Original Research Article

CHEMOTHERAPEUTIC POTENTIAL 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

Running Title :- Role of endogenous serotonin in lung cancer prevention

ABSTRACT:

BACKGROUND:

Lung cancer remains the leading cause of cancer related death worldwide. Physical activity and exercise are non pharmacological methods that have been shown to improve quality of life and it gives results for the population with lung cancer. Populations with lung cancer frequently do not present sufficient levels of physical activity and exercise and these can contribute to quality of life.

AIM:

To analyse Chemotherapeutic potential of endogenous serotonin replica mediated through β-catenin/Wnt signaling in lung cancer cell lines (A549)

METHODS AND MATERIALS:

Human lung cancer cell line (A549) was purchased from the National Centre for Cell Sciences (NCCS),Pune,India. Cell viability test was done by MTT assay. β-Catenin mRNA, Wnt mRNA and GSK mRNA Gene expression analysis was done by Real Time-PCR. The obtained data were analysed statistically by one-way analysis of variance and Duncan's multiple range test with Graph Pad Prism version 5 to analyse the significance. The significance was considered at $p<0.05$ level in Duncan's test.

RESULT:

The Results suggest that maximum inhibition of cell growth was at concentration (2-4mM/ml) used in this study when compared to control. The cancer cells were significantly inhibited and it was found that there was significant reduction in mRNA expression of GSK, β-Catenin, Wnt when compared to control at a dose of 2mM/ml.

CONCLUSION:

From the results of the analysis and within the limitations of the study.It can be concluded 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

INTRODUCTION:-

Lung cancer remains the leading cause of cancer related death worldwide. Populations with cancer were advised to rest, recover and save energy, avoiding engaging in tiring physical activity. Nevertheless,starting in the late 1980(1), new data progressively emerged, supporting the notion that physical activity defined as any bodily movement produced by skeletal muscle that results in energy expenditure and exercise may provide relevant benefits in production of endogenous serotonin. Lung malignancies are the leading cause of cancer related death(2). Human lung cancer cells (A549) constitute a leading cause of cancer and related mortality world wide(3). Serotonin produces chemical nerve cells and sends signals between your nerve cells. It is found in the digestive system too. It is made from the essential amino acid tryptophan(4). Serotonin stabilises our mood and feeling of well being, happiness and hormones impacts your entire body and enables brain cells and other nervous systems to help in sleeping,eating and digestion(5).

Physical activity and exercise are non pharmacological methods that have been shown to improve quality of life and it gives results for the population with lung cancer. P with lung cancer frequently do not present sufficient levels of physical activity and exercise and these can contribute to quality of life. There is no specific exercise and guidelines for the population with lung cancer are available(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 in gene expression favours cancer cell proliferation. Caspase 3 mRNA is an apoptosis gene and increase in gene expression reduces the cancer cells. 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 team has extensive knowledge and research experience that has translate into high quality publications (18–25),(26),(27),(28),(29,30),(31),(32),(33–37) Thus, the aim of the study is to analyze the

chemotherapeutic potential of endogenous serotonin replica in lung cancer prevention and prognosis in lung cancer cell line.

MATERIAL AND METHODS:

This is an in vitro - experimental study conducted in a private dental college and hospitals, in chennai. The study is approved by the institutional review board.

PROCEDURE

Serotonin dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Pvt Ltd, USA. Trypsin-EDTA, fetal bovine serum (FBS), antibiotics-antimycotics, RPMI 1640 medium and phosphate buffered saline (PBS) were purchased from Gibco, Canada. (5,5,6,6-tetrachloro-1,1,3,3 - tetraethylbenzimidazolocarbocyanine iodide) and Real Time PCR kit was purchased TAKARA (Meadowvale Blvd, Mississauga, ON L5N 5S2, Canada).

Cell lines and cell culture

Human lung cancer cell line (A549) was purchased from the National Centre for Cell Sciences (NCCS), Pune, India. Cells were cultured in DMEM medium (Thermo Fisher Scientific, CA, USA) containing 10% fetal bovine serum (Thermo Fisher Scientific, CA, USA), 100 U/ml penicillin and 100 µg/ml streptomycin (Thermo Fisher Scientific, CA, USA) at 37°C with 5% CO_2.

