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UNVEILING SEROTONIN'S HEALING CONCERT: EXPLORING IN-VITRO ANTI-INFLAMMATORY POTENTIAL ON MCCOY CELLS

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Abstract

Cancer affects around 0.2 % of world population every year. The cancers are characterized by malignant tumor and prolonged inflammation. An important factor in the development of tumors is inflammation. McCoy cells or the mouse fibroblast cells exhibit easy isolation and abundant yield and are a useful tool to study inflammation. Cancer prevention and therapy may benefit from anti-inflammatory drugs. Serotonin is a neurotransmitter that controls several bodily physiological functions and secreted mainly during exercise. It has been demonstrated that serotonin can have pro-inflammatory or anti-inflammatory effects on the body, depending on the situation and the particular receptor subtype that it activates. It was observed that serotonin exhibited concentration dependent cytotoxicity. The results showed that serotonin strongly induced the expression of TGF-beta whereas it suppressed the pro-inflammatory gene IL-6. The Bcl-2 genes, which prevent apoptosis by lengthening the time to death and increasing inherent cell-to-cell differences in the mitochondrial mechanism of cell death, were also found to be reduced by serotonin, according to research findings. Hence it can be stated that it could have a negative effect on HIF-alpha expression, a known positive regulator of inflammation. From the findings of the current study it can be concluded that exercise induced serotonin secretion exhibits anti-inflammatory characteristics. Thus serotonin could act as an instrumental anti-inflammatory agent in prevention and treatment of cancer.

Keywords: Serotonin, anti-inflammation, McCoy cells (fibroblast cells), Bcl2, IL-6, TGF-beta

INTRODUCTION

The prevalence of cancer has been rising over time, making it a serious worldwide concern[1]. There are over 100 different kinds of cancer, which are often categorized according to the type of cell they develop from[2]. Among them, the most widespread cancers are carcinomas, sarcomas, leukemias, lymphomas, CNS cancers, melanoma, thyroid, renal, kidney, liver, ovarian and testicular cancers. Incidence of lymphomas has increased in recent years. Lymphoma can be classified into 2 major types namely HL (Hodgkin lymphoma), NHL (Non-Hodgkin Lymphoma). Depending on the exact type (HL or NHL) and location, lymphoma prevalence varies[3]. Compared to Hodgkin lymphoma, non-Hodgkin lymphoma is more prevalent. White blood cells called lymphocytes, which are crucial in the fight against infections, increase abnormally in lymphomas[4]. There are several factors which lead to development of lymphomas like increasing age, weak immune system, viral infections, autoimmune diseases, obesity, etc. Inflammation has been found to be a potential risk factor in development of certain types of lymphomas[5].

When the immune system of the body detects possible dangers, such as viruses, wounds, or damaged cells, inflammation is a normal and vital biological reaction that takes place. It is a sophisticated defense system made to safeguard the body and start the healing process. Nevertheless, persistent or chronic inflammation may play a role

in the onset of a number of illnesses, several types of cancers including lymphomas[6]. It can be caused by a number of things, including obesity, irritant exposure, autoimmune disorders, chronic infections, and smoking[7]. Chronic inflammation is associated with a number of chronic disorders, including heart and vascular diseases, diabetes, arthritis, several malignancies, etc[8]. Addressing the underlying causes of chronic inflammation is crucial for managing it, including managing autoimmune illnesses, addressing infections, eating a balanced diet, getting regular exercise, and abstaining from smoking and excessive alcohol use[9]. Furthermore it has been stated that inflammation can be reduced by regular and moderate exercise, by promoting healthy immune system function.

