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Role of Endogenous Serotonin Replica Mediated Through β-Catenin/Wnt Signaling in Lung Cancer Prevention and Prognosis - an *in-vitro* Study in A549 Cancer Cell Lines

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: Lung cancer is still the most common cause of carcinoma-related death in the globe. Non-pharmacological treatments such as physical activity and exercise have been shown to improve quality of life and provide better results in lung cancer patients. Lung cancer patients usually lack adequate amounts of physical activity and exercise, both of which can improve quality of life.
Aim: To analyse Chemotherapeutic potential of endogenous serotonin replica mediated through β-catenin/Wnt signaling in lung tumor cell lines (A549).
Methods and Materials: The National Centre for Cell Sciences (NCCS), Pune, India, provided the human lung cancer cell line (A549). MTT assay was used to determine cell viability. Real-time PCR was used to examine -Catenin mRNA, Wnt mRNA, and GSK mRNA gene expression. The significance of the acquired data was determined using one-way analysis of variance and Duncan's

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multiple range test with Graph Pad Prism version 5. Duncan's test was used to determine significance at the 0.05 level.
Results: The Results suggest that maximum inhibition of cell growth was at concentration (2-4 mM/ml) used in this study when compared to control. The cancer cells were significantly inhibited and it was found that there was notable reduction in GSK mRNA gene expression, β-Catenin, Wnt when compared to control at a dose of 2 mM/ml.
Conclusion: It may be concluded, based on the analyses' findings and the study's limitations, that the role of exercise induced endogenous serotonin may act as the regulator of wnt/β-catenin signaling in lung cancer cells. The exercise may help in maintaining the equilibrium of the gene expression by modeling Wnt/β-catenin signaling pathway and act as a protective factor in prevention.

Keywords: β-Catenin; lung cancer; exercise; serotonin; Wnt signaling; innovative technique.

1. INTRODUCTION

Lung carcinoma is still the most usual cause of tumor-related death in the globe. Cancer patients were urged to rest, heal, and conserve energy by avoiding strenuous physical exercise. Nonetheless, fresh evidence began to emerge in the late 1980s [1], supporting the idea that physical exercise, defined as any mobilization of the body induced by skeletal muscle and resulting in energy usage and exercise, may give relevant improvements in endogenous serotonin synthesis. The most usual cause of carcinoma - related death is lung cancer [2]. Human lung cancer cells (A549) are the most usual cause of cancer and death globally [3]. Serotonin is a neurotransmitter that generates nerve cells and transfers information between them. It can also be present in the digestive system. Tryptophan, an important amino acid, is used to make it [4]. Serotonin regulates our mood, happiness, and hormones throughout the body, allowing brain cells and other nerve systems to assist us in sleeping, eating, and digestion [5].

Non-pharmacological treatments such as physical activity and exercise have shown to enhance quality of life and provide better results in lung carcinoma patients. Patients with lung cancer usually lack adequate amounts of physical activity and exercise, both of which can improve quality of living. For those with lung tumor, there are no specific exercise advice or guidelines [6,7]. Cell viability MTT are functions to test cell viability and decreased percentage of cells denote the protective role of test compounds against cancer cells. Wnt are regulated cell growth and increase in gene expression favours cancer cell proliferation. Beta catenin aids cell-cell adhesion increase Proliferation of cancer cells, aided by changes in gene expression. Caspase 3 mRNA is an apoptosis gene whose expression causes cancer cells to die. The experiences from our previous studies [8,9,10,9,11,12,13,14,12,14,15,16,17] have led us to focus on the current topic. Our researchers has elaborate stuff and research experience that has translate into good quality research papers [18–31,32,33–37]. Thus, the goal of the research is to analyze the chemotherapeutic role of endogenous serotonin replica in lung cancer prevention and prognosis in lung cancer cell line.

2. MATERIALS AND METHODS

This is an in vitro - experimental study that was carried out in a private dentistry college and hospital in Chennai. The institutional review board has given its approval to the study.

3. PROCEDURE

Sigma Chemical Pvt Ltd, USA, provided serotonin dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetr azolium bromide (MTT). Gibco, Canada, provided trypsin-EDTA, foetal bovine serum (FBS), antibiotics-antimycotics, RPMI 1640 medium, and phosphate buffered saline (PBS). TAKARA provided the (5,5,6,6-tetrachloro-1,1,3,3-tetraethylbenzimidazolocarbocyanine iodide) and Real Time PCR kit (Meadowvale Blvd, Mississauga, ON L5N 5S2, Canada).

