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RESEARCH ARTICLE

In-vitro Cytotoxicity assay of Betel quid extract against HeLa cells

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ABSTRACT:
HeLa cells are broadly used for in-vitro studies in bringing out the medicinal value of plants against some dreadful diseases. From time immemorial, it is well proven that plant extracts play a vital role in treating many diseases including cancer. The plant extract contains copious varied phenolic compounds and flavonoids that are majorly responsible for its medicinal properties. In our study we have focused on the extraction of betel quid (betel leaves, areca nut and slaked lime in an appropriate proportion) using the solvent hexane. The hexane extract of the betel quid was evaluated for in vitro studies of apoptosis of HeLa cells. The results revealed that hexane extract of the betel quid at the maximum concentration of 200 µg/mL was very effective and only 20.89 % of cells were viable. The cytotoxic effect against HeLa cells was significant with IC_{50} of 96.33µg/ml. Further studies can be done on concentrating the bioactive compounds responsible for the anticancer activity. The bioactive active compounds can be formulated in the treatment of cancer in the upcoming research.

KEYWORDS: HeLa cells, Betel quid, Cytotoxic effect, Anticancer

INTRODUCTION:
Cancer is the second dreadful disease in the biosphere that leads to death if precise treatment is not taken at appropriate time. The cancer cells are capable of extensive proliferation and has the potential to spread to various organs from its site of origin. It is the second prevalent disease that causes death worldwide after cardiovascular diseases[1]. Aggressive treatments for cancer includes surgical resection, chemotherapy and radiotherapy. They are used either alone or in combination depending upon the different stages of malignancies. These treatments have many side effects. Henceforth, research should be focused on effective curative measures with least possible side effects. The safe, nontoxic method of treatment involves the usage of plant extract for its antitumor properties. Plants are proven to exhibit potential resources for production of novel anti-cancer drugs[2].

The International Agency for Research on Cancer has reported in 2018 that, cervical cancer is the second most prevalent cancer in India. It contributes to about 6–29% of the cancer cases in women. Indian women are more prone to cancer when compared to other parts of the world as they lack awareness towards screening[3]. In India every year 96,922 new cases of cervical cancer are detected. HeLa cells is the first immortalized human cancer cell line. These cells are capable of proliferating and growing indefinitely and are derived from HPV-transformed cervical adenocarcinoma cells. It is used as a tumor model in several studies. HeLa cells are widely used to investigate the anticancer activity of the phytochemical compounds that are extracted from various parts of plants[4]. Various phenolic compounds are extracted from the plants to help in the apoptosis of the HeLa cells. Piper betel leaves is a reservoir of phenolic compounds which helps in fighting against cancerous cells Plant extracts contain bioactive compounds which are effective in the treatment of various diseases including cancer. Piper betel leaf is an evergreen perennial plant which is reported to have anti mutagenic, anticancer and anti-inflammatory properties[5]. The habit of chewing betel leaves with areca nut and slaked lime (betel quid) is a traditional culture in south India. All the major religious festivals and celebrations end up with the habit of taking betel quid after a heavy meal. Betel quid including tobacco is dangerous and many reports have proven that ingestion of betel quid with tobacco leads to cancer[6]. But in ancient times from time immemorial, betel quid was consumed without tobacco and our ancestors were

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hale and healthy devoid of any dreadful disease in their life span. Considering this point, we decided to extract the phenolic compounds of this betel quid with the solvent hexane. The focus of our study is to evaluate the activity of hexane extract of betel quid upon apoptosis of the HeLa cells. The composition of betel quid varies according to the regions. In our study we focused on the preparation of betel quid which is used locally in the southern regions of India especially Tamil Nadu.

MATERIALS AND METHODS:

Preparation of sample:

Fresh material of the betel quid (an appropriate ratio of betel leaves, areca nut and slaked lime) was crushed in pestle and mortar for greater surface area. The crushed material was subjected to soxhlet extraction using hexane as solvent for a period of 16 hours.

Preparation of HeLa cell suspension:

A subculture of HeLa cell lines in Dulbecco's Modified Eagle's Medium (DMEM) was trypsinized separately, after discarding the culture medium. To the disaggregated cells in the flask 25 mL of DMEM with 10% FCS was added. The cells were suspended in the medium by gentle passage with the pipette and the cells were homogenized.