A number of immune cells are activated during inflammation, inflammatory mediators (including cytokines and chemokines) are released, and signaling pathways are controlled. Cell culture models are frequently used by researchers to study particular facets of inflammation, such as the reaction to infections, the function of particular receptors, or the results of anti-inflammatory medication[10]. One of the most often used treatment approaches to treat cancer is chemotherapy. However, cancer chemotherapy drugs have frequently been linked to immune system dysfunction, which might ultimately increase the risk of autoimmune diseases like rheumatoid arthritis. A combination of genetic and environmental variables may contribute to the genesis of rheumatoid arthritis, despite the fact that the precise molecular aspects of its induction have not yet been fully understood[11]. The fact that people with rheumatoid arthritis have a greatly increased chance of getting several forms of cancer makes the situation more difficult. It's interesting to know that various cancer chemotherapy medications are frequently utilized to manage rheumatoid arthritis symptoms. Innate immunity and inflammation are well-established processes that are regulated by nuclear factor kappa B (NF-κB). Critical processes linking inflammation and the development of cancer also include NF-κB.

Serotonin (5-hydroxytryptamine / 5-HT) is a neurotransmitter and hormone that is essential for controlling a number of bodily functions, including physiology and behavior. It is frequently discovered in the thrombocytes, gastrointestinal tract (GIT) and also the central nervous system (CNS) [12]. Serotonin plays a variety of activities, including those of a neurotransmitter, a mood regulator, a regulator of gastrointestinal rhythms[13], and a vasoconstrictor that promotes clotting. Serotonin secretion is influenced by different factors like mood, emotions, sleep, diet, nutrition and exercise[14]. Serotonin is one of the neurotransmitters that are stimulated by exercise to be released in the brain. During exercise, nerve cells (neurons) activate, releasing serotonin into the synapses to improve neurotransmission.Serotonin levels that are in balance assist control emotional reactions, improve wellbeing, and may lessen the symptoms of despair and anxiety[15]. Antidepressant drug classes that target serotonin levels in the brain comprise both serotonin-norepinephrine reuptake inhibitors (SNRIs) and selective serotonin reuptake inhibitors (SSRIs). They function by preventing serotonin from being reabsorbed, which allows more of it to stay in the synapses between nerve cells (neurons) [16]. The availability of serotonin is enhanced, which improves mood control and neurotransmission[17]. Numerous studies have proven SSRIs to be more palatable and safe than earlier antidepressants. These antidepressants are known to possess anti-inflammatory characteristics in addition to their main function of treating depression and anxiety[18]. The immune system is known to be modulated by serotonin, a neurotransmitter that is the target of SSRIs and

SNRIs. These drugs may indirectly affect the immune system and its inflammatory processes by affecting serotonin levels[19]. Many studies have stated that anti-inflammatory strategies help in preventing carcinogenesis and progression of cancer.

Inflammation can easily be studied with the help of McCoy or the mouse fibroblast cells.

McCoy cells are a collection of mesenchymal-derived resident cells with a variety of locations, varied appearances, and unique functions, fibroblasts are heterogeneous in nature. These cells can be used to examine inflammation and immunity due to their widespread distribution as tissue cells and ability to react to substances generated by recently activated innate immune cells[20].

The current study thus tries to explore the anti-inflammatory potential of serotonin on McCoy or mouse fibroblast cells. This study can open new avenues in development of anti-inflammatory therapies and cancer treatments.

MATERIALS AND METHODS

MTT assay

The McCoy cells were purchased from the National Centre for Cell Sciences (NCCS) in Pune and cultured in a CO2 incubator (5%) using RPMI media with 10%FBS. Serotonin was procured from Sigma, (India). The cells were raised in Modified Essential Medium (E), which was supplemented with Non-Essential Amino Acids, 10% vol/vol inactivated fetal calf serum, 100 U ml of 1 penicillin, and 10 mg ml of 1 streptomycin at 37°C, 5% CO_2. 8000 cells were inoculated and left for adherence (24 h) [21]. Then they were treated with serotonin (50, 100, 250, 400, and 500 g/ml). Doxorubicin was used as positive control. After 48 h, MTT (0.5 mg ml^{-1}) was added to each well and kept for 4 h. Then 100 µl of acidic isopropanol (in 0.08 N HCl) was added and kept for 15 min. Then MTT-formazan's absorbance was measured at 570 nm. The ratio of treated to untreated cells' absorbance, reported as a percentage, was used to measure viability. The IC_{50} concentration was applied to the cells[22].

