In vitro examination of the role of exercise-induced serotonin replication in the prevention and advancement of liver cancer via the intrinsic apoptotic signalling pathway.

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ABSTRACT:

Background: Serotonin is an important mediator of variety of physiological processess including fibrogenesis,steatosis,cell proliferation. It functions as a neurotransmitter and vasoconstrictor. In this study we explained the influence of physical exercise which induces serotonin in modulating liver cancer and its functions as a neurotransmitter that inhibits the growth of tumors in a concentration dependent. Hence they are less likely to develop liver cancer through an intrinsic apoptotic signaling pathway through three genes (Bcl-xl, Bcl-2, p53).
Aim: To analyze Influence of exercise induced serotonin replica in prevention and progression of liver cancer through intrinsic apoptotic signaling pathway.
Materials and methods:

Human liver cancer cell line (HEPG2) was brought from NCCS, Pune, India. Cell viability test for seratonin and Gene expression analysis were carried out for The p53, Bcl-xl, Bcl-2 mRNA gene expression using MTT and PCR respectively. The results were analyzed using appropriate statistical tools.

Results: The p53 mRNA gene expression is increased on induction of 2mM, 4mM dosage of serotonin. Bcl-xl, Bcl-2 and % of cell viability decreases on induction of serotonin. Hence the compound has anti - cancer activity.
Conclusion: The results of the study concluded that people who exercise regularly have endogenous secretion of serotonin that inhibits tumor growth through modulating apoptotic and signaling pathways and hence they are less likely to develop liver cancer.

Keywords: Serotonin; neurotransmitter; apoptotic pathway' Bcl-2; Bcl-xl' p53; Innovative method

INTRODUCTION:

Liver cancer is the fourth most prevalent cause of cancer death worldwide, and it is on the rise due to the "diabesity epidemic."[1] . Exercise induces serotonin which functions as a neurotransmitter and vasoconstrictor[2,3]. Population data suggests Regular exercisers have a lower risk of developing liver cancer, through intrinsic apoptotic signaling pathway three of which were tumor suppressor gene p53, Bcl-2, Bcl-xl mRNA gene expression[4]. It has equal effects on reducing intrahepatic fat. Decreased hepatic lipid content improves cardiorespiratory fitness, this effect disappears when adjusted for weight loss[5]. The progression of certain tumors were accompanied by serotonin receptor expressions by a dysregulated pattern. [5,6]. It contributes to the development of human malignancies. The experience from our previous studies [7] [8,9] [8][10][11][12][13][11,13][14][15] [16] have led us to concentrate on the study.

The Bcl-2 family is the most well-studied set of apoptosis-mediating factors; their biological functions are divided into apoptosis-promoting (Bcl-xl, Bcl-2, p53) and apoptosis-inhibiting (Bcl-xl, Bcl-2, p53) features, and it is linked to cancer pathogenesis and progression.[17,18] ,[17]. Studies at molecular levels were performed by our team of researches which insisted us to proceed this study [19–26],[27],[28],[29],[30,31],[32],[33],[34–38] The current study's goal is to assess the role of exercise-induced serotonin replication in the prevention and advancement of liver cancer via the intrinsic apoptotic signaling system.

MATERIALS AND METHODS:

Cell line centre, Pune, India, provided the human liver cancer cell line (HEPG2). Tissues were cultured in RPMI media with 10% foetal bovine serum, 100 U/ml penicillin, and 100 g/ml streptomycin at 37 degrees Celsius and 5% CO2.The MTT test was used to measure cell growth. (HEPG2) tissues were sown in 96-well plates with 5x104/200l and grown overnight. Six duplicate wells were used in each treatment. All of the tissues were then grown for another 48 hours. The experiment was carried out three times. The

MTT absorbance in negative control tissues was employed as a 0 percent cell inhibition measurement. The expression status of m RNA was analyzed by Polymerase chain reaction for identifying the fold change of p53, Bcl-2, Bcl-xl mRNA Gene expression over control samples. The samples were assessed using (ANOVA) and Duncan's multiple range test with p value at 0.05.

RESULTS:

Seratonin was found to reduce the abnormal proliferation of cells by reducing its cell viability at concentration (2-4µg/ml). [Fig 1]. Effect of seratonin on BCL-2, BCL-XL, the cells were reduced in its proliferation and the reduction in in mRNA expression is observed. The P53 gene expression was also increased in accordance with the dose. [Fig 2] [Fig 3] [Fig 4]

