Role Of Exercise Induced Dopamine Replica On The Expression Of Intrinsic And Extrinsic Apoptotic Signalling In Human Melanoma Cell Line (A375)- In-Vitro Study

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ABSTRACT:

BACKGROUND:

Exercise has been implicated in modulating dopamine in a major amount as neurotransmission, increasing blood flow, etc. The role of exercise in tumor risk reduction, there is now convincing and vital evidence that exercise reduces melanoma and also other tumors. This has to be practiced in each and every place where tumor has grown. The goal of the study is to study the impact of exercise induced dopamine replica in modulating the apoptotic signaling pathway in human melanoma.

MATERIAL AND METHODS:

The melanoma cell line of human cell (A375) was brought from NCCS, Pune, India. Cell viability test and Gene expression analysis were carried out using MTT and PCR respectively. The results were analyzed using appropriate statistical tools using ANOVA and Duncan's test.

RESULT:

The Bcl-2 mRNA gene expression is increased on induction of 200μM, 400μM dosage of dopamine. BAD mRNA gene expression has no significant difference when compared to control and in p53, results are in down regulation compared with control.

CONCLUSION:

The study's findings reveal that dopamine has a favourable and persuasive effect on Bcl2 through lowering the gene's activity. However, when it came to BAD and p53, it yielded no meaningful results. To get a compelling conclusion, more investigations need be undertaken on a wide scale.

KEYWORDS: Melanoma; exercise; dopamine; Bcl-2; BAD; p53; Innovation

INTRODUCTION:

More than 10 million individuals are diagnosed with tumor each year, and an increasing percentage of patients may expect to live five years after their diagnosis (1). According to the International Agency for Tumor Research, obesity and a sedentary lifestyle are responsible for 25% of tumor cases. Physical activity has sparked interest in oncology patients in general and palliative care in particular (2). Exercise has been shown to have psychological and quality-of-life advantages for tumor survivors. (3). Dopamine is majorly associated with Alzheimer's disease is a neurological illness that has a complicated origin (4). The dopaminergic system, a major neurotransmitter involved in emotion and cognition, has been researched (5). According to studies, dopamine plays a significant role in synaptic plasticity mechanisms (6). Melanoma of the skin is a malignant tumour of the melanocytes. In the United States, roughly 76,100 new instances of melanoma of the skin are expected to be diagnosed in 2014, according to estimates. 9,710 people died due to this(7) .

The incidence of malignant melanoma has increased 3-6% over the last few decades (8), which makes this a fast growing tumor in the human race (9). By comparing this study with other previous articles these are some articles which go similar to the content. Physical activity - induced soreness, the role of serotonergic and dopaminergic system was reported (10). Exercise-induced neuroprotection of the

Nigrostriatal Dopamine system in Parkinson's disease also reported (11). Epidemiological studies indicate that exercise reduces the risk of developing Parkinson's disease. Tumour progression in the human melanocytic system(12) . Biology of tumour progression in human melanocytes.The experience from our previous studies (13) (14,15) (14)(16)(17)(18)(19)(17,19)(20)(21) (22) have led us to focus on the current topic.

This research is needed inorder to reduce the mortality of melanoma tumor tissues which also affects the normal tissues. It is termed as black tumour and the most dangerous type of skin tumor. It grows quickly and has the ability to spread to any organ. So we have taken an initiative by introducing exercise which induces dopamine to the tumorous tissues by curing them to normal tissues. Moreover there are less number of articles in this study. Studies at molecular levels were performed by our team of researches which insisted us to proceed this study. (23–30),(31),(32),(33),(34,35),(36),(37),(38–42) Thus the aim of the present study is to analyse the impact of exercise induced dopamine replica in modulating the apoptotic signalling pathway in human melanoma.

MATERIALS AND METHODS:

The study was carried out in a private dentistry college and hospital in Chennai, and it was an in vitro experimental study. The institutional scientific review board has given its approval to the project.

PROCEDURE:

The human melanoma cell lines (A375 were purchased form cellline centre, pune, India. At 37 degrees Celsius and 5% CO2, tissues were grown in RPMI media containing 10% foetal bovine serum, 100 U/ml penicillin and 100 g/ml streptomycin. The MTT test was used to measure cell growth. (A375) tissues were sown in 96-well plates with 5x104/200l and grown overnight. Untreated control A375 tissues are in Group 1, A375 + Dopamine treated tissues are in Group 2, A375 + Dopamine treated tissues are in Group 3, A375 + Dopamine treated tissues are in Group 4, and A375 + Dopamine treated tissues are in Group 4. As a vehicle control, tissues were treated with dimethyl sulfoxide (0.1 percent DMSO). 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 analysed by Polymerase chain reaction for identifying the fold change of BCL2, BAD and P53 m RNA expression over control samples. The obtained date were analysed for its significance using one-way analysis of variance (ANOVA) and Duncan's multiple range test with significance at the 0.05 level.