Cell viability by MTT assay

Cell viability was assayed using a modified colorimetric technique that is based on the ability of liver cells to convert MTT, a tetrazolium compound into purple formazan crystals by mitochondrial reductases (Mosmann, 1983). Briefly, A549 lung cancer cells (1×10^4/well) were exposed to different concentrations of serotonin (100, 200 and 400µM) with A549 cells for 48 h. At the end of the treatment, 100 µl of 0.5 mg/ml MTT solution was added to each well and

incubated at 37 ◦C for an hour. The crystals formed were dissolved in dimethyl sulfoxide (100 µl) and incubated in dark for an hour. Then the intensity of the color developed was assayed using a Micro ELISA plate reader at 570 nm. The number of viable cells was expressed as the percentage of control cells cultured in serum-free medium. Cell viability in the control medium without any treatment was represented as 100%. The cell viability is calculated using the formula: % cell viability = [A570 nm of treated cells/A570 nm of control cells] × 100.

Gene expression analysis by Real Time-PCR

Samples from each group were submerged in 2 ml Trizol (Invitrogen, Carlsbad, CA, USA) for RNA extraction and stored at −80°C until further processed. cDNA synthesis was performed on 2 µg RNA in a 10 µl sample volume using Superscript II reverse transcriptase (Invitrogen) as recommended by the manufacturer. Real-time PCR array analysis was performed in a total volume of 20 µl including 1 µl cDNA, 10 µl qPCR Master Mix 2x (Takara, USA) and 9 µl ddH_2O. Reactions were run on an CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA) using universal thermal cycling parameters (95°C for 5 min, 40 cycles of 15 sec at 95°C, 15 sec at 60°C and 20 sec at 72°C; followed by a melting curve: 5 sec at 95°C, 60 sec at 60°C and continued melting). For quality control purposes, melting curves were acquired for all samples. The specificity of the amplification product was determined by melting curve analysis for each primer pair. The data were analyzed by comparative CT method and the fold change is calculated by 2−ΔΔCT method described by Schmittgen and Livak (2008) using CFX Manager Version 2.1 (Bio Rad, USA).

Statistical analysis

The obtained data were analyzed statistically by one-way analysis of variance (ANOVA) and Duncan's multiple range test with a computer-based software (Graph Pad Prism version 5) to analyze the significance of individual variations among the control and experimental groups. The significance was considered at $p<0.05$ level in Duncan's test.

RESULTS:

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 dose 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 compared to control. (Figure 1)

Effect of GSK mRNA expression on the A549 cancer cells (Fold change over control)

The mRNA expression of GSK was assessed in a dose dependent manner. The cancer cells were significantly inhibited and it was found that there was significant reduction in mRNA expression of GSK when compared to control at a dose of 2mM/ml. Further there was significant reduction in mRNA expression of GSK when compared to control at a dose of 4mM/ml. Thus the decrease in gene expression was in dose dependent manner.(Figure 2)

Effect of β-Catenin mRNA 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 reduction in mRNA expression of β-Catenin when compared to control at a dose of 2mM/ml. Further there was significant reduction in mRNA 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. (Figure 3)

Effect of Wnt mRNA expression on the A549 cancer cells (Fold change over control)

The mRNA expression of Wnt was assessed in a dose dependent manner. The cancer cells were significantly inhibited and it was found that there was significant reduction in mRNA expression of Wnt when compared to control at a dose of 2mM/ml. Further there was significant reduction in mRNA expression of Wnt when compared to control at a dose of 4mM/ml. Thus the decrease in gene expression was in dose dependent manner. (Figure 4)