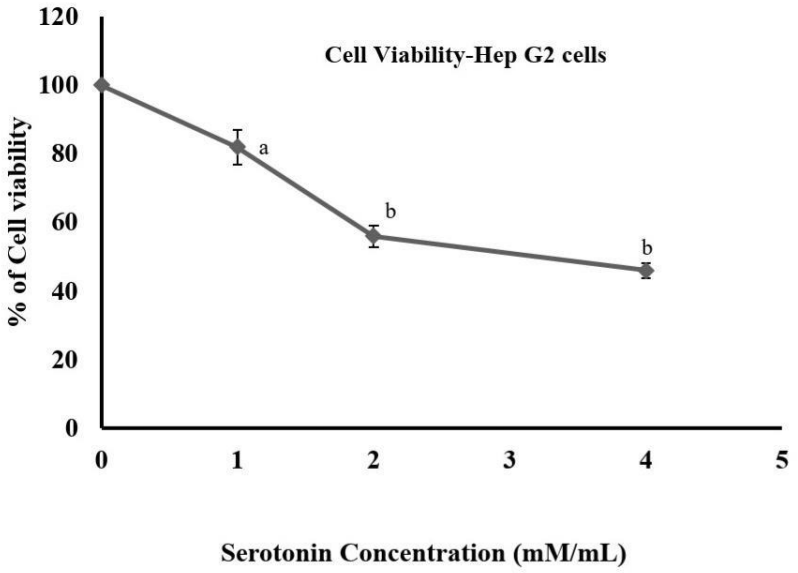

Figure 1: Effect of serotonin on cell viability in HepG2 cells.

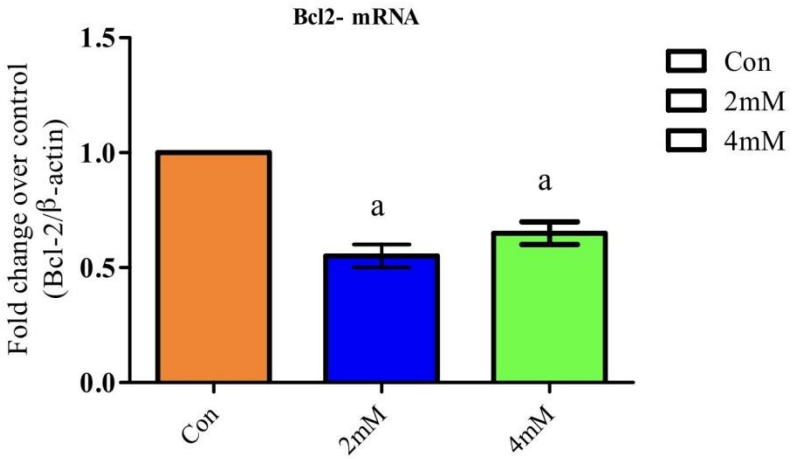

Figure 2: Effect of serotonin on Bcl-2 mRNA expression in HEPG2 cells.

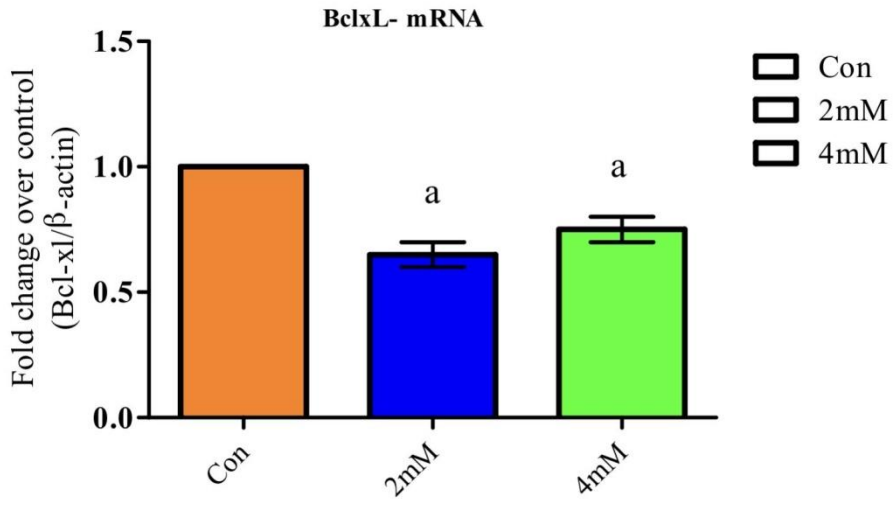

Figure 3: Effect of serotonin on Bcl-xl mRNA expression in HepG2 cells

P53 mRNA expression (Fold change over control):

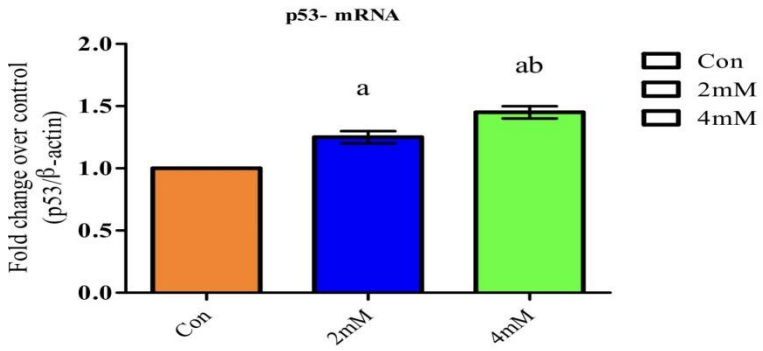

Figure 4: Effect of serotonin on p53 mRNA expression in HepG2 cells.