Seeding of cells:

One mL of the homogenized cell suspension was added to each well of a 24 well culture plate along with different concentration of sample (Hexane extract) (0 to 200 µg/mL) and incubated at 37°C in a humidified CO_2 incubator with 5% CO_2. After 48 hours of incubation the cells were observed under an inverted tissue culture microscope. With 80% confluence of cells cytotoxic assay was carried out.

Cytotoxicity assay:

The assay was carried out using (3- (4, 5-dimethyl thiazol-2yl) -2, 5- diphenyl tetrazolium bromide (MTT). MTT is cleaved by mitochondrial Succinate dehydrogenase and reductase of viable cells, yielding a measurable purple product formazan. This formazan production is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity. After 48 h incubation the wells were added with MTT and left for 3 h in room temperature. All wells were removed the content using pipette and 100µl SDS in DMSO were added to dissolve the formazan crystals, absorbance's were read in Lark LIPR- 9608 micro plate reader at 540 nm

RESULTS AND DISCUSSION:

The results of *in-vitro* cytotoxicity activity of the Hexane sample extract against HeLa cells revealed that the sample was effective against the HeLa cells and its growth was highly inhibited. The observation of control cells is presented in (Fig.1).

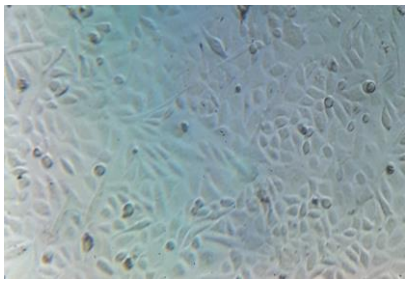

Figure 1: Hela cells- Control before treatment

Upon addition of sample concentration up to 100 µg/ml showed high increment of cytotoxicity and cell death as shown in (Fig.2). Our studies prove that hexane extract of the betel quid is capable of leading to apoptosis of the Hela cells at lower concentration of µg/ml. The cytotoxic effect of *Cocos nucifera* cells against HeLa cells was reported [7]. It revealed that the crude extract of *Cocos nucifera* induced apoptosis of the HeLa cells and the gene expression P53, Bcl2 was revealed. The anticancer cytotoxic activity of the lead compound pentane-2,4-dione from *Cordia sebestena* leaves is due to inhibition of Kras protein in HeLa cells [8].

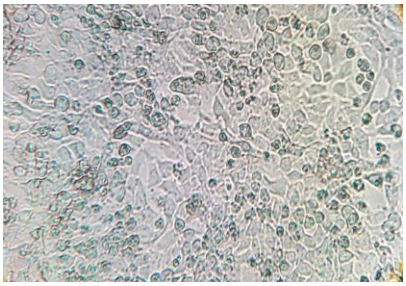

Figure 2: Hela cells- apoptosis after addition of 100 µg/ml hexane extract treatment

It was evident from the results that samples tested at maximum concentration of 200 µg/ml showed cytotoxicity activity as high as 20.89% against HeLa cell lines as shown in (Fig.3). The high cytotoxicity effect of the test sample showed cell disintegration after 48 h of treatment against the selected tested cell lines.

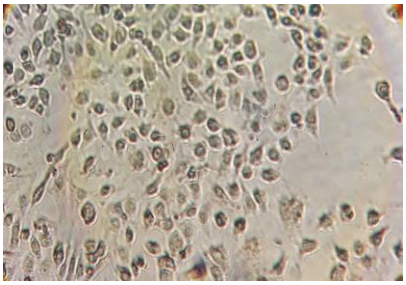

Figure 3: Hela cells- apoptosis after addition of 200 µg/ml hexane extract treatment

Almost 50 % of the drugs used for cancer contain plant alkaloids. Hence more focus can be procured from plant based drugs[9]. From the results we can clearly witness the apoptosis of the HeLa cells. Numerous vegetables, fruits and spices we use in our day today life are now proven to have anticancer effects. The habit of consuming vegetables, fruits and the follow up the food habit by our ancestors paved way for the prevention of cancer[10]. The intake of betel quid might also be a part of it. Keeping these points in our mind the studies were conducted and the results are evident that the betel quid possess anticancer activity. *Curcuma longa* is used as a spice from time immemorial and is proven to have many medicinal effects inclusive of anti-cancer activity[11]. The pharmacological evaluation of *Maytenus emarginata* revealed that leaves extracts were more potent than the stems extracts against anticancer cells with methane as solvent[12].

