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Anti-Cancer Activity of *Adhatoda vasica* Flowers Against Human Liver Cancer

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ABSTRACT

Adhatoda vasica Nees (Acanthaceace) is a well known medicinal plant. Its diverse medicinal activities include cardiovascular protection, abortifacient, antitubercular, antimutagenic, antiulcer, antiasthmatic activities, hepatoprotective, antibacterial and anti cancer activities. It is commonly used in original and traditional folk medicine system. In the prior times, many people used plants to cure diseases just by experimental data without knowing anything about its compounds. A number of evidence has been documented to demonstrate the probability of these plants in pharma industry. In recent years Adhatoda vasica an important medicinal plant that has been extensively used in pharmacological analysis and toxicological studies was found and these phytochemicals were considered to be greater importance in pharma industry. The present study was carried out to evaluate the anticancer activity of the compound isolated from ethyl acetate fractions of Adhatoda vasica and it was found to very good anticancer action against liver cancer. The compound isolated from ethyl acetate fraction of Adhatoda vasica flowers was tested for its anti cancer activity against liver cancer HePG2 cell line by MTT assay. The CTC50 value of sample was 228.5µg/ml against liver cancer HePG2 cell lines. Significant results were observed thereby proving the use of this plant in the traditional system of medicine.

Keywords:MTT assay, anticancer activity, Adhatoda vasica, Liver cancer HePG2, pharmacological actions etc.,

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INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. A wide range of medicinal plant extracts are used as raw drugs and they posses varied medicinal properties. Adhatoda vasica has been used for a multitude of disorders including; bronchitis, leprosy, blood disorders, heart troubles, cancer, thirst, asthma, fever, vomiting, loss of memory, leucoderma, jaundice, tumors, mouth troubles, sore-eye, fever, and gonorrhea. Over the past decade, herbal medicines have become a topic of global importance, making an impact on both world health and international trade. Medicinal plants continue to play a central role in the healthcare system of large proportions of the world's population [1]. This is particularly true in developing countries, where herbal medicine has a long and uninterrupted history of use. Recognition and development of the medicinal and economic benefits of these plants are on the increase in both developing and industrialized nations [2]. Continuous usage of herbal medicine by a large proportion of the population in the developing countries is largely due to the high cost of Western pharmaceuticals and healthcare. In addition, herbal medicines are more acceptable in these countries from their cultural and spiritual points of view [3]. Every year, millions of people are diagnosed with cancer, leading to death in a majority of the cases. According to the American Cancer Society deaths arising from cancer constitute 2–3% of the annual deaths recorded worldwide [4].

Recently, a greater emphasis has been given towards the researches on complementary and alternative medicine that deals with cancer management. Plants have long history of use in the treatment of cancer [5-7]. Several studies have been conducted on herbs under a multitude of ethnobotanical grounds [8-11]. For example, Hartwell has collected data on about 3000 plants, those of which possess anticancer properties and subsequently been used as potent anticancer drugs. Plants derived components have played an important role in the development of several clinically useful anticancer agents. These include vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, etoposide, derived from epipodophyllotoxin and paclitaxel (taxol). Several promising new agents are in clinical development based on selective activity against cancer related molecular targets, including flavopiridol and combretastin A4 phosphate, and some agents which failed in earlier clinical studies are stimulating renewed interest. Sixty percent of currently used anticancer agents are derived in one way or another from natural sources [12].

Use of plants for medicinal remedies is an integral part of the Indian cultural life, and this is unlikely to change in the years to come. Many traditional healers and herbalists in the Chhattisgarh region of India have been treating cancer patients for many years using various medicinal plant

species[13,14]. Despite the long history of cancer treatment using herbal remedies, the knowledge and experience of these herbalists have not been scientifically documented. Information on traditional herbal practice in the cancer is passed from one generation to the other through oral tradition. Considering the rapid rate of deforestation and loss of biodiversity, there is a need for accurate scientific documentation of the knowledge and experience of these herbalists. Adhatoda vasica is used in against ferric nitrilotriacetate (Fe-NTA)-induced renal oxidative stress, hyperproliferative response, and two-stage renal carcinogenesis.

The oxidation induced by reactive oxygen species (ROS) can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular disorders.[15] Reducing power is associated with antioxidant activity and may serve as significant reflection of the antioxidant activity[16]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes[17]. The present study has been undertaken to investigate the anticancer potential of the compound isolated from ethyl acetate fraction of Adhatoda vasica flowers.

MATERIALS AND METHOD

Extraction and fractionation

Fresh flowers (1kg) of Adhatoda vasica were collected at O.Koothur village, Ariyalur district, during the month of August and identified by Dr.John Britto, Director, Rabinat Herbarium and Center for Molecular Systematics, St.Joseph's College (Campus), Trichirappalli-2, Tamilnadu. India. The flowers were extracted with 90% ethanol (5x500ml). The combined alcoholic extract was concentrated in vacuo and the aqueous extract was successively fractionated with petroleum ether (60-80^{0}C) (6x250ml), Peroxide free diethyl ether (4x250ml) and ethyl acetate (8x250ml). Petroleum ether fraction and diethyl ether fraction did not yield any isolable material. Ethyl acetate fraction was taken for anti-cancer activity against human liver cancer.

