EVALUATION OF ANTI TUMORIGENIC ACTIVITY OF Boswelliaserrata USING KB CELL LINE

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Abstract

Background: B.serrata is commonly known as Indian olibanum, it is mainly used in the production of Indian frankincense. B.serrata is used for many other purposes including asthma diabetes. The medicinal plant Boswelliaserrata has great potential for treating cancer. Since many cancer therapies have their origins in natural ingredients, this study aims to explore the anti-tumorigenic effect of B.serrata using KB cell line.

Materials and methods: Seed of B.serrata was collected and extraction was done. Then the extract was added to the culture and the oral cancer cells were obtained from Pune. The cultured oral cells were incubated in a CO2 incubator. Treated oral cancer cell sample was done MTT assay and gene expression analysis.

Result: The collected data from MTT assay and gene expression analysis tabulated using SPSS software. MTT assay results showed gradual decrease in cell viability with increase in concentration of Boswelliaserrata and the gene expression showed up regulation of caspase 3 and 9 expression.

Conclusion: From the study, Boswelliaserrata has a significant effect on anti-tumorigenic activity; this might be due to the active bio components present in Boswelliaserrata extract.

Keywords: Anti tumorigenic, B.serrata, KB cell line, apoptosis pathway, caspase 3 and 9.

INTRODUCTION

The sixth most frequent cancer in the world is oral squamous cell carcinoma, which is also one of the top causes of death, particularly in developing nations like India (1). Chemotherapeutic chemicals, which are typically used to treat it, are harmful in many different ways to both normal and malignant cells. Cancer is a multipotent disease that is often treated with a variety of chemotherapeutic drugs that are hazardous to both normal and cancer cells in various ways. These substances are also quite expensive and cannot be used to prevent cancer (2).

Natural products have received much interest since they are rich sources of various chemicals that can be used as biologically active medications to treat a variety of chronic conditions. These chemicals from plants have greatly improved the current medical system(3)(4). For instance, the use of phytomedicines, which are crucial to the health management system of a developing country like India, benefits about 65% of the population. The WHO estimates that approximately 80% of people worldwide utilise phytomedicines to treat a variety of illnesses (5)(6). Boswelliaserrata is an abundant species that is easier than two derived drugs; it will also be cost effective. Boswelliaserrata is one such by a chemical obtained from the gum resin of the posterior species that possibly add in the treatment of different chronic diseases. It is one of the most essential and commonly used components in conventional Ayurvedic and Unani medicines which have proven to be extremely effective in relieving numerous inflammatory gastrointestinal hormonal and microbial diseases (7). Various reports subscribe to the antimicrobial anticancer antiasthmaticantidiabetic, hypolipidemic, antidiarrheal, hepatoprotective, hyperglycemic and even antiviral effect of

different Boswellia species (8,9). Additionally, it has been established that more than 50% of cancer treatment substances are also present in plant products and have the ability to induce apoptosis (10)(11).

Our team has extensive knowledge and research experience that has translated into high quality of publications (12)(13–26).

The current need is to create medications that might specifically target cancer cells rather than healthy cells. This research was done to determine whether Boswelliaserrata extract had an inhibiting effect on the KB cell line.

MATERIALS AND METHODS:

Chemicals

Trypsin-EDTA, fetal bovine serum (FBS), antibiotics-antimycotics, Dulbecco's modified Eagle's medium (DMEM) and phosphate buffered saline (PBS) were purchased from Gibco, Canada. JC-1 (5,5 ,6,6 - tetrachloro-1,1 ,3,3 - tetraethylbenzimidazolocarbocyanine iodide) and real time PCR kit (MESA Green) were purchased from Invitrogen, USA. All the chemicals used were extra pure of analytical grade.

Extract preparation:

The Boswelliaserrata were soxhlet extracted with 70% ethanol. The extract was then filtered with Whatman no.1 filter paper and the solvent evaporated at reduced pressure by using a Rotary evaporator apparatus to get a viscous mass, which was then stored at 4°C until used.

Procurement and culture of KB cells:

The oral squamous cell carcinoma cell line (KB) was obtained from The National Centre for Cell Science (NCCS), Pune, India and cultured according to the cell culture instructions provided. Briefly, KB cells were grown in MEM containing 10% FBS at 37˚C in an atmosphere containing 5% CO2.