Two concentration, 50 and 100 µg/ ml (< IC_{50}) was selected for gene expression analysis. RNA was isolated using the TRIZOL method, and samples of synthesized cDNA (from the Takara Primscript kit for RNA isolation) were diluted 30 times in nuclease-free water and stored at 20 °C until needed. Using qRT-PCR and a TaKaRa Thermal Cycler Dice TP 800 real-time system, the gene expression of many inflammatory gene representatives, including IL-6, TGF- and -actin, was investigated[23].

Statistical Analysis

The specified error limits and the depicted error bars indicate straightforward standard deviations from the mean. The most exact numerical result can usually be to indicate the least significant digit. Results were deemed significantly different when P 0.05 was used to compare data from several samples. With the help of the GRAPHAD Prism, a two-way Anova was conducted[25].

RESULTS AND DISCUSSION

Cytotoxic effect of serotonin

McCoy cells (mouse fibroblast cells) were treated to various doses (50 and 100µg/ ml) of serotonin for 24 hours in order to detect the cytotoxic effect of serotonin. The cell population was shown to decrease with increasing serotonin concentration, but the cell population was larger in the serotonin-free control group (Fig. 1 and 1A). The doxorubicin and serotonin showed cytotoxicity as 27.56 ± 2.15% and 23.36 ± 0.87 % at 50 µg/ ml respectively. At 500 µg/ ml, they showed 96.23 ± 1.95 % and 86.75 ± 1.09 % as cytotoxicity respectively. The results showed that that the serotonin exhibited its cytotoxicity on concentration dependent manner. Compared with the control, the results were statistically significant. IC_{50} was calculated as 180.56 ± 1.25, 240.56 ± 1.56 µg/ ml for doxorubicin and serotonin respectively[26].

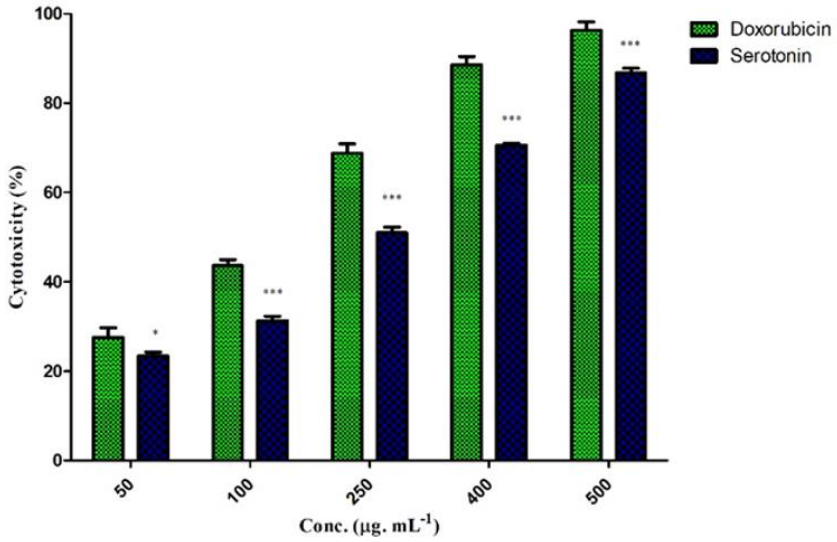

Figure 1: The cytotoxic effect of serotonin on McCoy cells. Doxorubicin was used as control. The cytotoxicity was dose- dependent manner. (Two Way ANOVA, Bonferroni post- hoc test, *- 0.05, *** - 0.001)