MTT Assay method

MTT-Assay-Chemicals

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India.

Cell Lines and Culture Medium

HePG2 (Liver cancer cell line) cell cultures were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in Dulbecco's modified Eagle's medium (DMEM). Medium was supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of Test Solutions

For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serially two fold dilutions were made from this for carrying out cytotoxic studies.

Determination of Cell Viability by MTT Assays

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using medium containing 10% FBS and were used for the determination of cell viability by MTT assays as described by Francis and Rita (1986) respectively. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

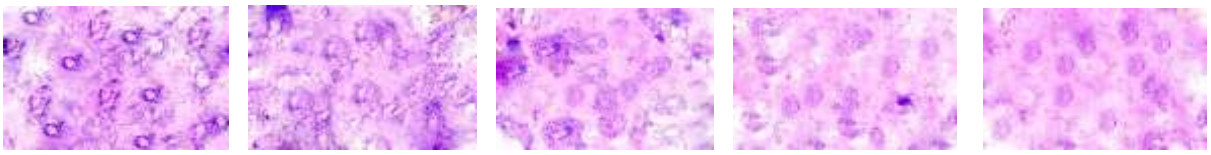
$$\% \text{ Growth inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

RESULT AND DISCUSSION

The MTT assay is based on the reduction of MTT (3-(4,5- dimethyl thiazolyl)-2,5-diphenyl-tetrazolium bromide) by mitochondrial dehydrogenase to purple formazan product. The different concentration of isolated ethyl acetate fraction of Adhatoda vasica flowers were subjected for MTT assay and results are presented in Table.1and Figure .6. The photographs (Figure. 1 to Figure. 5) show the effect of test drug on human Liver cancer HePG2 cell line.

MTT Assay method

HePG2 cell line figures:



1.62.5 µg/ml **2**.125µg/ml **3**. 250µg/ml **4**.500 µg/ml **5**. 1000 µg/ml

Figures(1-5) of the compound isolated from ethyl acetate fractions of Adhatoda vasica flowers against human Liver cancer HePG2 Cell line in different concentrations.

Table.1: The CTC50 values of the compound isolated from ethyl acetate fractions of Adhatoda vasica flowers against human Liver cancer HePG2 Cell line

S.No	Concentration of extracts (µg/ml)	% CTC_{50} Cytotoxicity (µg/ml)	CTC_{50}
1	1000	74.90	228.5 **µg/ml**
2	500	65.28	
3	250	55.17	
4	125	43.72	
5	62.5	34.01	

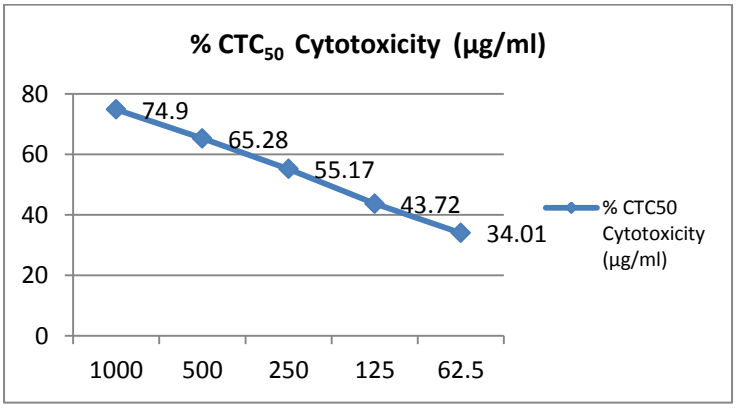


Figure.6 Graphical representation of the CTC_{50} values of the compound isolated from ethyl acetate fractions of Adhatoda vasica flowers against human Liver cancer HePG2 Cell line.

CONCLUSION

The MTT assay of isolated ethyl acetate fractions of Adhatoda vasica shows that all concentrations are having anticancer activity. The sample concentrations of 1000µg/ml, 500 µg/ml, 250µg/ml, 125µg/ml and 62.5µg/ml show 74.90µg/ml, 65.28µg/ml, 55.17µg/ml, 43.72µg/ml, 34.01µg/ml of IC_{50} value against the human Liver cancer HePG2 cell line respectively. These concentrations were able to induce apoptosis on human cancer cell lines and its anticancer activity was found to be precise. Further work is required in order to establish the identity of the chemical component responsible for anticancer activity. Studies are in progress in our laboratory to elucidate the molecular structure of that component. This contributes towards the development of valuable anticancer drug.

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