

## SYNERGISTIC ROLE OF FOOD BIO-MOLECULES IN CELLULAR PROLIFERATION AND CYTOTOXIC ACTIVITY

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### Abstract

Nutrigenomics is the study of molecular relationships between nutritional stimuli and the response of the genes. The metabolic signals that the nucleus receives from internal factors (hormones) and external factors (nutrients) are responsible for maintaining the functional integrity of genes, with the latter being more influential of environmental stimuli. In response to many types of environmental stimuli including nutrition, the human genomes evolve. Herbs promote proliferation of tissue progenitor cells. Identification of multi-potential progenitor population is important for understanding the developmental process and tissue homeostasis. Progenitor population are ideal targets for analysing the cellular metabolism, gene therapy, cell transplantation, and tissue engineering. Chicken has been used as a favourite model in embryology and developmental biology and it serves as a good model for nutrigenomic studies, in other mammalian system including humans due to its closeness to the mammalian genome. In the present study progenitor cell population from the chicken tissues such as muscle, bone marrow, heart and liver were isolated and cultured for analysing the interactions with food biomolecules. Preliminary studies showed that  $11.11 \pm 0.05$  million cells (heart),  $9.63 \pm 0.05$  million cells (liver),  $1.66 \pm 0.05$  million cells (muscles). The progenitor population were subjected for different concentrations of isolated food biomolecules derived bio-peptide combinations.

### Keywords:

chicken, food biomolecules, progenitor cells, tissue engineering, *Ocimum sanctum*, *Phyllanthus amarus*

### Introduction

Nutrigenomics is the study of how naturally occurring chemicals in foods alter molecular expression of genetic information in each individual particularly nutrition on the individual level, which also helps to focus on disease prevention. Diet is one of the serious factor for many genetic diseases. Bioactive food components are very important in understanding the effect of individual variability on the response to dietary change. Among the plants known for medicinal value, the plants of genus *Ocimum* belonging to family *Labiatae* are very important for their therapeutic potentials. The botanical name, *Ocimum sanctum* (Latin) of basil depicts that it is a sacred plant not only in India but also throughout the world because of the outstanding medicinal and purifying virtues contained in its essential volatile oils in the leaves. Tulsi, the Queen of herbs is one of the most potent general adaptogens known to modern science, strengthening the body's natural capacity to adapt to a wide variety of stresses, and restore and maintain healthy homeostatic equilibrium. 'Adaptogens' are still new to western medicine, and like other adaptogens, contain many nutrients and active phytochemicals, which act synergistically to bring about a state of balance in almost all of the body's systems. Research indicates that tulsi has a very high safety margin with exceptionally low toxicity, providing general beneficial effects at doses without adverse reactions or other undesirable side effects.

Karthikeyan *et al.* (1999) investigated the medicinal properties of *Ocimum sanctum* against human fibrosarcoma cells (HFS cells) in culture. The cell treated with the ethanolic extract of the plant showed shrunken cytoplasm and

condensed nuclei. The aqueous and ethanolic extract of *O. sanctum* was administered to mice bearing tumour led to the reduction in tumour volume and increased the life span of the animal. It was reported that the extracts possess anticancer activity. Ibrahim *et al.* (2015) reported the cytotoxic effect of volatile oil of *Ocimum sanctum* on different human cell lines and on different cancer cell lines including; HU60 (human lung adenocarcinoma), MCF7 (human breast adenocarcinoma), Hep G-2 (human hepatocellular carcinoma), HeLa (human cervical carcinoma) and U251 (human brain tumour cell lines). Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) to purple formazan. A comparison on the effect of the volatile oil from tulsi leaf and flower demonstrated that the volatile oil significantly suppresses growth and induces apoptosis in HeLa, HepG-2 and HU60 cell lines.