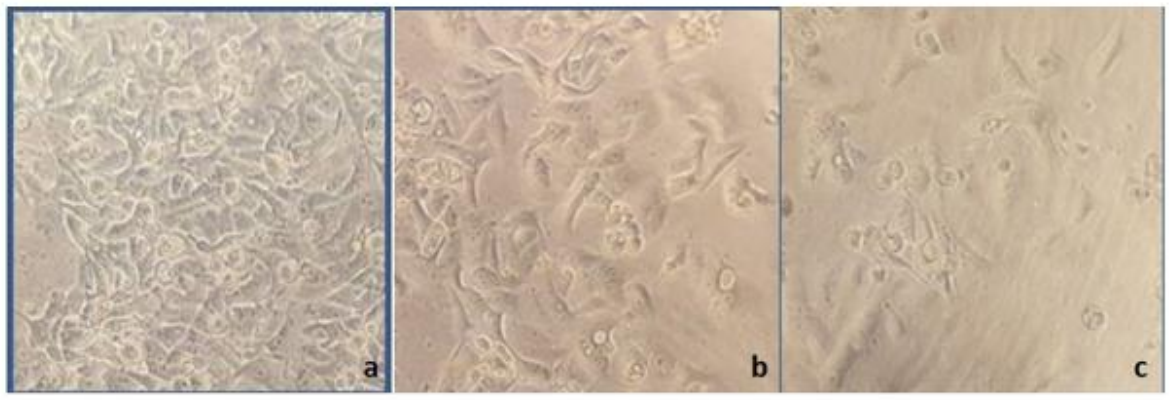

Figure 1a: The cytotoxic effect of serotonin on McCoy cells. Doxorubicin was used as control. The cytotoxicity was dose- dependent manner. a.Control, b. Cells with serotonin (50µg/ml), c. cells with serotonin (100µg/ml)

Serotonin promoteted anti-inflammatory gene profile

The results showed that the serotonin promoted the anti-inflammatory gene expression in treated McCoy cells, meanwhile it reduced pro- inflammatory genes. The pro inflammatory genes such as IL- 6, BCl-2, p38 were down regulated. Serotonin upregulated TGF-β.

Serotonin reduced IL-6 expression in McCoy cells

IC_{50} of the serotonin was calculated as 240.56 ± 1.56 µg/ ml for serotonin. The McCoy cells were treated with lesser concentration (50 and 100 µg/ ml) for 48h. Their total RNA was isolated and analyzed for gene expression with specific primers using RT-PCR. The results showed that serotonin decreased IL- 6 expression. Compared to control, approximately, 1.75-fold reduction was recorded at 100 µg/ ml followed by 50 µg/ ml respectively. The results were statistically significant[27].

p38 gene reduced under serotonin treatment

Serotonin showed a drastic reduction on p38 expression of McCoy cells. The cells were treated with 50 and 100 µg/ ml and the p38 was down- regulated by dose-dependent activity. At, 100 µg/ ml it showed higher reduction than 50 100 µg/ ml treatment. The results were evaluated using One Way ANOVA and found to be statistically significant ($p < 0.05$).

