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Original Article

Inhibitory property of aqueous extract of *Ricinus communis* leaves on proliferation of melanoma treated against A375 cell lines

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ABSTRACT

Some medicinal plants are a root of new drugs in order to hunt for the treatment of melanoma cancer whose treatment is still a big confront to the whole world. This study was intended to evaluate the anti-tumor activity of aqueous extract of *Ricinus communis* leaves on the human melanoma cancer cell lines (A375). The percentage viability of cancer cell lines was carried out by using trypan blue dye exclusion method and cytotoxicity assay was screened by applying MTT [3-(4, 5-Diamethylthiazol-2-yl)-2, 5-Diphenyl tetrazolium Bromide] assay. *Ricinus communis* aqueous extract has depicted momentous and potent cytotoxic effect on A375 cell lines with IC_{50} 48µg/ml in concentration ranging between 25µg/ml to 100µ/ml and acted as best agent to reduce the number of cancer cells to minimum. Therefore impending growth inhabiting activity of aqueous extract should be well thought-out for further phytochemical analysis and marker compound identification, so that it should be used for promoting expansion as a cancer therapeutic agent against human melanoma cancer cell lines alone or adjuvant to other chemotherapeutic drugs.

Key Words: *Ricinus communis,* antitumor activity, A375 cell line, MTT assay Trypan blue dye.

INTRODUCTION

Since last many years, plants have flattering activity in different type of diseases producing in human beings. As per WHO calculate that about 80% of the world's inhabitants which should be treated by medicinal herbal drugs for their prime health care[1]. Plants have long history used in the treatment of cancer. Active constitutes of *Catharanthus roseus, Angelica Gigas, Podophyllum peltatum, Taxus brevifolia, Podophyllum emodii, Ocrosia elliptica,* and *Campototheca acuminata* have been used in the treatment of sophisticated stages of various malignancies [2]. There are various medicinal plants reported to have anti-cancer as well as anti-inflammatory activity in the Ayurvedic system of medicine. *Ricinus comunis* are one of them with proven anti-cancer as well as anti-inflammatory activity [2] and sometimes have been proven as anti-oxidant agent [3]. The *Ricinus communis*, also known as castor oil plant is a species of flowering plant in the spurge family, Euphorbiaceae. It belongs to a monotypic genus, Ricinus, and subtribe, Ricininae. The evolution of castor and its relation to other species is currently being studied [4-5]. Its seed is the castor bean which, despite its name, is not a true bean. Castor is indigenous to the southeastern Mediterranean Basin, Eastern Africa, and India, but is widespread throughout tropical regions (and widely grown elsewhere as an ornamental plant) [5-6]. Castor seed is the source of castor oil, which has a wide variety of uses. The seeds contain between 40% and 60% oil that is rich in triglycerides, mainly ricinolein. The seed contains ricin, a toxin, which is also present in lower concentrations throughout theplant. Alcoholic extract of the leaf was hepatoprotective in rats [6-8]. Aqueous extracts of the leaves of *Ricinus communis* were used for Antimicrobial testing against pathogenic and dermatophytic bacteria and showed potent antimicrobial properties [9]. Pericarp of *Ricinus communis* showed CNS stimulant effects in mice at low doses. At lower doses, the extract Improved memor consolidation. At high does mice quickly died [10]. A water extract of the root bark showed analgesic activity in rats [11]. Antihistamine and anti inflammatory properties

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were also found in ethanolic extract of *Ricinus communis* root bark. [12]

The endeavor of the present study was to travel around the potential anti-tumor activity of *Ricinus comminus* leaves on melanoma tumor. The study was not only supportive in determining the optimum dose of ricin toxin employed against melanoma tumors but also in the development of a new and a potential anti-cancer drug. The aqueous extract of *Ricinus communis* leaves is an effective and potent antitumor agent against human malenoma cancer cell lines so it may provide a poor man friendly and a drug of preference to the world.

METERIALS AND METHODS

Methods Preparation of Plant Extract:

Accurately weighed 2Kg of *Ricinus communis* leaves were meticulously washed with tap water and kept for drying in shade at room temperature and thoroughly air dried leaves were pulverized to coarse particles (40-60 mesh) [13] weighted and stored. The coarse particles of leaves were subjected for auxiliary extraction by applying subsequent technique.

By using Maceration Technique: The coarsely powdered dried leaves of selected plant were put in a container with 1000ml petroleum ether solvent for defating and permissible to stand at room temperature for a period of at least 3 days with recurrent agitation until the soluble matter has dissolved. Similarly, the defatted leaf extract was further placed in the same container after utterly washing of container with distilled water. The leaf extract was soaked in the container with five volumes of distilled water and the pH was adjusted to 4.0 by adding dilute acetic acid. The suspension was homogenized at a maximum speed in a grinder mixture at 40C in ten sequences of 1min. each with 30 min. interval and left over night. The homogenate was then centrifuged at 8000g for 10min. Supernatant was adjusted to 60% ammonium sulphate saturation, left over night at 40C and centrifuged at 10,000g for 30min. in refrigerated centrifuge. Pellet was collected and dissolved in 0.005M sodium phosphate and exhaustively dialyzed against PBS till complete removal of ammonium sulphate. The dissolved pellet was concentrated by lyophilization and referred as crude ricin [14].

