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ANTI CANCER ACTIVITY OF *DATURA STRAMONIUM* (FLOWERS) AGAINST HUMAN LIVER CANCER

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Abstract:

Our present investigation is focused on the anticancer activity of the compound isolated from the ethyl acetate fraction of flowers of Datura stramonium against human liver cancer HePG2 cell line by MTT assay using in-vitro method. The CTC_{50} value of the sample was 131.53 µg/ml against liver cancer HePG2 cell lines. Significant results were observed there by explaining the use of this plant in the traditional system of medicine.

Keywords: *MTT assay, anticancer activity, Datura stramonium, Liver cancer HePG2*

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INTRODUCTION:

Medicinal plants have various effects on living systems which include sedative, analgesic, antipyretic, cardioprotective, antibacterial, antiviral and antiprotozoal among others [1]. The specific constituents which impact medicinal values on the plants can be derived from whole or parts of the plant such as stems, leaves, fruits, flowers, seeds and roots [2]. The growing public interest and awareness in herbal medicine have led the pharmaceutical industry and biomedical researchers to give more attention on medicinal plants [3]. Cancer is one of the ailments which cannot be completely subdued by chemotherapy. The chemotherapeutic agents though effective against various types of tumor, they are not totally free from side effects [4].

Datura stramonium (family: *solanaceae*) is a wild-growing herb, known as Jimson weed. It also has several other names: thorn apple, angel's trumpet, loco weed, etc. The incidence of *D. stramonium* poisoning is sporadic with a cluster of poisoning cases occurring mostly among adolescents. Some medicinal uses of the plant are its anti-inflammatory property of all part of the plants, stimulation of the central nervous system (CNS), respiratory decongestion, treatment of dental and skin infections and also in the treatment of toothache and alopecia [5-12].

MATERIALS AND METHODS:

Collection of Flowers

Fresh flowers of *Datura stramonium* were collected from S. Pudur, Sivagangai (Dt), Tamil Nadu, India, during the month of January and identified by Dr.S.John Britto, Director, The rapinat Herbarium and Centre for Molecular Systematics (Authentication No. AR001 dated: 08/01/2016). St.Joseph's College (Campus), Trichy, Tamil Nadu, India.

Extraction and fractionation

Fresh flower (3 kg) of *Datura stramonium* collected at S. Pudur, Sivagangai (Dt), Tamil Nadu, India 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 on concentration yielded a dry powder which was dissolved in DMSO to get various concentrations and were used for further study.

MTT Assay method

HePG2 cell line figures:

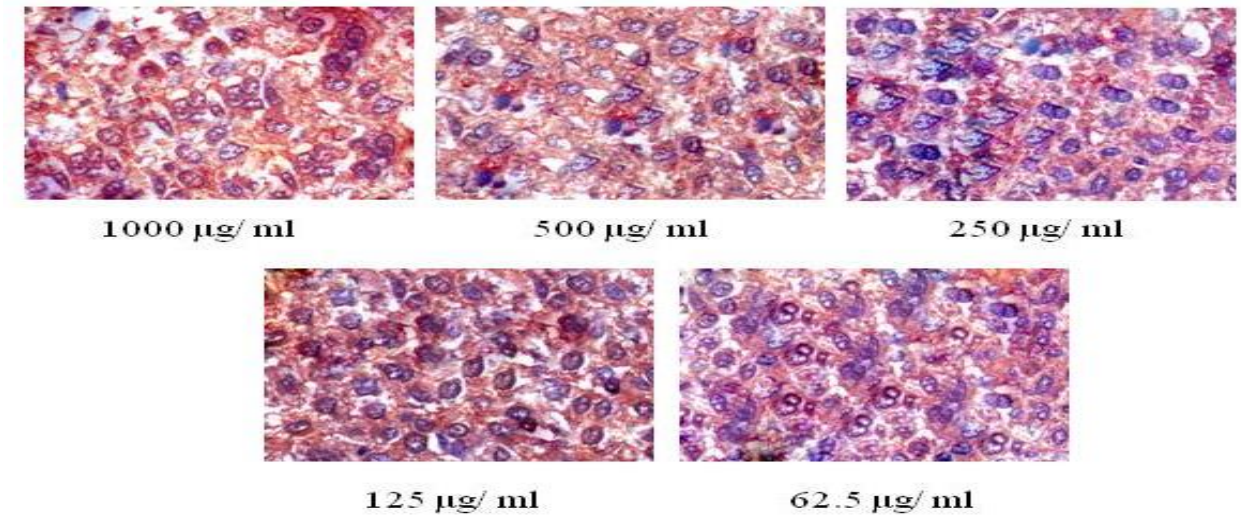
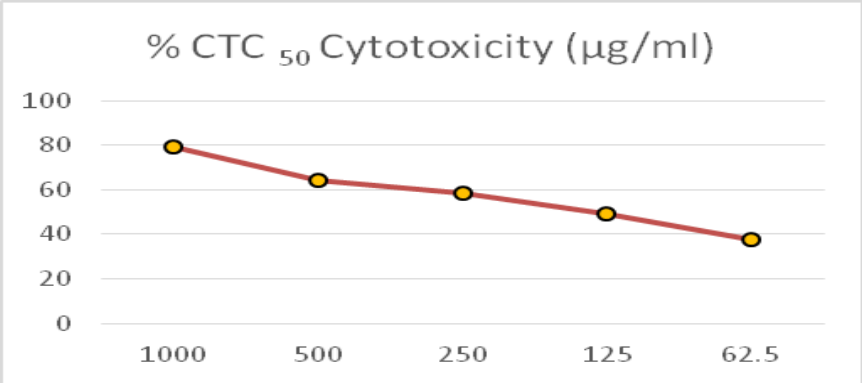


Fig: 1-5 Effect of the compound isolated from the ethyl acetate fraction of *Datura stramonium* flowers against human Liver cancer HePG2 Cell line in different concentrations

Table 1: The CTC_{50} values of the compound isolated from the ethyl acetate fraction of *Datura stramonium* flowers against human Liver cancer HePG2 Cell line

S. No	Concentration (µg/ml)	% CTC_{50} Cytotoxicity (µg/ml)	CTC_{50}
1	1000	79.43	131.53
2	500	64.15	
3	250	58.63	
4	125	49.52	
5	62.5	37.48	



Graphical representation of the CTC_{50} values of the compound isolated from the ethyl acetate fraction of *Datura stramonium* flowers against human Liver cancer HePG2 Cell line.

MTT Assay:

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 an 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 prepared 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 different concentration of the compound isolated from the ethyl acetate fraction of *Datura stramonium* flowers were subjected for MTT assay and results are presented in table.1. The photographs (Fig. 1 to Fig. 5) show the effect of the compound on the human liver cancer HePG2 cell line. The sample concentrations of 1000µg/ml, 500 µg/ml, 250µg/ml, 125µg/ml and 62.5µg/ml show 79.43 µg/ml, 64.15 µg/ml, 58.63 µg/ml, 49.52 µg/ml, 37.48 µg/ml CTC_{50} value against the human liver cancer HePG2 cell line respectively.

CONCLUSION:

The MTT assay of the compound isolated from the ethyl acetate fraction of flowers of *Datura stramonium* shows that all concentrations are having anticancer activity. So, it could be concluded that the compound to have a high anticancer potential.

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