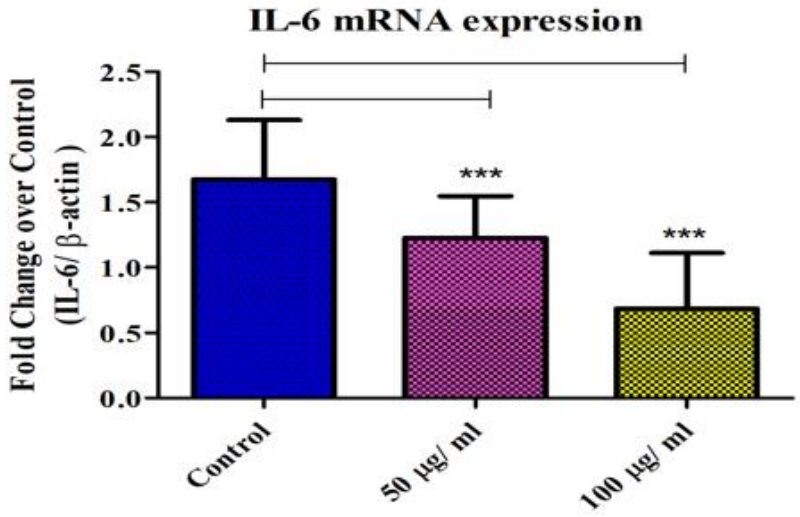

Figure 2: Serotonin inhibited IL-6 expression on McCoy cells. The serotonin at 50 and 100 µg/ ml reduced IL- 6 expression on concentration dependent manner (One Way Anova –Newmann Keuls post hoc test) with p value $p < 0.001$- ***).

Serotonin reduced BCl-2 expression in McCoy cells

The McCoy cells were incubated with 50 and 100 µg/ ml of serotonin respectively. The concentrations were selected based on the IC_{50}. The gene expression analysis showed that it reduced proinflammatory gene, BCl-2. The reduction was dose-dependent manner. It showed higher reduction at 100 µg/ ml than 50 µg/ ml respectively. Compared to control, two-fold reduction was recorded at 100 µg/ ml.

Serotonin increased TGF-β expression

McCoy cells showed increased expression of TGF-β compared to the control cells. At 100 µg/ ml, it increased the expression of anti- inflammatory gene, TGF-β. Comparatively, it showed lower expression at 50 µg/ ml than 100 µg/ ml. The results were statistically significant.

The PDB databank was accessed to download the protein structure, G protein signaling homology (RH) domain (PDB: 7WF9), which includes the structure of the imidazolbenzoxepin molecule linked to protein (Figure. 6a). The binding site detector was used to locate the protein's binding pockets following the protein wizard's refinement of the protein structure.

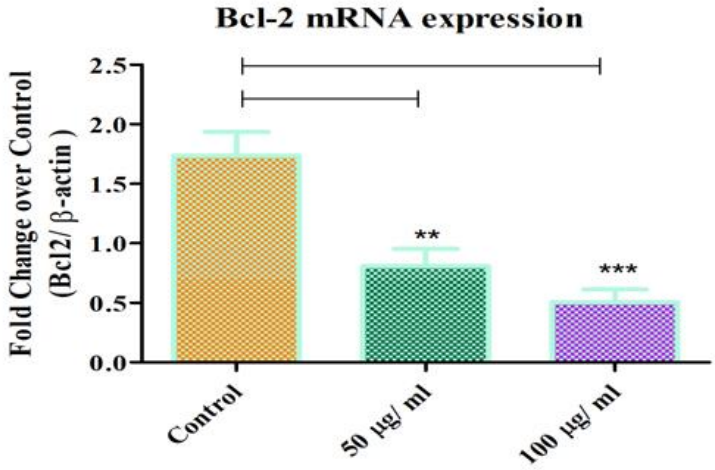

Figure 3: Serotonin prevented McCoy cells from expressing BCl-2. Serotonin inhibited BCl-2 expression in a concentration-dependent manner at 50 and 100 g/ml. (One Way Anova –Newmann Keuls post hoc test) with p value p< 0.001- ***)

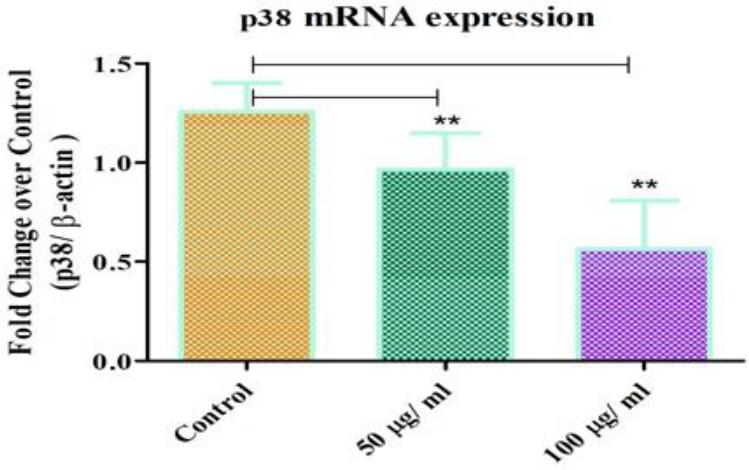

Figure 4: The expression of p38 in McCoy cells was inhibited by serotonin. At 50 and 100 g/ml, serotonin suppressed BCl-2 expression in a concentration-dependent manner.

