeks.

The *Hedera* extract showed antitumour activity *in vivo* because the serum of the host must have neutralized the cytotoxic effect of saponins as was seen in *in vitro* experiments.

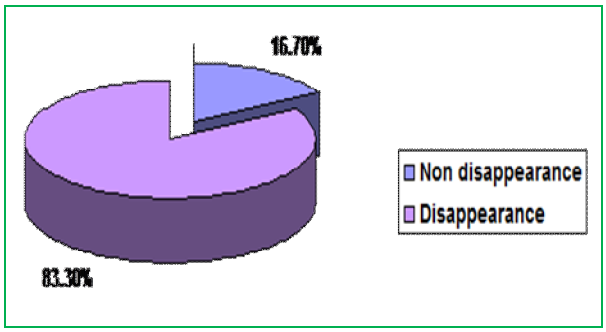

Figure 6: Percentage of tumour disappearance

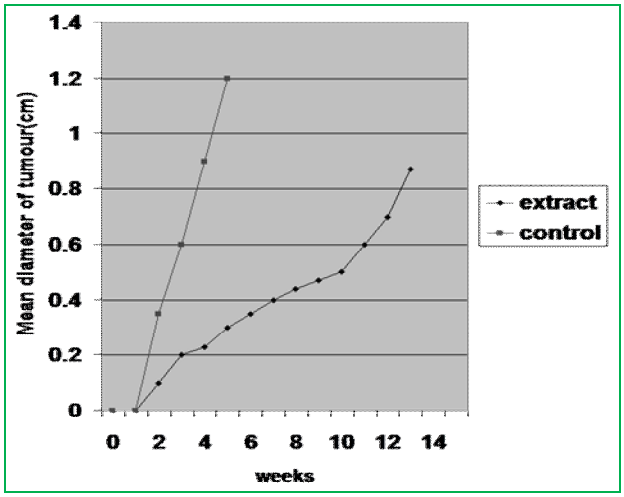

Figure 7: Rate of tumour growth after 25µl injections of alcoholic Hedera plant extract intravenously in mice for 5 times after each week. The result is statistically significant at $p<0.001$.

This shows that *Hedera* plant extract possesses a profound antitumour activity *in vitro* and *in vivo.* Though some work in plant extraction and identification of various components of the plant has been carried out[23], the cytotoxic effect of saponins as an important antitumour component is ruled out because its neutralization still leads to the death of tumour cells alone and the exact component that brings about this profound antitumour activity could be isolated and its effect established and this would be a cost effective source of a potent herbal medicine and an effective herbal cure for malignancy.

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