DISCUSSION:

The observation of the current study suggested that exercise-induced endogenous serotonin secretion may have acted against cancer cell proliferation by modulating the apoptotic signaling pathway. The Bcl-2 mRNA gene expression is decreased on induction of 2mM, 4mM dosage of serotonin with significant difference in comparison with control. The Bcl-xl mRNA gene expression is decreased on induction of 2mM, 4mM dosage of serotonin with significant difference in comparison with control. The p53 mRNA gene expression is increased on induction of 2mM, 4mM dosage of serotonin with significant difference in comparison with control. The % of cell viability is decreased on induction of 2mM, 4mM dosage of serotonin with significant difference in comparison with control. This compound does have anti-cancer properties. We observed that people who exercise have induced serotonin that helps prevent liver cancer like hepatocellular carcinoma through intrinsic apoptotic signaling pathway.

Anne Christine Piguet et al in the year 2015 provided a previous study on predisposition of non-alcoholic steatohepatitis due to an unhealthy lifestyle. The condition further led to the development of hepatocellular carcinoma. The author studied the role of exercise against Hepatocellular carcinoma in male hepatocyte-specific PTEN-deficient mice and that reported mean number of tumor per liver, total tumor volume was found to be reduced in the exercise group. The author concluded that exercise decreases cell proliferation. Exercise through its influence in phosphorylation of AMPK, it decreases the kinase activity of mTOR [39].

Kirsteen J Camphell in his study in 2018 reported that mitochondrial apoptosis is regulated by the balanced expression of pro-apoptotic and anti-apoptotic gene expression modulation. This ensures the equilibrium of programmed cell death and modulates the cell division in moderation. The author reported targeting the BCL-2 gene expression can aid in the prevention of the occurrence of cancer and also can have a good prognostic effect in early-stage tumors. Deregulation of BCL2 is becoming a target activity in various types of cancer and targeted cancer therapies can aid in maintaining the quality of life of cancer survivors[40].[41]

Ma Ming et al in their study attempted to identify the role of mRNA-125a-5p regulation in controlling hepatocellular carcinoma by targeting BCL2 gene expression. The study concluded the mRNA-125a-5p expression is downregulated in patients with hepatocellular carcinoma. Its upregulation is observed to inhibit the proliferation of hepatocellular cancer cells and its metastasis by targeting BCL2 gene expression[40].

JiaNan Ji et al in the year 2019 reported Tetrandrine (Tet) a compound isolated from Menispermaceae species to have an anti-cancer effect by targeting BCL2 gene expression. The study demonstrated the effect of seratonin in controlling the cell proliferation in cancer cells in a dose-dependent manner by targeting BCL2 mRNA gene expression[40,42].[43].[44]

Whereas Danielle Haack et al in their study reported the role of exercise in neuroprotection against the impact of chronic stress, which is not in agreement with the results of the current study. The results suggest that voluntary exercise with chronic stress upregulates the PI-3 kinase activity that aids in cell survival and promotes the upregulation of Bcl 2 expression. The upregulation of PI-3 kinase activity also induced the phosphorylation of Akt, which in turn upregulates Bcl2 expression and down-regulates GSK - 3 beta which is a pro-apoptotic protein that stimulates Bax protein expression, thus the results suggest the decrease in the ratio of Bax/Bcl2 ratio in the context of neuroprotection [45]

Gabriella Marfe et al in 2010 studied the effect of marathons on mRNA expression of anti-apoptotic and pro-apoptotic gene expression in recreational long-distance runners. The results suggest strenuous exercise load post marathon impacts the expression of the bcl2 family. The study results suggested that the impact of exercise on the bcl2 family, upregulates caspase-3, caspase -9 and increases the bax:bcl2 ratio. Pro-apoptotic BaxmRNA Gene

expression found to be decreased after marathon and the mRNA gene expression of Bcl2 is increased post-marathon. [46].

CONCLUSION:

In this in-vitro study we have concluded that people who exercise regularly induce serotonin that inhibits tumor growth through modulating the genes and hence they less likely develop liver cancer. Further research on serotonin is increasing day by day because of its potent pharmacological uses. However further investigations are needed for further confirmation of various potential benefits of serotonin.

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CONFLICT OF INTEREST:

The author declares that there was no conflict of interest in the present study

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