Cai *et al.* (2015) reported that the *in vitro* stem cell models would be suitable for studying the effects induced by new chemicals or therapeutic agents. The application of mesenchymal stem cells would be a very good model for cytotoxicity screening as bone marrow derived MSC on mutagenic stimulation could predict exactly the models of fibrosarcoma. *Phyllanthus* has been subjected for the cellular protective properties on normal liver and bone marrow cells. The *P. amarus* extract significantly decreased the number of cancer cells (HEPG2) in a dose dependent manner in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The cellular system for screening protective and anti-proliferative activity of drugs applying stem cells was used to validate the activity of *Phyllanthus amarus*. Abhyankar *et al.* (2010) studied the establishment of hairy root cultures of *Phyllanthus amarus* using *Agrobacterium rhizogenes* and cytotoxic effects of methanolic extract of hairy roots on human adenocarcinoma cell line, MCF-7. Most of the chemotherapeutic agents are known to induce apoptotic cell death in the cancer cells. They drive cells to over-generate the reactive oxygen species (ROS) thereby inducing apoptosis. The anti-proliferative activity of hairy root extract on breast cancer cells may be attributed to the major constituent, amarone. Since apoptosis has been established as a distinctive therapeutic target in cancer treatment, the accrued results amply substantiate the importance of *P. amarus* hairy root extract as a plausible source of anticancer drug in breast cancer therapy. Londhe *et al.* (2012) examined the ability of ellagitannins namely geraniin and amariin isolated from *Phyllanthus amarus* protects the mouse against liver toxicity.

Genes are turned on and off according to the metabolic signals the nucleus receives from internal factors (hormones) and external factors (nutrients, which are among the environmental stimuli). As the evolution of simple organisms into complex forms of life took place, they retained the ability to respond to nutrient or nutrient/hormonal signals that govern the expression of genes encoding the proteins of energy metabolism, cell differentiation and cell growth (Amin *et al.* 2012). Based on the above details an attempt has been done for studying the effect of nutrient biomolecule synergistic activity in tissue progenitor cells..

## Materials and methods

### Materials

The material used for the study were purchased from ISO certified siddha referred pharmacy.

### Medicinal herbs

The medicinal herbs used for the cell proliferation are *Ocimum sanctum* and *Phyllanthus amarus*.

### Methods

#### *Ethanol extraction of the medicinal herbs*

5 g sample of each sample was extracted with 100 mL 80% ethanol at 35° C for 24 hr in a shaking bath.

#### *Cell culture*

Chicken tissue progenitors were isolated from the muscles tissues, liver tissues, and heart tissues and were maintained in cell culture growth media, Dulbecco's Modified Eagle's Medium (DMEM) containing 10% Fetal Bovine serum (FBS) (Sigma chemicals, USA), and 1% penicillin (Sigma). Cells were cultured as adherent monolayer and incubated at 37± 0.5 °C and 5% CO<sub>2</sub>

**Treatment of cells**

Cells were plated ( $1 \times 10^4$  /well) in 96-well plates in complete medium and cell viability was ascertained by trypan blue. After 24 hours, medium was removed and replaced by the fresh medium (control) or supplemented with various doses of the extracts. The percentage of liveability was estimated by MTT assay.

**MTT Assay for cell viability**

After 24 h incubation, with different concentrations of the extracts, MTT 3-(4,5- dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide (5 mg/ml) 100 $\mu$ l/well was added, at appropriate time and incubated for 4 hours at 37°C. Viable cells had intact mitochondria and dehydrogenases present there which convert the tetrazolium salt to insoluble formazan violet crystals. The formazan crystals were dissolved in 200 $\mu$ l of dimethyl sulfoxide (DMSO). The absorbance was read at 570nm using an absorbance microplate reader.

**The food biomolecule activity on microbial cells**

*E.Coli* colonies checked by multiplex PCR for pathogenicity by specific amplification of *eae* (618bp) and *stx 1* (256 bp) genes were used for this study. Damage to the cells in supplemented cultures (*O. sanctum* and *P. amarus*) was observed by performing comet and DNA fragmentation assays.