RESULTS:

EFFECT OF DOPAMINE ON THE CELL VIABILITY:

After delivering various dosages of dopamine, the cell viability of Human Melanoma (A375) was assessed using the MTT test. When compared to control, it was discovered to inhibit melanoma tumor tissues by reducing the degree of viability of tumor tissues in a different doses. In comparison to control, the concentration (200-400 M/ml) employed in this investigation showed the greatest reduction of cell growth. (Figure 1).

EFFECT OF GENE EXPRESSION ON THE A375 TUMOR TISSUES

Bcl-2 mRNA expression at a dose of 200 g/ml, the tumor tissues were considerably suppressed, but there was no statistical significance in comparison to untreated. At a dosage of 400 g/ml, the Bcl-2 mRNA expression was decreased in comparison to untreated. As a result, the reduction in gene expression was based on dosage. (Figure 2).

The level of BAD mRNA expression was analysed. At a concentration of 200 g/ml, the tumor tissues were significantly suppressed with no statistical signifiance. At a dosage of 400 g/ml, there was no significant difference in BAD mRNA expression as compared to untreated group. As a result, there is no discernible variation in gene expression in a dose-dependent way. (Figure 3).

The level of p53 mRNA expression was measured in different doses In comparison to the control group, at a dose of 200 g/ml, there was a considerable drop in p53 expression, although this did not reveal a protective effect. At a dose of 400 g/ml, there was a drop in p53 mRNA expression. As a result, m RNA expression was reduced in based on dose. (Figure 4).

ASSESSMENT OF CELL VIABILITY:

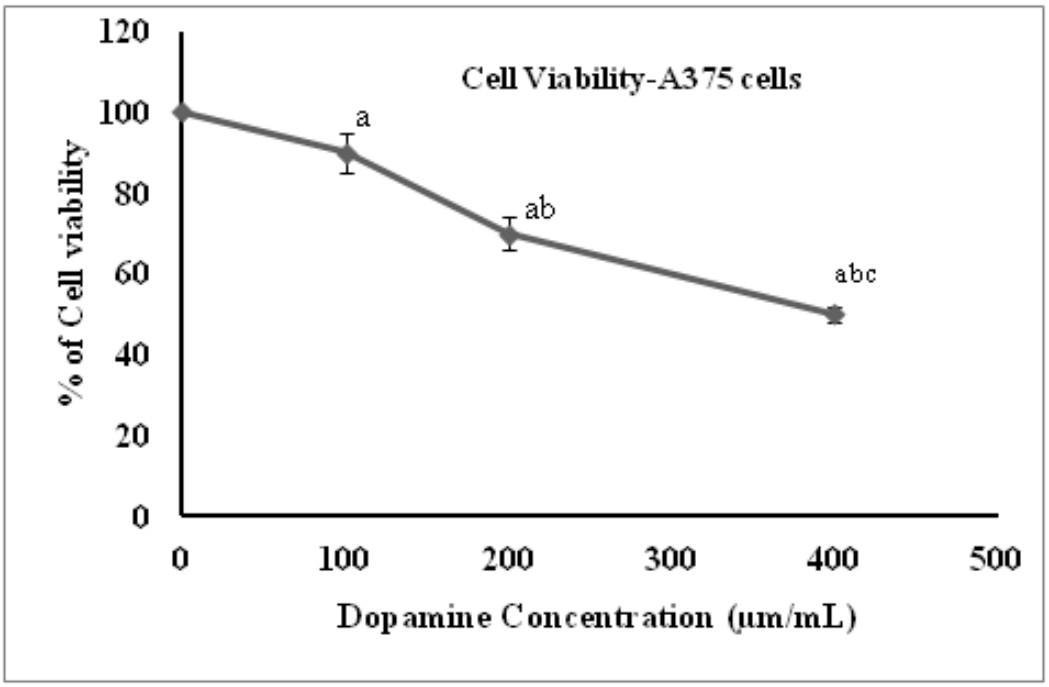

Fig : 1 Impact of dopamine on cell viability in human melanoma tissues.

