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

Anuradha Rai*  
Head of the Dept. of Zoology, St. Joseph’s College, P.O. North Point, Darjeeling 734104, West Bengal, India.  
*Corresponding author’s E-mail: anuradha_62@hotmail.com

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ABSTRACT

The alcoholic 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 Hedera4, its antispasmodic properties5 and its allergic effect after contact6. The effect of dry extracts on respiratory functions of children with chronic bronchial asthma has been carried out7,8. Some antitumour activities of the Hedera plant extract has also been reported9-12. The plant is reported to contain saponins in its leaves and stems13-16. The saponins have been reported to be cytotoxic to cells17. 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.

Asctic 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 tumour 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 tumour growth after induction having palpable tumor growth were taken as tumour-bearing mice18.

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 with a pestle. The mixture was then filtered through Millipore filter paper and the final solution obtained was stored at 4°C for further use19.

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 NH4Cl (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 serum20.

In vitro viability assay

Splenic lymphocytes and ascitic fibrosarcoma cells were transferred to culture plates at a density of 1x10^5 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% CO₂ 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.

**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 cytotoxicity 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. 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.](image)

![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.](image)

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. (Fig 7). 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](image)

![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.](image)

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\(^\text{[23]}\), 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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REFERENCES


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