Cell counting was performed by applying trypan blue dye: Calculated the number of cells per ml, and the total number of cells, [15] using the following formula:-

%Viability = (Live Cell Count/ Total Cell Count x 100)

Micro culture tetrazolium (MTT) assay Procedure: The monolayer cell culture was trypsinized and the cell count was adjusted to 3-lakhcells/ml using medium containing 10% newborn calf serum. To each well of 96 well microtitre plates, 0.1ml of diluted cell suspension was added. After 24 hours, when the monolayer formed the supernatant was flicked off and 100 µl of different test compounds were added to the cells in microtitre plates and kept for incubation at 37ºC in 5 % CO2 incubator for 72 hour and cells were periodically checked for granularity, shrinkage, swelling. After 72 hour, the sample solution in wells was flicked off and 50µl of MTT dye was added to each well. The plates were gently shaken and incubated for 4 hours at 37oC in 5% CO2 incubator. The supernatant was removed, 50 µl of Propanol was added, and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 490 nm [16]. The percentage .growth inhibition was calculated using the formula below: The percentage growth inhibition was calculated using following formula,

$$\%\text{Cell Inhibition}=100-\{(At-Ab)/(AcAb)\}\times 100$$

Where, At= Absorbance value of test compound, Ab= Absorbance value of blank, Ac=Absorbance value of control

Percentage cell survival rate was calculated by applying formula,

$$\%\text{Cell Servival} = \{(At-Ab)/(Ac-Ab)\}\times 100$$

Where, At= Absorbance value of test compound, Ab= Absorbance value of blank, Ac = Absorbance value of control, % cell inhibition= 100-cell survival

RESULT AND DISCUSSION

Viability and Characterization of Cell Lines:

Cell lines derived from NCCS, Pune were free from any kind of bacterial and fungal contamination. *In-vitro* studies on *Ricinus communis* extract depicted significant antitumor activity against human melanoma cancer cell line (A375). Percentage of viable cell was obtained by performing trypan blue dye exclusion technique. The cytotoxicity activity was carried out by using MTT assay. To measure the cytotoxicity of aqueous extract of *Ricinus communis* leaves in human melanoma cell line (A375), A375 cells were cultured with (25, 50, 100, µg/ml) and without extract for 12hrs to 96hrs. Cell viability was evaluated by trypan blue dye exclusion method. By using trypan blue test, aqueous extract exhibited a remarkable reduction against A375 cell viability in a concentration dependent manner with $p<0.05$ Vs

control, these results are in concordant with MTT assay. Thus inhibition of cell growth by aqueous extract was more pronounced at concentration of (100ug/ml). However, the blend combination of 100µg/ml + cisplatin standard (1µg/ml) enhance cytotoxic potential. Graph 1-2 exhibits significant activity of aqueous extract of *Ricinus communis* leaves against A375 cell line. The extract showed activity against cell viability in a dose dependant manner. There was considerable decline in cell viability at given extract concentration of 100µg/ml. But when the extract was given at the concentration of 100µg/ml adjuvant with the 1µg/ml cisplatin, a synthetic antitumor drug, there was remarkable turn down in the cell viability. Graph 1-2 exhibits cytotoxic upshot of aqueous extract of *Ricinus communis* leaves against melanoma cell line (A375), when it was being incubated for 72hrs with 3 (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT). The aqueous extract showed cytotoxicity in a concentration dependant manner. IC_{50} value was experimentally calculated to be 48µg/ml. The percentage cell survival rate decreased significantly at 50µg/ml extract treatment. When the A375 melanoma cells were treated with 1µg/ml cisplatin alone, there was considerable reduce in the percentage of cell survival rate. But when the same cells were treated with 100µg/ml extract adjuvant with 1µg/ml cisplatin, there was remarkable turn down in the percentage of cell survival rate. An important fact of biomedical research is to provide a practical approach for identifying potentially useful inhibitors of tumor development from mother nature, and natural products has long been used to prevent cancer, thus act as good candidates for the development of anticancer drugs [17]. The present study evaluated the potent antitumor activity of aqueous extract of *Ricinus communis* leaves against human melanoma cancer cell lines. The toxicity of *Ricinus communis* may be due to the presence of ricin present in *Ricinus communis*. Ricin is a potent plant glycoprotein toxin. Ricin binds to cell surface carbohydrates containing N-acetyl galactosamine or β-1,4- linked galactose residues. In addition ricin contains large number of mannose residues by which it can bind to the limited number of cell lines that bear mannose receptors. Similarly Lin *et. al*. 1986 [18] observed that ricin A is a newly isolated lectin from *Ricinus communis* which has a strong inhibitory effect on the growth of tumor cells. By using cell cultures, it was demonstrated that the tumor cells were more sensitive to lectin than non-transformed cells, and that this could be caused by the higher binding affinity of lectin to tumor cells than to non-transformed cells. Alike results were contributed by Wang *et. al*. 2007 [19]; Liu *et. al*. 2006 [20]; Simpson *et. al*. 1996 [21]; Svinth *et. al*. 1998 [22]; Vago *et. al*. 2005 [23].