Bcl-2 mRNA expression (Fold change over control)

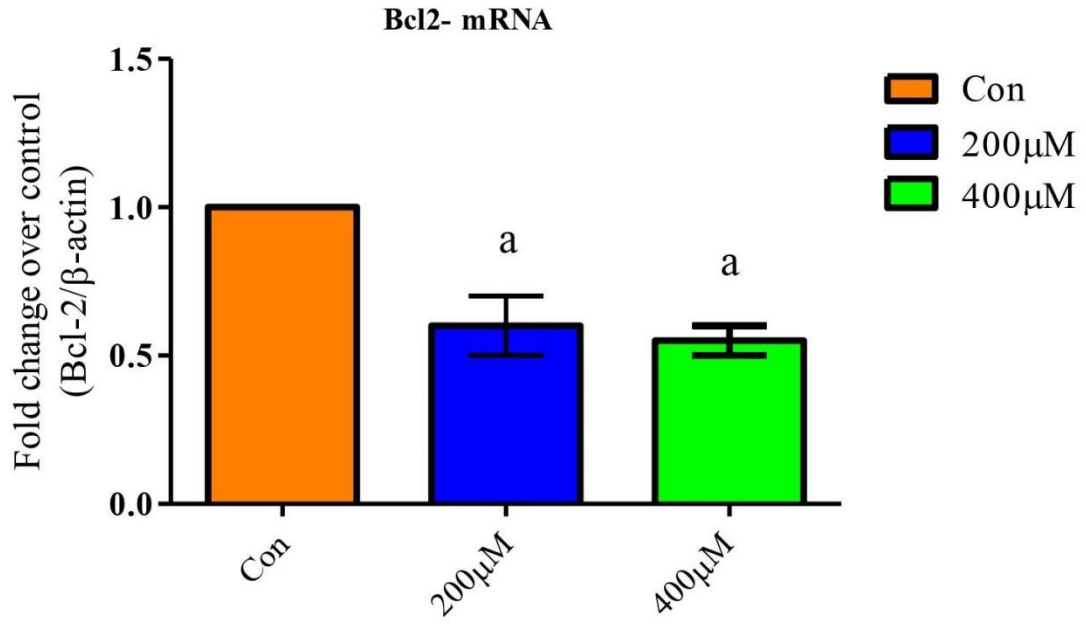

Fig : 2 Effect of dopamine on Bcl-2 mRNA expression in A375 tissues.

BAD mRNA expression (Fold change over control)

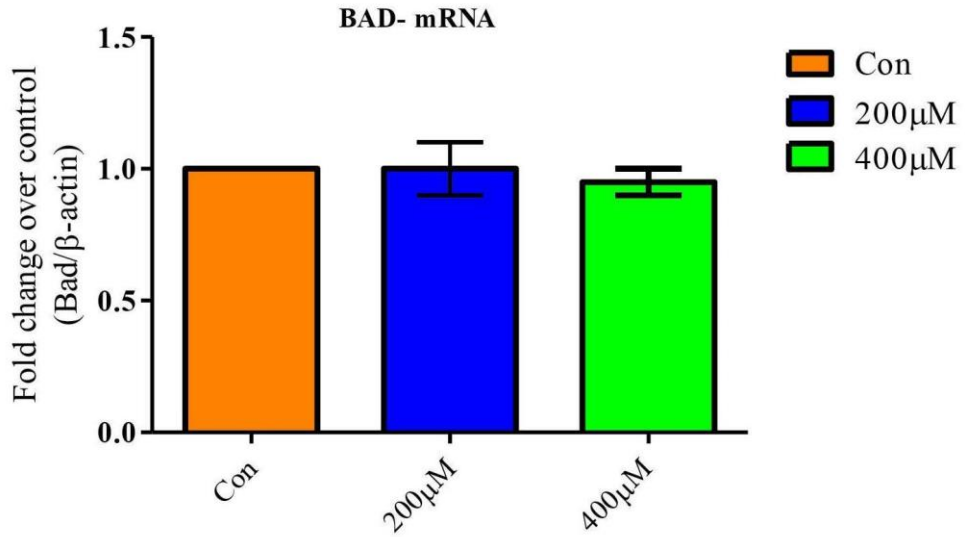

Fig 3: Effect of dopamine on BAD mRNA expression in A375 tissues..