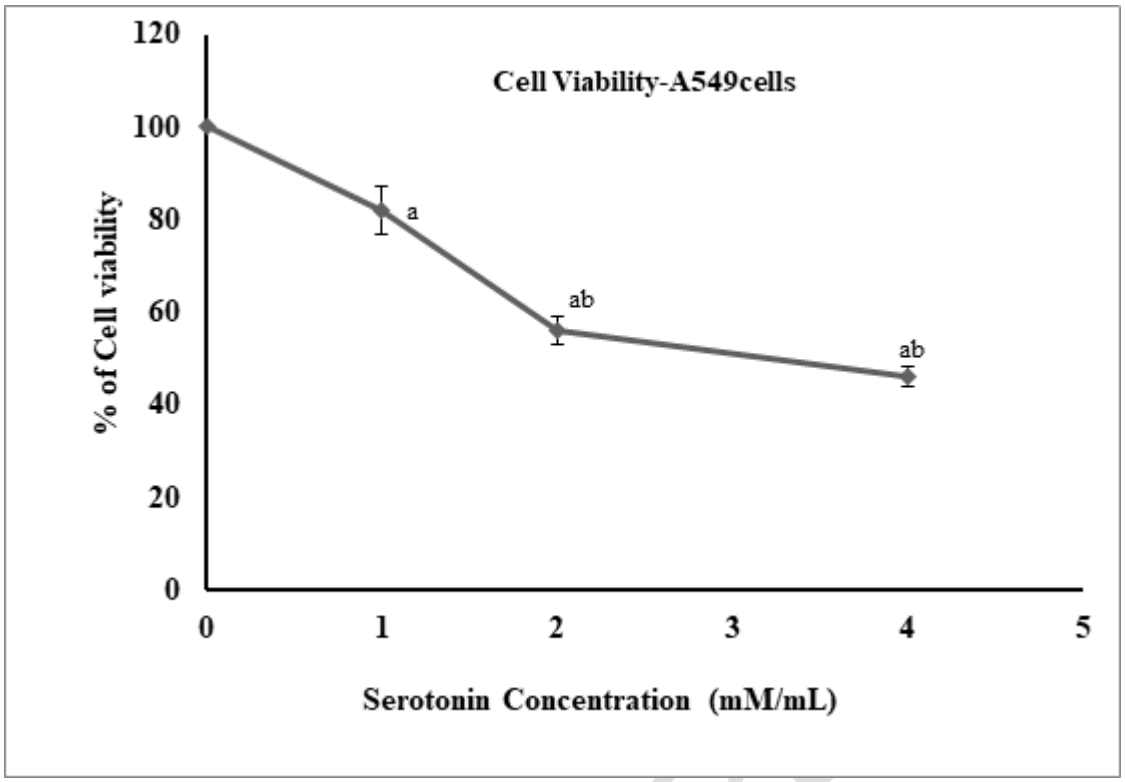

Fig 1: Effect of serotonin on cell viability in human A549 cells. X axis represents serotonin concentration and Y axis represents % of cell viability. a-compared with untreated control cells, b-compared with 1mM treated A549 cells.Statistically Significant difference is observed in comparison with control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

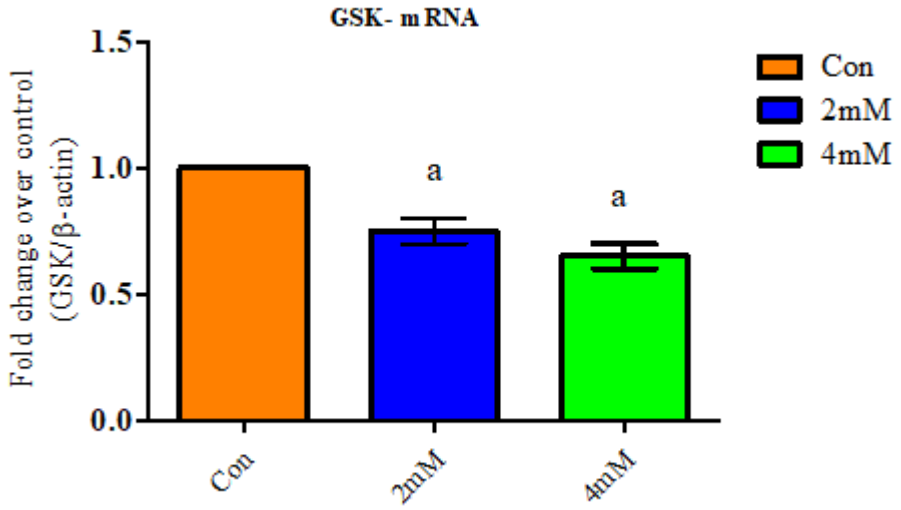

Fig:2 Effect of serotonin on GSK mRNA expression in A549 cells. X axis represents serotonin concentration, Y axis represents fold change over control. Orange colour represents control, blue colour represents 2mM, green colour represents 4mM.

Statistically Significant difference is observed in comparison with control in dose dependent manner with p< 0.05.