Figure 1: Single –gel migration assay (COMET)

Normal melting point (NMA) agarose-thin layer was made in frosted microscopic slides and was air dried. A mixture having 10  $\mu$ l of *E. coli* added with 75  $\mu$ l of LMPA was overlaid on NMA and rested on ice-pack. Third layer was made using 80  $\mu$ l of LMPA and hardened. The slides were treated with lysing solution for 3 hrs as shown above

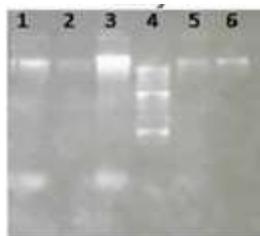


Figure 2: DNA fragmentation analysis

Apoptosis can be visualized as a ladder pattern of 180-200 bp due to DNA cleavage by the activation of a nuclear endonuclease by standard agarose gel electrophoresis (Chen, 1996; Matalalova and Spanova, 2002). From left to right the samples were treated in 5 lanes. In lane 1-50  $\mu$ g of the sample was treated. In lane 2- 50 $\mu$ g of the sample was treated. In lane 3- 100 $\mu$ g of sample was treated. In lane 1 Kb DNA ladder was loaded. Lane 5 and 6 were used as Control shown above

**Table 1: Determination of Cell Viability of treated cells by Trypan Blue Dye Exclusion Test (mean  $\pm$  SD)**

Sl.No	Sample	Synergistic activity of herbs in Treated cells(Cells/ml) live	Cell viability(%) of the treated cells
1.	S1-Heart	20.75 $\pm$ 0.12x10 <sup>6</sup>	93.2%
2.	S2-Heart	39.75 $\pm$ 0.14 x10 <sup>6</sup>	84%
3.	S3-Heart	20.25 $\pm$ 0.22 x10 <sup>6</sup>	80.1%
4.	S4-Liver	23 $\pm$ 0.32 x10 <sup>6</sup>	88.46%
5.	S5-Liver	45.75 $\pm$ 0.22 x10 <sup>6</sup>	87.14%
6.	S6-Liver	39.79 $\pm$ 0.15 x10 <sup>6</sup>	74.6%
7.	S7-Muscle	13.2 $\pm$ 0.13 x10 <sup>6</sup>	76.8%
8.	S8-Muscle	15.2 $\pm$ 0.15 x10 <sup>6</sup>	73.4%
9.	S9-Muscle	16.5 x10 <sup>6</sup>	78.5%

Data from three different set of experiments were analyzed and expressed as mean  $\pm$  SD. significant difference were observed for treated cells analyzed using Duncans's multiple comparison method. A value of  $p < 0.05$  was considered to be significant.

## Results and discussion

The result indicated that the herbal supplementation had an inhibitory activity on the growth of bacterial cells while proliferation on tissue progenitor cells was recorded. The cytotoxicity in herbal supplemented cultured were appreciated by the appearance of typical comets. The therapeutic action of *O. sanctum* and *P. amarus* could be attributed to its antibacterial activity by its cytotoxic principles as evidenced by this study. The results of this study support the traditional culinary uses of *Ocimum sanctum* and *Phyllanthus amarus*. Tulsi has health benefitting effects by reducing stress and improving both cellular and humoral immunity. It has been shown that tulsi exhibits anticancer activity in animal models, and studies were carried out in human cancer *in vivo* like human cell fibrosarcoma and *in vitro* in human cervical cancer cell line (HeLa) and (Hep-2) and it was found to be effective which supports our research findings (Singh *et al.* 2012). The cytotoxic effect of volatile oil in *Ocimum sanctum* on different human cell lines and on different cancer cell lines which suppressed the growth of the cancer cells which is similar to our research findings (Ibrahim *et al.* 2015). The ability of ellagitannins namely geraniin and amariin isolated from *Phyllanthus amarus* protects the mouse against liver toxicity which proves that the herb is effective in analysing the cytotoxic activity which supports our findings (Londhe *et al.* 2012). The food biomolecules synergistic activity may be attributed to the proliferation of tissue progenitor cells which warrants further molecular analysis (Gaboon, 2011)

## Conclusion

The food biomolecules synergistic activity may be attributed to the proliferation of tissue progenitor cells which warrants further molecular analysis. The ultimate aim of science is prevention is better than cure. This is very similar to the dictum of Hippocrates - Father of Medicine (460–360 BC), who said “Leave your drug in the chemist's pot if you can heal the patient with food”.

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