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Research Article

A CYTOTOXIC APPROACH OF *JUSTICA GENDARUSSA* BURM.F AGAINST HUMAN CANCER CELL LINES

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ABSTRACT

India has about several thousand medicinal plants which possess notable cancer curing properties. The raw usage of these plants species have been found widely in rural herbal remedies for the treatment of cancer. Many research works have been carried out concurrently in last few decades on these plants for cancer treatment and emphasized the importance of clinical support for these plants. One of such traditionally important plant is *Gendarussa Vulgaris*. In connection with that, the present study is aimed to give a surge of interest about cancer preventing potential of the plant *G.vulgaris* by *in-vitro* cell culture. The Chloroform, ethyl acetate and ethanol extract of the plant were used to analyse the cytotoxicity against the human cancer cell lines –human liver carcinoma cancer cell line(HepG2) and cervical cancer cell line(HeLa) by MTT assay. The results obtained showed that the ethanol extract of the plant showed better cytotoxic activity against all the tested lines and more prominent against HepG2 with the IC_{50} value 19.8µg/ml. The effectiveness of the ethanol extract was confirmed by comparing the experiment results with the previously analysed datas of literature. The comparison study brought a key attention on the notable cytotoxic efficacy of the plant *G.Vulgaris* against many cancer cell lines. However an *in-vivo* clinical support and isolation of active phyto constituents are needed for the reliance of the plant in chemotherapeutic use.

Key words: Acanthaceae, MTT assay, Cytotoxicity, IC_{50} value, chemotherapeutic use.

INTRODUCTION

Cancer is the predominant cause of human mortality which comprise of 2-3% of annual death worldwide. Breast cancer is the very common form of cancer in women worldwide. Hep G2 is also one of the most frequent and lethal maliganancies**.** But the chemotherapeutic agents usually cause various side effects like digestion problems, hair loss, etc by affecting normal cell along with cancer cell[1]. They cause immune toxicity by affecting patient's immune system. Even though the natural resources provide 60% of the approved cancer drugs, their safety and efficacy are still a question mark and to be confirmed[1]. Many more plants that are having folk medicinal background for the treatment of cancer are under study and their experimental evidences are yet to be documented. One of such plant is *Gendarussa Vulgaris* for which a concrete experimental support is needed to improve its therapeutic use in cancer treatment.

Gendarussa Vulgaris Syn.*Justica gendarussa*.L.is a medicinal herb, belonging to the family Acantheceae. It is an evergreen shrub growing in India, China, Malaysia, Indonesia, SriLanka, Philippines and Bangladesh[2]. It has been utilized to treat chronic rheumatism, inflammations, bronchitis, vaginal discharges, dyspepsia, eye diseases, muscle pain, lumbago, headache, earache, injuries and fever[3-6]. The plant leaves has been found to possess anti-inflammatory activity which was confirmed *in-vivo* and i*n-vitro* studies using alcohol and aqueous extracts[7].

J.gendarussa leaves and stem extracts were reported to have antioxidant, antibacterial, anti-fungal, anti-angiogenic, anthelmentic and hepato protective activities[8-15]. The chemical constituents present in *J.Gendarussa* are flavonoids, alkaloids, triterpenoidal saponins, amino acids, aromatic amines, stigmasterol and lupeol[16-20]. The presence of two flavonoids-Kaempferol and Naringenin are also determined[11]. The methanolic extracts of *J.vulgaris* leaves and roots showed cytotoxic activity against brine shrimp in the brine shrimp lethality assay with IC_{50} values of 48.71 µg/ml and 93.25µg/ml respectively[11]. In addition, the cyto toxicity of the plant against human breast cancer cell lines (MDA-MB-231 and MDA-MB-468) was studied[21].

Based on the literature survey, the present study was focused to explore the enhanced cytotoxicity of *J.Vulgaris* against other human cancer cell lines so as to increase its therapeutic offers against cancer treatment.

MATERIALS AND METHODS

Preparation of plant extract

The plant was collected from Anaimalai hill area of Western Ghats region in the month of January 2016 and was identified by the Botanical Survey of India, Coimbatore. A Voucher specimen was displayed in the host institute laboratory with the specimen number: 16CHG10. The leaves were washed with fresh water and shadow dried for ten days. After crushing into fine sieves, it was soaked into Chloroform, Ethyl Acetate and then with Ethanol sequentially. The solvents were removed under reduced pressure by rotatory evaporator to get the crude extracts. The extracts were 8 g/kg, 8.3g/kg and6g/kg of the plant material respectively which were then stored in desiccator for further studies.

Selection of cancer cell lines

HepG2 cell line is a human liver carcinoma cell derived from

the liver tissue of adult male. The morphology of HepG2 cell is epithelial and is the fifth most common cancer worldwide. They can be grown successfully at large scale and are suitable for *in-vitro* model system for the cytotoxic and genotoxic studies. Human cervical cancer (HeLa) is the second largest cancer commonly found at the global level.

Methodology

The human cancer cell lines (HepG2 and HeLa) were obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fatal bovine serum (FBS)[22]. All cells were maintained at an experimental condition of 37^0C and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice in a week[22,23].

Cell treatment procedure [22,23]

The monolayer cells were isolated with trypsin-ethylene diamine tetra acetic acid (EDTA). The number of viable cells was then counted using haemocytometer and the density of $1x10^5$ cells/ml is made by the dilution with 5% FBS medium. One hundred micro litres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated for a day for cell attachment. A temperature of 37^0C and 100% relative humidity is maintained throughout the experiment. The dimethyl sulfoxide (DMSO) is used for extracts dissolution and double dilution with serum free medium is done to get the required concentration. Five sample concentrations were obtained by additional four, 2fold serial dilutions. A serial concentration of the crude extract samples were treated with the tested cells. 100 µl of each extract dilutions were added to the appropriate wells and the plates were incubated for 48 hrs under the same experimental condition. The blank medium without samples was also maintained as control. Triplicate was maintained for all concentrations.