3.1 Cell lines and Cell Culture

The National Centre for Cell Sciences (NCCS), Pune, India, provided the human lung cancer cell line (A549). Cells were cultured at 37°C with 5% CO2 in DMEM media, 10 percent foetal bovine serum, 100 U/ml penicillin, and 100 g/ml streptomycin in medium (Thermo Fisher Scientific, CA, USA).

3.2 Cell Viability by MTT Assay

The ability of mitochondrial reductases in liver cells to change MTT, a tetrazolium compound, into purple formazan crystals was measured using a modified colorimetric approach (Mosmann, 1983). A549 lung cancer cells (1 104/well) were treated for 48 hours to varied doses of serotonin (100, 200, and 400 M). After the treatment, each well was filled with 100 l of 0.5 mg/ml MTT solution and incubated for an hour at thirty seven degree Celsius. The crystals were diluted in 100 litres of dimethyl sulfoxide and let to sit in the dark for one hour. A Micro ELISA plate reader was used to measure the intensity of the colour generated at five seventy nanometer. The quantity of viable cells was calculated using the proportion of control cells grown in serum-free media. Cell viability in the control ground plate was reported to be 100% without any treatment. Percent cell viability = [A570 nm of treated cells/A570 nm of control cells] x 100 is the formula for measuring cell viability.

3.3 Gene expression analysis by Real Time-PCR

Each group's samples were immersed in 2 mL Trizol (Invitrogen, Carlsbad, CA, USA) for RNA extraction and maintained at 80°C until further processing. The manufacturer recommended using Superscript II reverse transcriptase (Invitrogen) to make cDNA from 2 g RNA in a 10 l sample volume. A total volume of 20 l was used for real-time PCR array analysis, which included 1 l cDNA, 10 l qPCR Master Mix 2x (Takara, USA), and 9 l ddH2O. Reactions were carried out using a Bio-Rad CFX96 Touch Real-Time PCR Detection System with universal thermal cycling conditions followed by a melting curve: @ 95°C for 5 seconds

Melting curves are obtained for all samples for quality control objectives. Melting curve analysis was used to determine the specificity of the amplification results for each primer pair. Using CFX Manager Version 2.1, the data were processed through the comparative CT approach, and the fold change was deter mined using the 2CT method provided by Schmittgen and Livak (2008). (Bio Rad, USA).

3.4 Statistical Analysis

The collected data were statistically analysed using one-way analysis of variance (ANOVA) and Duncan's multiple range test with computer-based software (Graph Pad Prism version 5) and one-way analysis of variance (ANOVA) and Duncan's multiple range test to determine the significance of individual results among the control and experimental groups. At the 0.05 level, Duncan's test was performed to evaluate significance.

4. RESULTS

4.1 Effect of Serotonin on the Cell Viability

Cell viability of human lung cancer cells (A549) was determined using MTT assay administering the different doses of serotonin. It was found to exhibit inhibition of lung cancer cells by decreasing the percentage of viability of cancer cells in a dosage dependent manner when compared to control. It was found that maximum inhibition of cell growth was at concentration (2-4mM/ml) used in this study when in comparison to control [Fig. 1].

4.2 Effect of GSK m RNA Expression on the A549 Cancer Cells (Fold Change over Control)

GSK mRNA expression was measured in a dose-dependent fashion. At a dosage of 2mM/ml, the cancer cells were severely inhibited, and there was a considerable drop in GSK mRNA expression when compared to control. Furthermore, at a level of 4mM/ml, there was a substantial reduction in GSK mRNA expression when compared to control. Thus the decrease in gene expression was in dose dependent manner [Fig. 2].

4.3 Effect of β-Catenin m RNA Expression on the A549 Cancer Cells (Fold Change over Control)

The mRNA expression of β-Catenin was assessed in a dose dependent manner. The cancer cells were significantly inhibited and it was found that there was significant decrease in mRNA expression of β-Catenin when compared to control at a dose of 2mM/ml. Further there was significant decrease in m RNA expression of β-Catenin when compared to control at a dose of 4mM/ml. Thus the decrease in gene expression was in dose dependent manner [Fig. 3].

4.4 Effect of Wnt m RNA Expression on the A549 Cancer Cells (Fold Change over Control)

The mRNA gene expression of Wnt was assessed in a dose dependent manner. The cancer cells were significantly inhibited and it was found that there was significant decrease in m RNA expression of Wnt when compared to control at a dose of 2mM/ml. Further there was significant decrease in m RNA expression of Wnt when in comparison to control at a dose of 4mM/ml. Thus the decrease in gene expression was in dose dependent manner [Fig. 4].