In this cell lines studies of our research, cytotoxicity effect was observed in tested sample concentrations in 48 hours' treatment. It also revealed that all tested concentration of test samples shown certain cytotoxicity over the tested cell line (Figure 4).

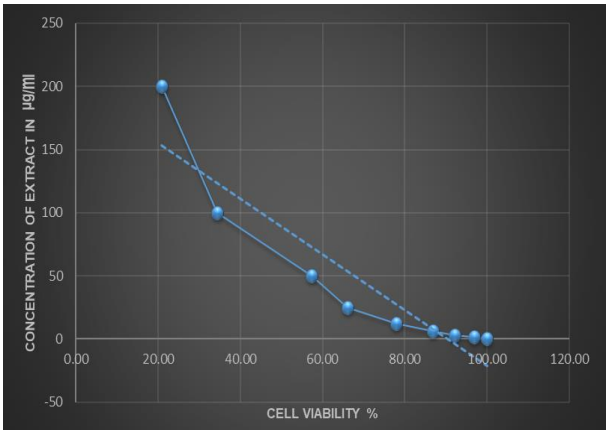

Fig. 4: Cytotoxic activity of extract against HeLa cell lines

The IC50 of the tested sample Hexane extract against HeLa cells was calculated as 96.33µg/ml. IC50 denotes the value of cytotoxicity with 50 % decrease in cell viability. Methanol extracts of *Rosa damascene* was reported to have IC50 of 265 µg/ml[13]. Ethanolic extracts of fruits of *Tribulus terrestris* and leaves of *Bougainvillea spectabilis* are reported to have potential anticancer activity[14]. The apoptosis inducing activity was reported to be in the casein fraction of human milk and was characterized as a multimeric form of human α-lactalbumin (MAL). MAL induced apoptosis in transformed and non-transformed cell lines[15]. Many anticancer drugs are derived from the marine environment too[16]. Methanolic and aqueous extract of *Avicennia marina* are reported to exist cytotoxicity against HL-60 and NCI-H23 cell line with proficient IC50 value[17]. Piper betel leaves are said to exhibit various medicinal properties: antimicrobial, antidiabetic, gastro protective, platelet inhibitory, oral care agent, antioxidant hepato protective and anticancer activity. Aqueous extract of the leave has cytotoxic effect against Hep 2 cell line[18]. Betel leaves are reported to exhibit anti-ulcer properties[19] and is potential in preventing colon cancer[20]. Numerous studies indicate a relation between telomerase and sensitivity of cancer cells to therapy. Betel leaves possess various nutritive benefits and the phyto components present in the betel leaves namely hydroxychavicol is reported as an antimutagenic agent[21]. Telomerase is a diagnostic and therapeutic biomarker because it is absent from most somatic cells and is present in most cancer cells. The relationship between telomerase and cancer is complex, which makes it a distinctive target for cancer therapy. Telomerase synergistically with natural products may play a crucial role in the development of a drug for cancer therapy. natural compounds such as polyphenols, alkaloids, triterpenes, and xanthones are potential chemo preventive and chemotherapeutic agents for the treatment of cancer[22]. Areca nut is traditionally masticated either alone or along with a large variety of ingredients, such as betel leaf and slaked lime for traditional ceremonial cultural purposes in Indonesia. Previous study showed that the antioxidant activity of areca nut extract has the potential to prevent oxidative damage in normal cells. The apoptotic activity of areca nut was repoerted[23]. Many reports have confirmed that the phenolic compounds in betel leaves and areca nut are antimutagenic and anticancer activity. Hence a combination of these might effectively prove as a potential inhibitor of HeLa cells.

CONCLUSION:

It is evident from our results that betel quid without tobacco has the potential for apoptosis of HeLa cells. Less than 100 µg/ml is capable of killing almost 50 % of the cells. Hence further studies can be done to extend this work on different cell lines. The biologically active compound responsible for the anticancer activity can be isolated and included in pharmacological combinations in the invention of new drugs that are plant based to fight against cancer.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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