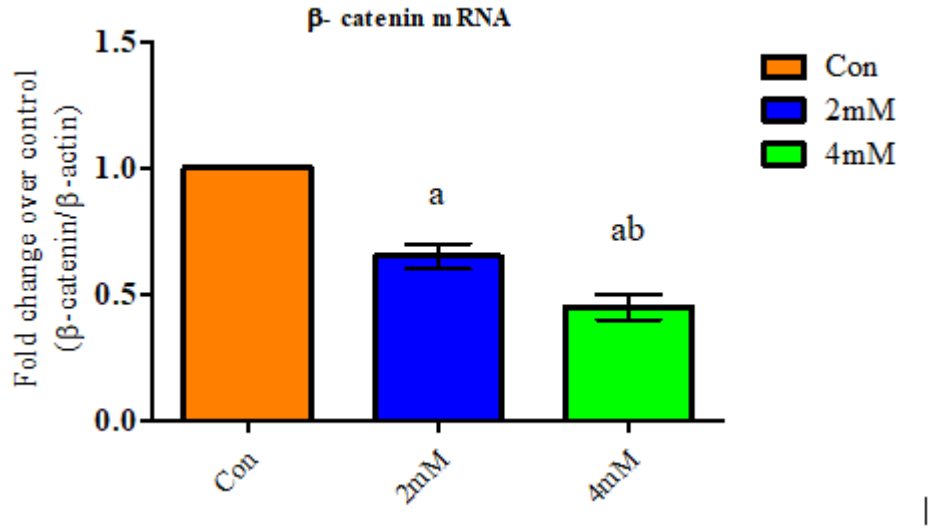

Fig 3:Effect of serotonin on β-catenin mRNA expression in A549 cells. X axis represents serotonin concentration, Y axis represents fold change over control.Orange colour represents concentration, blue colour represents 2mM, green colour represents 4mM. A-compared with untreated control cells. B-compared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

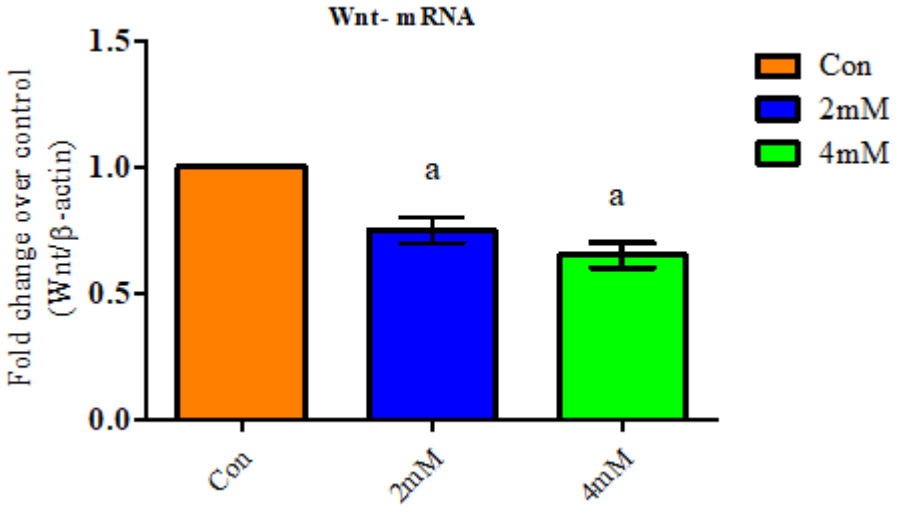

Fig: 4 Effect of serotonin on Wnt mRNA expression in A549 cells. X axis represents serotonin concentration, Y axis represents fold change over control.Orange colour represents concentration, blue colour represents 2mM, green colour represents 4mM. Statistically significant difference is observed in comparison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

DISCUSSION:

The results of the present study may act 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 cancer. As the function of Beta catenin is to aid cell-cell adhesion, from that concentration level of serotonin increases level by level, it will decrease mRNA gene expression of cancer cell proliferation level of beta catenin.

As the function of WNT is to regulate cell growth, from that concentration level of serotonin increases level by level, it will decrease mRNA gene expression of cancer cell proliferation level of beta catenin which is compared to that signaling is initiated by the secreted wnt proteins(38), which bind to a class of seven pass transmembrane receptors encoded by the frizzled genes, activation of the receptor leads to the phosphorylation of the dishevelled protein which, through its association with axin, prevents glycogen synthase kinase from phosphorylating critical substrate. 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 results of the current study evidence the dose-dependent response of apoptotic signaling pathway on the induction of serotonin. The limitation 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.

CONCLUSION:

From the results of the analysis and within the limitations of the study, It can be concluded 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.

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Conflict of interest :

The authors declare no conflict of interest

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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

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