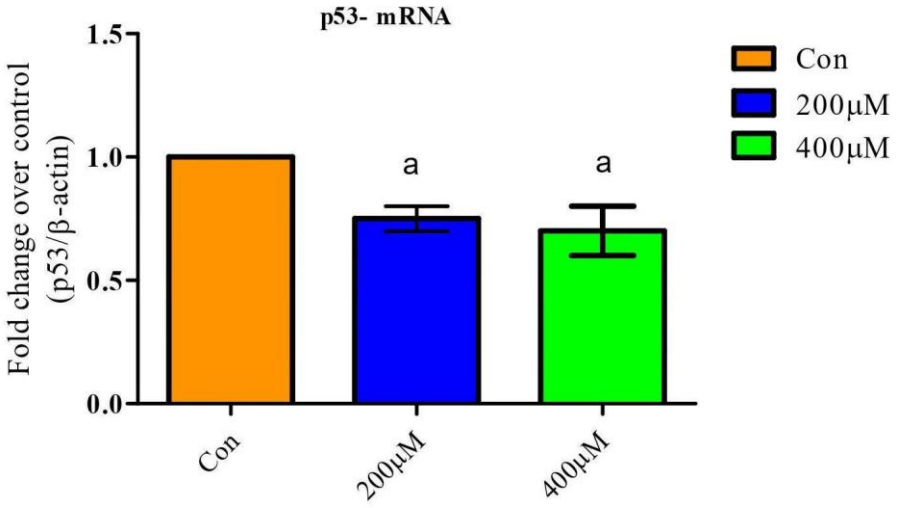

Fig 4: Effect of dopamine on p53mRNA expression in A375 tissues.

DISCUSSION:

The results of the current study suggested that exercise-induced endogenous dopamine secretion may have acted against tumor cell proliferation by modulating the apoptotic signaling pathway. The Bcl-2 mRNA gene expression is decreased on induction of 200mM, 400mM dosage of dopamine with significant difference in comparison with control. The BAD mRNA gene expression has no significant change on induction of 200mM, 400mM dosage of dopamine with significant difference in comparison with control. The p53 mRNA gene expression is decreased on induction of 200mM, 400mM dosage of dopamine with significant difference in comparison with control. We observed that people who exercise have induced dopamine that helps to prevent Melanoma through intrinsic apoptotic signaling pathway. Therefore the results which have been obtained are inconvinsive when dopamine is induced because only Bcl-2 has a protective role where, BAD and p53 does not have a protective role when dopamine is induced in this study.

In this study, since Bcl-2 is an anti apoptotic gene, its expression was reduced on induction of dopamine. It clearly proves that it has a protective effect against tumor tissues. Where in previous article, evident study which took place in 2009 shed light to the role of pro and anti-apoptotic Bcl2 family members in tumour pathogenesis and mediating the the effects of novel as well as classical anti-tumor agents, which allows the development of more efficient targeted treatments(43). By comparing both the studies it clearly shows Bcl2 has its role in treatment of different malignant disease in humans.

In the case of BAD, as it being a pro-apoptotic gene, there is no impact of its role in tumor when dopamine is induced. Evidence in a previous study, which was done in 2015, the author used PCA modelling, by using this the author assumed a positive role of BAD against tumor tissues(44). Therefore it has its role against the prevention of tumor.

When it comes to p53, in our study it did not increase when compared to control when dopamine is induced in a dose-dependent manner. Evidence in a previous study which took place in 2011, the author Conducted a study to analyse the role of mutation of p53 gene in tumour initiation and progression(45). The author reported around 50% of tumors to have mutation in the P53 tumour suppressor gene. The author concluded that there was an improvement in novel strategies to re-

activate mutant p53 tumour suppressor gene is required to provide clues to effectively treat malignant tumors bearing *p53* mutations. therefore the positive result was seen in the previous study.

In our study, the role of dopamine showed a positive and convincing result in Bcl2 by reducing the genes activity. But, it didn't show any significant result when it comes for BAD and p53. This makes the role of dopamine inconclusive against tumor tissues but, we can obtain different results when this experiment is done under a large population. By comparing this study with the previous study which was done in the year 2017, the authors conclude the same result of this study by telling dopamine has an inconclusive role against any tumor cell(46).

CONCLUSION:

The study's findings reveal that the role of dopamine showed a positive and convincing result in Bcl2 by reducing the genes activity. But, it didn't show any significant result when it comes for BAD and p53. Physical exercise may have an impact in controlling melanoma and other tumors too in humans by releasing neurotransmitters such as dopamine from the human brain. Future studies should be conducted on a large scale to have a convincing conclusion.

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Conflict of interest:

All the authors declare that there was no conflict of interest in the present study.

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