Cell viability assay:

KB cells were seeded at a density of 5x105 cells/well in 96-well plates and allowed to attach to the well overnight. After incubation, cultured cells were stimulated with various concentrations of BoswelliaSerrataextracts in triplicate and incubated at 37˚C in a 5% humidified CO2 incubator for 24 h. Subsequently, MTT was added to each well, and incubation was continued for a further 4 h at 37˚C. To dissolve the formazan formed from MTT, the cells were resuspended in 200 µl dimethyl sulfoxide (DMSO), and the optical density (OD) of the solution was determined using a spectrometer at a wavelength of 570 nm. The experiments were repeated 3 times, independently. The mean optical density (OD) ± SD for each group of replicates was calculated. The entire procedure was repeated 3 times.

The inhibitory rate of cell growth was calculated using the equation:

% Growth inhibition = (1 – ODextract treated)/OD negative control x 100.

Gene expression analysis by Real Time PCR:

mRNA expression levels were examined using real-time PCR. The total RNA was isolated by using Tri Reagent (Sigma). Total RNA (2 µg) from each sample was reverse transcribed using a commercial SuperscriptIII first strand cDNAsynthesis kit (Invitrogen, USA) according to the manufacturer's protocol. Real time-PCR was carried out in a MX3000p PCR system (Stratagene, Europe). Reaction was performed using MESA Green PCR master mix (It contains all the PCR components along with SYBR green dye.) Eurogentec, USA. The specificity of the amplification product was determined by melting curve analysis for each primer pair. The data were analysed by comparative CT method and the fold change is calculated by 2−CT method described by Schmittgen and Livak (2008) using CFX Manager Version 2.1 (Bio Rad, USA).

Statistical analysis:

Data were expressed as the means ± SD of 3 individual experiments performed in triplicate. Statistical analysis was performed using the one-way ANOVA and $p<0.05$ was considered to indicate a statistically significant result.

RESULT AND DISCUSSION:

The results from the MTT assay showed that with increased concentration of B.serrata the cell viability of the KB cell line gradually decreases. It is also found through results that caspase 3 mRNA level and caspase 9 mRNA level are increased with decreased cell viability.Therefore,it can be concluded that caspase 3 and 9 mRNA are two of the many pathways followed by B.serrata to kill the cancer cells.

In this study, the MTT test was being used to investigate how the extract of B.serrata affected KB cells. The MTT assay technique's outcomes showed that B.serrata extract could dramatically lower the growth of KB cancer cells and also had a dose-dependent cytotoxic effect on KB cells' ability to survive. The extract concentration of 200µg/ml had a substantially greater inhibitory impact than other levels (Figure 1).

According to Huang et al, a B. serrata extract containing β boswellic acids and related compounds prevents mice from developing tumours when it induces tumour growth by preventing the up-regulation of pro-inflammatory cytokine proteins (27). Boswellic acids have been discovered to exert lethal effects on malignant glioma cells at low micromolar concentrations (28). The induction of intrinsic and extrinsic apoptotic pathways by B. serrata extract and boswellic acids has also been demonstrated (29). Numerous plant extracts have been shown to have the ability to stop cancer cells from proliferating, making them useful in the treatment of cancer (27,30). According to Huang et al, a B. serrata extract containing β boswellic acids and related compounds prevents mice from developing tumours when induces tumour growth by preventing the up-regulation of pro-inflammatory cytokine proteins. Boswellic acids have been discovered to exert lethal effects on malignant glioma cells at low micromolar concentrations (28).

The intrinsic death receptor-independent pathway and the extrinsic death receptor-dependent pathway, both of which necessitate the sequential activity of cysteine proteases known as caspases, respectively. These are the two separate processes that trigger apoptosis (31). When initiator caspases like caspase-8 and caspase-9 are activated, caspase-3 is proteolytically cleaved. Multiple cytoplasmic and nuclear proteins are cleaved as a result of the main apoptosis mechanism executor, caspase-3, being triggered (32). Caspase-3 measurement is an easy, trustworthy, and accurate way to identify apoptosis in its early stages (33). In the current investigation, the caspase 3 and caspase 9 gene expression level significantly upregulated when compared to the untreated KB cells (Figure 2). The induction of intrinsic and extrinsic apoptotic pathways by B.serrata extract and boswellic acids has also been demonstrated (29). Although the theories and findings of different research about apoptosis in oral epithelium were unclear and highly contentious, this discovery was consistent with the findings of the current study (34). Caspase-3 upregulation was a significant connection between the intensity of caspase staining and tumour staging. It has been recommended to use the cytoplasmic expression of caspase-3 in tumour cells as a metric to gauge the tumour's prognosis since it indicates the abnormal differentiation in OSCCs (35).

