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RESEARCH ARTICLE

ANTICANCER ACTIVITY OF HEMIDESMUS INDICUS ROOT BY DYE EXCLUSION METHOD

[1,*]Dr. Shalini, R. and [2]Dr. Rajan, S.

[1]Department of Microbiology, Imayam Arts and Science College, Thuraiyur-620 204, India
[2]Department of Microbiology, M. R. Government Arts College, Mannargudi-614 001, India

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ABSTRACT

The present article gave a variable idea for treating the cancerous disease by using the herbal drug. There was a desirable process for treating the carcinoma infection with chemotherapy and commercial modern medicine, but there was several limitations as revealed with side effects. The herbal drugs with anticancer activity formulated with *Hemidesmus indicus* root extract have been acclaimed for their therapeutic properties in the traditional system of medicine.

Key words: Anti-cancer activity, *Hemidesmus indicus* root, *Cytotoxicity* effect, Dye Exclusion method.

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INTRODUCTION

Cancer has been considered as a most threatened disease all over the world in both circumstances of developing and developed countries (Khaled Nabin Fashed, 2014). Due to cancer, it has been recorded that about 24.6 million people survive their life with is dreadful disease and 6.7 million people died every year (Ganguly, 1994). Malignant tumour and malignant neoplasm were the optional given for cancer. The word neoplasm indicates new growth of cells or tissues. To cure this fearful disease, people mind shift over to the natural green medicine due to lesser side effects. *Hemidesmus indicus* L Root, commonly called as Nannari, used to screen the anticancer activity. It belongs to the *Ascleipediaceae* family. The presence of secondary metabolites has been considered to have the curable property of Cancer. In view of the above fact, it is possible to assess the In-Vivo anticancer activity of *Hemidesmus indicus* root using standard methods.

MATERIALS AND METHODS

Plant Material

*Hemidesmus indicus*root was collected along with the aerial parts and authendicated by Dr.John Britto, the Department of Botany, Rapinet Herbarium, St.Joseph's College, Trichy-620002. The roots were allowed to dried in shade and powdered in a coarse manner using mechanical grinder.

Corresponding author: **Dr. Shalini, R.**
Department of Microbiology, Imayam Arts and Science College, Thuraiyur-620 204, India.

The ethanolicextract was prepared by dipping the root material in the respective solution for 3 days in the ratio of 1:10 (100gm of root powder in 1000ml of ethanol). The filterate was taken and allowed to condense at 50°C & refrigerate for further use (Jonathan, 2009).

Phytochemical Investigation: The alcoholic extracts of *Hemidesmus indicus* root were studied for their phytoconstituents using different phytochemical tests. (Ayoola *et al*., 2008).

Invitro Cytotoxicity Assay (Tryphan Blue Method): Short-term in-vitro cytotoxicity was assessed using Ehrlich Ascites Carcinoma cell lines by incubating different concentrations of the extracts of *Hemidesmus indicus* root at temperature for 3 hours. The tumour cells were aspirated from peritoneal cavity of tumor bearing mice using an insulin syringe and transferred to a test tube containing isotonic saline. The cells were then washed in normal saline and cell number was determined using a heamocytometer and adjusted at $10x10^6$ cells/ml. For the cytotoxicity assay, different concentrations of the extracts (100-1000µg/ml) were added to each tubes and the final volume was adjusted to one ml with normal saline. Control tubes were kept with the saline, tumor cells and without the drugs. All the tubes were incubated at 37°C for 3 hours. After incubation, 0.1ml of 0.4% tryphan blue dye in isotonic saline was added to each tube and the number of viable(unstained) and dead(stained) cells werecounted using haemocytometer (Sheeja *et al*., 1997).

$$\% \text{ Dead Cells} = \frac{\text{(Total Cells Counted-total Viable cells)}}{\text{Total Cells Counted}} \times 100$$

RESULT

Preliminary phytochemical screening of alcoholic extract answered positively for steroid, flavanoids, phenolic compounds, tannins and lignin. These secondary metabolites were considered to be one of the primary agents for using as anticancer drug (Table 1).

Table 1. Qualitative phytochemical analysis of Hemidesmus indicus root

S.No	Secondary Metabolites	HIREE
1	Steroids	-
2	Flavonoids	+
3	Phenol	+
4	Tannins	+
5	Lignin	+
6	Proteins	+
7	Carbohydrates	+
8	Aminoacids	+
9	Reducing sugars	+
10	Terpenoids	+
11	Alkaloids	+
12	Saponins	-

Cancer is the disease caused by abnormal multiplication of cells lead to the formation of tissue or other forms. This abnormal tissue growth was called under the medical term as "Tumour". This could treat with chemotherapy and may cause lots of side effects. This leads to lot of effects such as hair loss, weight loss, fatigue, abdominal pain according to the organ which it causes the cancer (Ashish *et al*., 2001). Due to these effects, plant derived products have been taken as medicine for the cancer treatment and it has the advantage of having lesser side effects and lower in cost. *In-Vitro* methods were used to assess anticancer potentials of *Hemidesmus indicus* extracts.