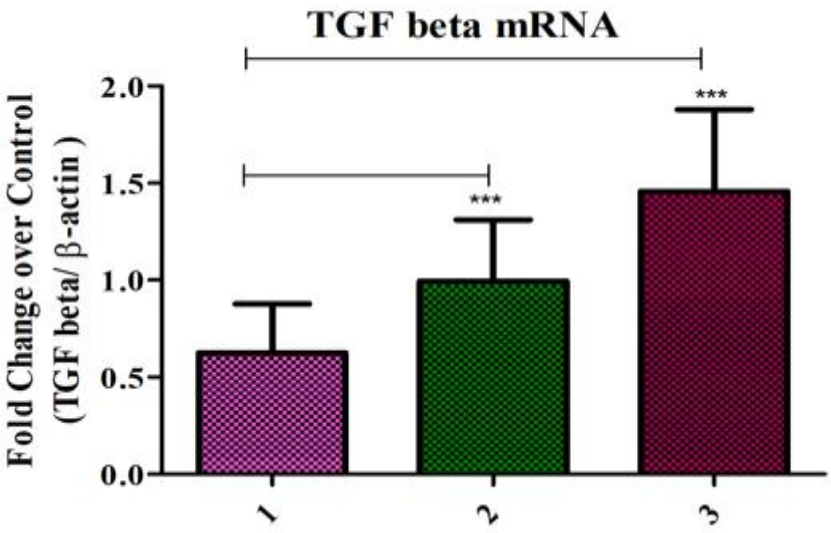

Figure 5: Serotonin increased the expression of TGF-β in McCoy cells. Serotonin upregulated TGF-β expression at concentrations of 50 and 100 g/ml in a concentration-dependent manner. (One-Way ANOVA with a Post Hoc Newmann-Keuls Test, p = 0.001-***).

An essential idea in drug development is the confirmation and positioning of the ligand-inhibitor combination at the active or docking site. Schrodinger docking program was utilized in the current investigation, and Figures 6b and 6c show the outcomes. Figure 6b depicted the pose with the most docking energy. Serotonin formed three intramolecular hydron bonds with 7WF9 (Figure.6c). We also looked at the existence of intermolecular interactions in protein-ligand complexes in this work using the LigPlot+ tool and the BIOVIA DS Visualizer (Figure. 8 b-c). The serotonin formed two hydrogen bonds with 7WF9 via histidine (HIS 322), serine (SER 319) and glutamine (GLU 318) respectively. The anticipated binding free energy of the 7WF9-serotonin complex was found to be -4.158 kcal/mol (Figure 7).

The intricate relationship between serotonin and inflammation has garnered significant attention in scientific research. Serotonin, primarily recognized for its role in mood regulation, has demonstrated intriguing properties in modulating inflammatory responses within the body. The interplay between serotonin and pro-inflammatory genes, such as IL-6 and p38, presents a complex yet promising avenue for understanding potential therapeutic interventions for inflammatory conditions. The findings of this study suggest a compelling association between serotonin levels and the suppression of pro-inflammatory gene expression. Serotonin, a neurotransmitter primarily associated with mood regulation and neurological processes, has been revealed to exert modulatory effects on immune responses. The observed suppression of pro-inflammatory genes, IL-6 and p38, with increasing concentrations of serotonin underscores the potential regulatory role of this neurotransmitter in dampening inflammatory pathways[28].