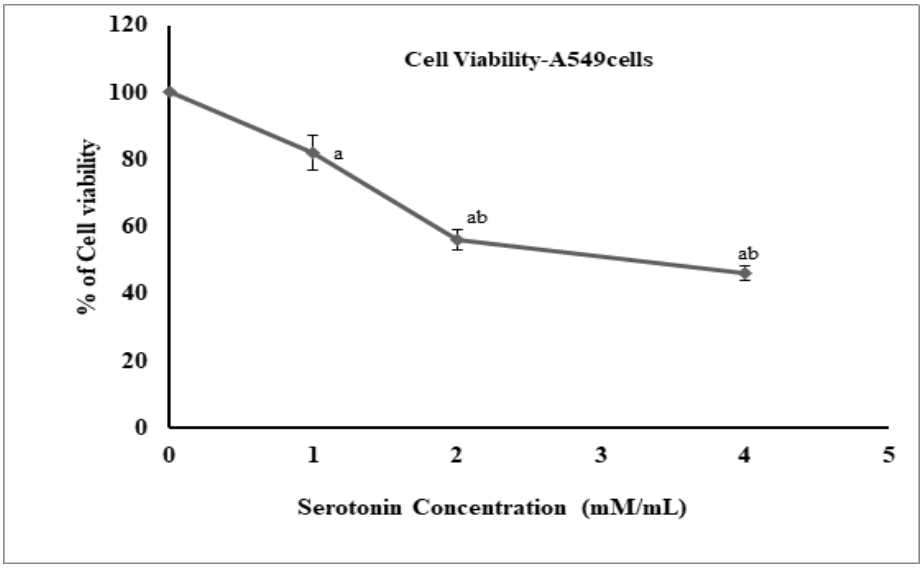

Fig. 1. Effect of serotonin on cell viability in human A549 cells. X axis represents serotonin concentration and Y axis represents % of cell viability. a-compared with untreated control cells, b-compared with 1mM treated A549 cells.Statistically Significant difference is observed in comparison with control in dose dependent manner with p< 0.05

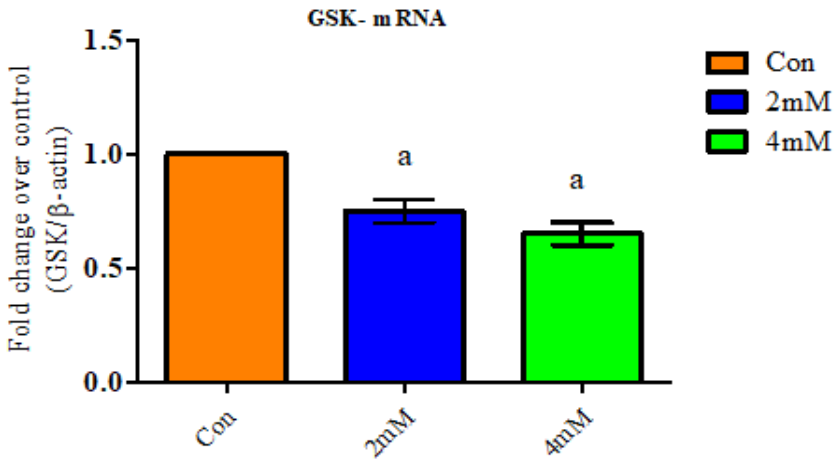

Fig. 2. Effect of serotonin on GSK mRNA expression in A549 cells. X axis represents serotonin concentration, Y axis represents fold change over control. Orange colour represents control, blue colour represents 2mM, green colour represents 4mM. Statistically Significant difference is observed in comparison with control in dose dependent manner with p< 0.05