Cytotoxicity Effect

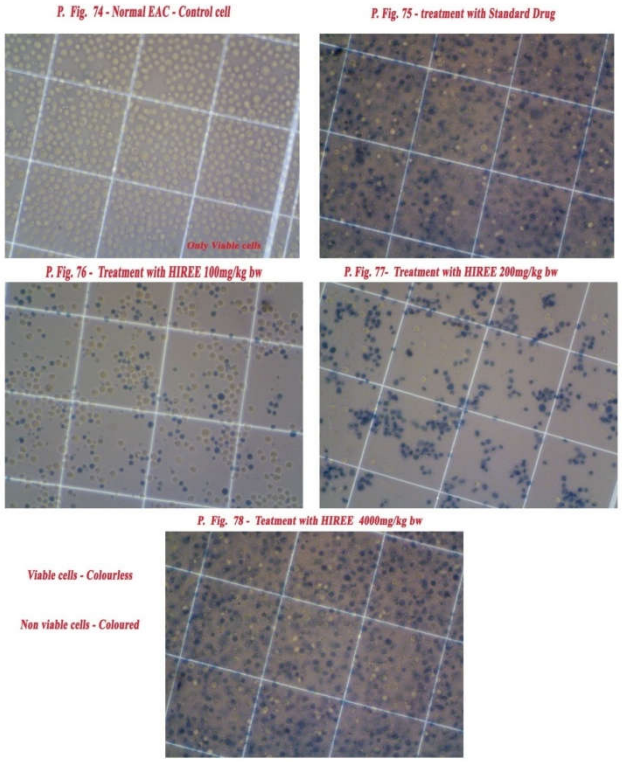
Ethanolic extracts of *Hemidesmus indicus* root were tested for their cytotoxicity effect against EAC cell lines using tryphan blue dye exclusion method. The Ascitic fluid was collected from the tumour bearing animals and the fluid was subjected to cytotoxicity assay. It is worked under the principle of dye exclusion mechanism. It describes the ability of living cells to prevent the entry of dye due to cell permeability and so the cell remain colourless. Dead cells lose its permeability and allow dye to enter inside of the cells. Hence living cells are colourless and the dead cells are blue to violet coloured; which was counted and calculated percentage of cell lysis. *Hemidesmus indicus* is rich in tannins, flavonoids, phenols, isovanillin, octadecanoic acid, dodecanoic acid etc, which may responsible for this cancer cell cytotoxic activity. Both the extracts showed the cytotoxicity activity against EAC lines (Plate XV). At 400µg of the extracts, the percentage of cytotoxicity was increased above 70% concentration (Table 2).

Table 2. Cytotoxic effect of Hemidesmus indicus against EAC cell lines (Trypan Blue Method)

Concentration (µg/ml)	No. of viable cells	No. of. Dead cells	Cytotoxicity (%)
Normal	-	-	-
Disease Control	368	290	21.19
Std.control	447	280	37.36
100 mg HIREE	886	284	67.90
200mg HIREE	708	218	69.20
400mg HIREE	766	224	70.48

Plate XV

Anticancer Assay - Viability Assay - Tryphan blue dye exclusion method



DISCUSSION

Cancer is the major burden having the duty of misguiding of cells or abnormal dividing of cells. High power of excess proliferation of cells without any relation to the normal physiological process indicated cancer. Cancer has been considered as the second largest disease causing death of the people in the world. During the 19th century, the disease severity was increased due to the unavailability of medicines. The commercial available drugs may cause lot of side effects like hair loss, loss of weight, painful effects and so on. The chemotherapy method has a drawback of impossibility of curing cancer to patients because it has the difficulty to differentiate the normal and tumour cells. By giving the chemical agent, both type of cells were damaged in the body, leads to apoptosis. So the present study was carried using of *Hemidesmus indicus* root extracts using Erhlich Ascites Carcinoma cells. The major element to keep in mind for judging the value of any anticancer drug to have the potential of increase the life span of animal and also have the capacity to reduce the leukemia cells in the blood (Jose Thomas *et al*., 2002). In this study, the induction of tumour cells in the mice leads to the decrease in the life span and increase body weight of the animal was noted. These changes were caused due to the increase in volume of Ascites which act as nutrients for the tumour cells for efficient growth. By administering the Ethanolic extract of *Hemidesmus indicus* root there is an opposite reaction to be caused such as the body weight increasement was come to the normal weight and the life span of the animal was also increased. The decrease in the viable cell count and increase in the rate of non-viable cell indicated the extracts stimulate the growth and activity of the immune cells leads to the production of interleukins. As the EAC was injected into mice first result in increased survival time indicate the delay in vascular permeability of the cells (Bhist *et al*., 2010).The standard drug 5- fluorouracil will arrest the cell

cycle and inhibit the nucleic acid synthesis and enroll about indirect cytotoxicity (Marklune *et al*., 1982).

Summary & Conclusion

Significant anticancer effect was noted with HIREE. Different effect was obtained from the same extract at 400mg/kg bw. Ethanolic extract at 400mg/kg bw clearly demonstrates the potential ability as an anticancer agent. Tryphan blue cytotoxic assay revealed that the extracts were found to be cytotoxic against EAC cell lines. *HIREE* treated group indicates its potential as an inhibitor of tumour induced intracellular oxidative stress.

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