Serotonin's ability to suppress pro-inflammatory gene expression aligns with existing literature indicating its involvement in immune modulation. Research has highlighted the presence of serotonin receptors on immune cells, implicating direct communication between the neurotransmitter and the immune system. This interaction allows

serotonin to exert regulatory control over immune cell activity, thereby influencing the expression of genes involved in the inflammatory cascade[29].

The downregulation of pro-inflammatory genes, such as IL-6 and p38, in response to increasing serotonin concentrations provides valuable insights into potential therapeutic implications. IL-6, a key cytokine involved in the inflammatory response, plays a pivotal role in initiating and propagating inflammation. Its suppression by serotonin suggests a potential avenue for mitigating excessive inflammation, which is often implicated in various pathological conditions, including autoimmune disorders and chronic inflammatory diseases. Furthermore, the observed suppression of p38, a critical signaling molecule in pro-inflammatory pathways, underscores the multifaceted impact of serotonin on modulating inflammatory responses. The inhibition of p38 signifies a potential mechanism by which serotonin mitigates the activation of downstream inflammatory cascades, contributing to the overall attenuation of the inflammatory process.

The implications of these findings extend beyond basic research, offering potential therapeutic avenues for managing inflammatory conditions. Strategies aimed at modulating serotonin levels or enhancing its activity could hold promise in regulating excessive inflammation. Pharmacological interventions targeting serotonin receptors or pathways involved in serotonin synthesis and signaling may represent novel approaches for developing anti-inflammatory therapies[30]. However, it is essential to acknowledge the complexity of the serotonin-immune system interplay and its implications for clinical translation. While the study demonstrates a correlation between serotonin levels and the suppression of pro-inflammatory genes, further investigations are warranted to elucidate the precise molecular mechanisms underlying this regulatory effect.

Understanding the specific pathways and receptors through which serotonin modulates inflammatory gene expression is crucial for developing targeted and effective therapeutic interventions[31]. Moreover, considering the diverse roles of serotonin in various physiological processes, potential side effects and unintended consequences of modulating serotonin levels must be thoroughly evaluated. The intricate balance of serotonin in neurological, gastrointestinal, and cardiovascular systems necessitates a comprehensive assessment of the systemic effects of manipulating serotonin for anti-inflammatory purposes. In conclusion, the study's findings shed light on the potential of serotonin as a regulator of pro-inflammatory gene expression, exemplified by the suppression of IL-6 and p38 with increasing serotonin concentrations. This discovery holds promise for advancing our understanding of immune modulation and suggests potential avenues for therapeutic interventions in inflammatory conditions. However, further research elucidating the underlying mechanisms and comprehensive evaluation of the therapeutic potential and safety profile of targeting serotonin in inflammation management are imperative for clinical translation. Serotonin suppresses pro-inflammatory gene expression. Serotonin decreases the action of pro-inflammatory cytokines. The expression of genes IL-6, p38 (proinflammatory genes) was suppressed with increasing concentrations of serotonin. This analysis explained that serotonin reduced the activity of pro-inflammatory genes and thus helped in reducing inflammation. Serotonin suppresses the activity of Bcl2, one of the anti-apoptosis gene. Thus this analysis showed that serotonin was one of the factors which caused cell death.

CONCLUSION

The study's outcomes suggested that the exercise-triggered serotonin release manifested the anti-inflammatory properties. This study revealed the nature of the serotonin as a potentially instrumental anti-inflammatory agent in cancer prevention and treatment. Harnessing exercise-induced serotonin, it presented a promising avenue for modulating inflammatory responses, potentially curbing cancer progression. Serotonin's multifaceted role, coupled with its capacity to suppress inflammation, offers a novel therapeutic approach in combating malignancies.

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Conflict of Interest

The authors declare no conflict of Interest

Funding Details

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Author contribution

SG and TM performed the majority of the experiments, MS performed in silico analysis, MS and LP gave technical support, analysed the data and wrote the manuscript. LP designed the study and finalizing the manuscript. All authors critically reviewed and approved the final manuscript.

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