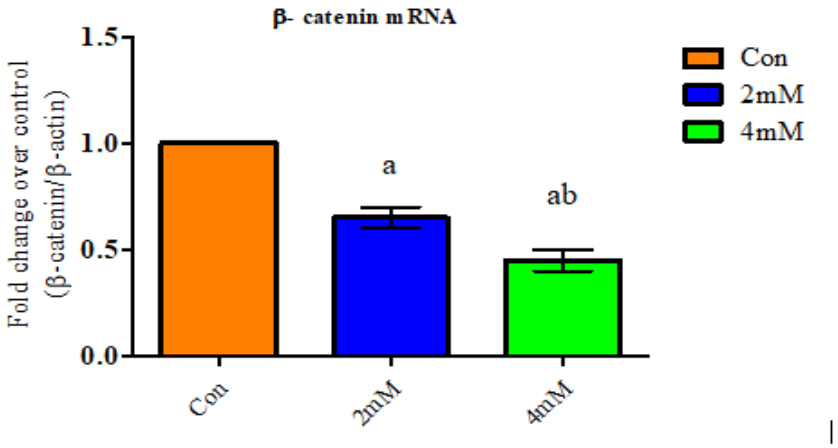

Fig. 3 Effect of serotonin on β-catenin mRNA expression in A549 cells. X axis represents serotonin concentration, Y axis represents fold change over control.Orange colour represents concentration, blue colour represents 2mM, green colour represents 4mM. A-compared with untreated control cells. B-compared with 2mM serotonin treated cells. Statistically significant difference is observed in comparison with control in dose dependent manner with p<0.05

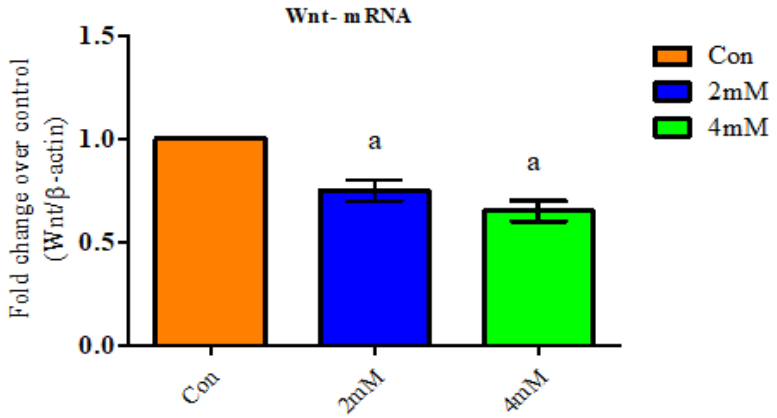

Fig. 4. Effect of serotonin on Wnt m RNA expression in A549 cells. X axis represents serotonin concentration, Y axis represents fold change over control.Orange colour represents concentration, blue colour represents 2mM, green colour represents 4mM. Statistically significant difference is observed in comparison with control in dose dependent manner with p<0.05

5. DISCUSSION

The study results may serve as supporting evidence to the context that exercise induced endogenous serotonin may act as a proactive compound against lung cancer cell progression. Understanding these types of interaction was used to design more selective and effective inhibitors. From this study, we confirmed that serotonin might act as a regulator for Wnt/beta catenin signaling pathway in lung carcinoma. The function of beta catenin is to aid cell to cell adhesion and increases the survival of cancer cells. Serotonin that is secreted endogenously as a result of chronic exercise practice may aid in regulating the m RNA gene expression of Beta catenin by reducing its expression in cancer cells there by reduces the cancer cell survival [Fig. 1,2,3,4].

As the function of WNT is to regulate cell growth, from that concentration level of serotonin

increases level by level, It will reduce beta catenin mRNA gene expression in cancer cells when compared to that signalling Secreted wnt proteins [38] bind to a class of seven pass transmembrane receptors generated by the frizzled genes, causing the dishevelled protein to be phosphorylated, preventing glycogen synthase kinase from phosphorylating essential substrate.

Through its association with axin. Catherine L Granger et al in the year 2017 announced that however we underscore the significance of actual exercise in different clinical conditions the proof isn't in any case changed into the clinical practice because of a few boundaries. The authorr tended to the patient-level elements like stationary way of life, natural variables to be considered to distinguish individualized exercise remedy that can help in the regulation of cellular breakdown in the lungs [39].

These results of the various studies emphasize the importance of the dosage of exercise in regulating the apoptotic signaling pathway. Vigorous exercise induces oxidative stress which in turn may attenuate the upregulation of GSK [40], whereas moderate exercise maintains the oxidant and antioxidant level in equilibrium which may down regulate the GSK gene expression [41]. The outcome of the present study evidence the dose-dependent response of apoptotic signaling pathway on the induction of serotonin. The limits of the study includes less sample size and in future the study can be carried out with more cell interaction in human models as a large scale study to make the context evident.

6. CONCLUSION

Within the constraints of the study, it may be inferred that exercise-induced endogenous serotonin may act as a regulator of wnt/-catenin signalling in lung cancer cells based on the results of the investigation. The exercise may help in maintaining the equilibrium of the gene expression by modeling Wnt/β-catenin signaling pathway and act as a protective factor in prevention.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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