MTT Assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow coloured tetrazolium salt. When MTT is added to the cells, a notable colour change from yellow to purple occurred due to the formation of formazan crystals. The intensity of the colour is measured by spectrophotometric method. 5-Florouracil was used as a positive control.
After 48 hrs of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for four hours. The formazan crystals formed were dissolved in 100µl of DMSO and then measured the absorbance at 570 nm. The % cell inhibition was determined using the following formula[24].

$$\% \text{ cell Inhibition} = \frac{100 - \text{Abs (sample)}}{\text{Abs (control)}} \times 100$$

The IC_{50} was determined using Graph Pad Prism software version 3.0025.

Statistical analysis

The Cytotoxic profile of the plant was assessed by analyzing the experimental data obtained by triplicate of the entire samples. The results are expressed as mean+SD and are analysed by one-way analysis of variance (ANOVA) using SPSS version 4.0.

RESULTS

This study evaluates the cytotoxic effect of Chloroform, ethyl acetate and Ethanol extracts of *J.Gendarussa* against two cancer cell lines-HepG2 and HeLa by performing MTT assay which is a simple and reliable technique. The MTT assay is explored based the reaction between the mitochondrial enzyme-Succinate dehydrogenase present in the living cells with the tetrazolium ring. The enzyme converts the MTT into formazan compound through the cleavage of tetrazolium ring. The yellow colour of MTT gets converted into purple due to the formation of formazon which is measured by spectro photometrically. Since the metabolically active cells only can able to produce formazan, the amount of formazon crystals is the measure of the number of viable cells which showed the activity level.

The cell viability of the three extracts against the selected cell lines was reported in table:1**.** From the data, it has been identified that incubation with different concentrations of the extract affected the viability of the cell lines. The IC_{50} values determined were tabulated in table 2**.** The best potential was obtained at certain optimal concentration of the extract. A remarkable cytotoxicity was observed with the ethanol extract and its IC_{50} values were determined as 43.8 and 19.8µg/ml against the HeLa and HepG2 cell lines respectively. A common cancer drug 5-Florouracil was used as the standard.

According to the datas provided by Zahidah Ayob et.al[21], the cytotoxicity of *Jedarussa vulgaris* was analysed by collecting the plant from different locations of Malaysia. Of these, the better results were taken into account for comparison with the present work (Table:3). The standard drug Tamoxifen is used as the positive control by Zahidah Ayob et.al[21]. On comparison, the methanol and ethanol extracts of the plant has the prominent effect against HepG2 cell line. The crude extract of the plant is more active pharmacologically which may be due to the presence of synergistic effects of the bio-constituents present in the extract.

According to the NCI plant screening programme, a plant extract is considered to have potential *in-vitro* cytotoxicity if the IC_{50} value on tumor cells is below 20µg/ml after 48-72 hrs incubation. Therefore the potential use of *J.Gendarussa* as therapeutic agent holds a great promise. The isolation of one or more phyto constituents with potent cyto toxicity from the crude extract and the judicious use of such constituents can control the progression of cancer.

DISCUSSION

The results obtained make a conclusion that the ethanol extract of the plant is found to have a remarkable cytotoxicity against all the tested cell lines. A notable effect was found against liver carcinoma cancer cell line (HepG2) with IC50 value 19.8µg/ml. On comparison with the literature datas, *Jendarussa vulgaris* extract has a remarkable effect against HepG2. A continuous study is under progress for identification of active ingredients present in the plant, the effect on non-tumor cells and in vivo efficacy in order to provide a clinical approval for the successful use of the plant *Jendarussa vulgaris* in the chemotherapeutic drugs.

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Table :1 Percentage viability of leaf extracts:

Extract	Conc in µl	HeLa	HepG2
Chloroform	6.25	30.0 ± 0.87	29.4± 0.40
	12.5	38.9 ± 0.89	32.86±0.81
	25	48.16± 0.38	22.16±0.47
	50	50.2 ±0.31	48.66±0.61
	100	51.9±0.55	49.26±0.31
Ethyl Acetate	6.25	28.3±0.30	30.23± 0.55
	12.5	35.23± 0.25	39.73± 0.31
	25	42.5± 0.46	49.56± 0.55
	50	66.56± 0.35	58.6±0.60
	100	74.56±0.49	71.53±0.31
Ethanol	6.25	26.36± 0.40	30.43±0.35
	12.5	32.46± 0.45	39.03±0.35
	25	58.93± 1.01	50.53±0.32
	50	66.40± 0.70	72.36±0.40
	100	85.06± 0.85	89.76± 0.71

The results are mean ± SD

Table:2 IC_{50} value of the extracts against the tested cell lines

Extract used	HeLa	HepG2
Chloroform	63.2	70.6
Ethyl acetate	60.9	73.9
Ethanol	43.8	19.8
Standard (Fluorouracil)	0.72	1.35

Table:3 Comparative study of IC_{50} values of the extracts

	Literature data		Experimental data	
Extract	**MDA-MB-468**	**MDA-MB-231**	**HepG2**	**HeLa**
Methanol	23	40	-	-
Chloroform	-	-	70.6	63.2
Ethyl acetate	-	-	73.9	60.9
Ethanol	-	-	19.8	43.8
Tamoxifen Std drug (Literature data)	27	12	-	-
Flouroracil Std drug (exp.data)	-	-	1.35	0.72

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