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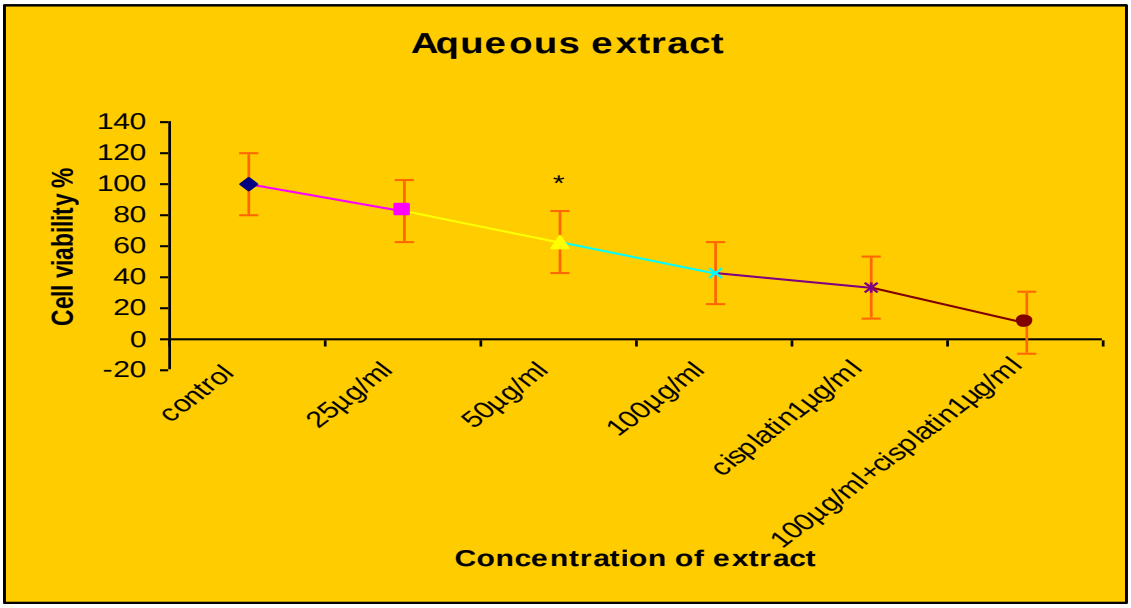
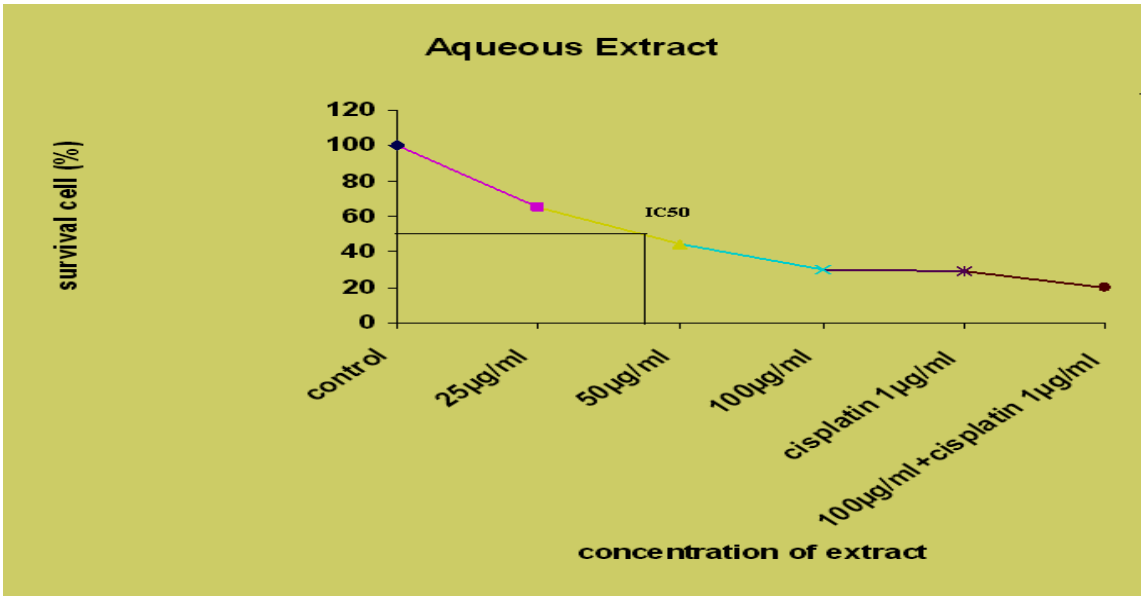


Graph 1: Showing effects of aqueous extract of *Ricinus communis L.* leaves on human melanoma cell line A375 (A dose –dependent effect of extract on cell viability, Mean±SEM, n =3, * p<0.05 vs. control)



Graph 2: Showing cyto-toxic effect of aqueous extract of *Ricinus communis* L. leaves on Human melanoma cell line A375 after 72h of incubation with MTT

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