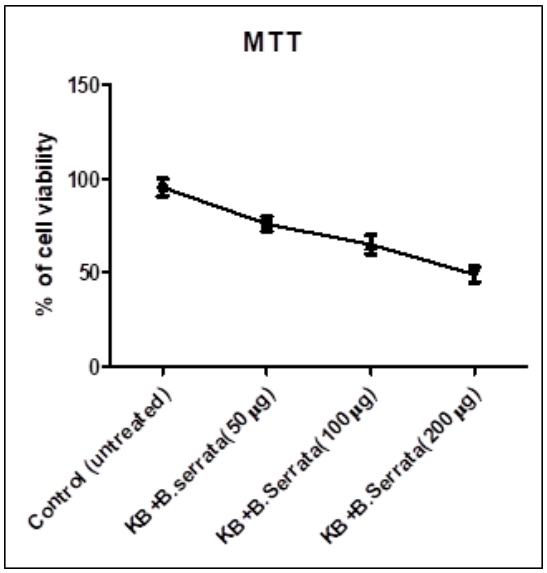

Figure 1: MTT assay

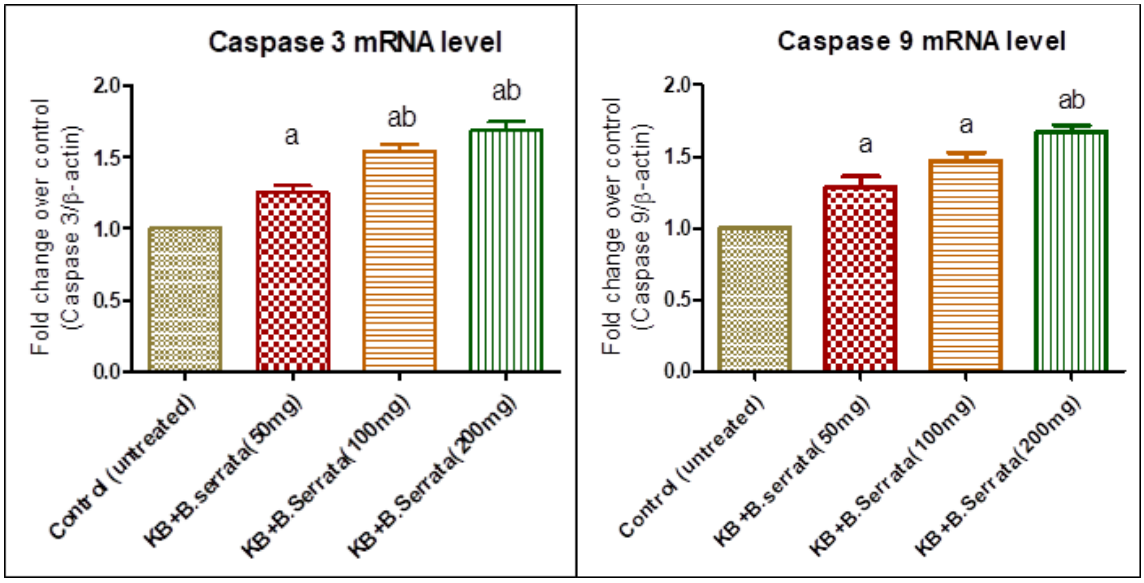

Figure 2: Caspase 3 and Caspase 9 mRNA expression

CONCLUSION:

It can be concluded from above results that Boswelliaserrata has anti tumorigenic properties against KB cell line. B.serrata uses caspase 3 and 9 mRNA pathways to decrease the cell viability of the cells. This property of the B.serrata can be used to synthesise anti-cancer drugs in the future.

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Author Contributions:

Tania Michael: Literature search, Data collection analysis, Manuscript drafting

Dr.R. Gayatri Devi: Data Verification, Manuscript draft

Dr.J.Selvaraj: Data collection analysis, Data Verification, Manuscript draft

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