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ROLE OF DOPAMINE ON REDUCING THE PRO-INFLAMMATORY MARKERS IN McCoy CELLS

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Abstract

Dopamine plays a crucial role as a neuroregulatory molecule and is secreted during exercise, although its full range of physiological functions is not yet fully understood. Recent research has uncovered a previously unknown role for dopamine in suppressing inflammation, which is particularly relevant in conditions like colon cancer, where prolonged inflammation can play a significant role in the development and progression of the disease. In this study, the anti-inflammatory effects of dopamine were investigated using a colon cancer model with McCOY cells. These cells were cultured using DMEM, and their IC50 (the concentration at which 50% growth inhibition occurs) was determined through MTT assay. The study assessed the expression levels of key markers, including BCl-2, TNF-α, NF-κB, and IL-6, at concentrations of 50 and 100 µg/ml, both below the IC50 value. To gain insights into the molecular interactions, Schrodinger software was employed for docking analysis, focusing on the interaction between NF-κB (PDB: 5T8O) and dopamine (CID 681). The results of this investigation demonstrated that dopamine exhibited significant growth inhibition in McCOY cells, with an IC50 value of 266.32±3.1 µg/ml. Furthermore, dopamine downregulated the expression of BCl-2, NF-κB, and IL-6, while paradoxically increasing the expression of TNF-α. Through docking analysis, it was revealed that dopamine formed bonds with NF-κB through two hydrogen bonds, specifically with ASP53 and ALA54. In summary, this study sheds light on the promising anti-cancer potential of dopamine by blocking the NF-κB signaling pathway in McCOY cells.

Keywords: Colon Cancer, Dopamine, Inflammatory Pathway, Good Health and Well Being, Sustainable Development.

INTRODUCTION

Cancer remains a formidable global health challenge, necessitating continual exploration of novel therapeutic approaches. In recent years, researchers have delved into the intricate interplay between neurotransmitters and cancer cells, uncovering potential links that may open new avenues for cancer treatment[1]. This manuscript seeks to introduce and elucidate the relevance of McCoy cells in cancer research, with a specific focus on investigating the anti-cancer activity of dopamine. Cancer, characterized by uncontrolled cell growth and proliferation, poses a significant threat to public health worldwide[2]. Despite advances in traditional treatments, the need for innovative and targeted therapeutic strategies persists. Emerging research has expanded the scope of investigation beyond conventional avenues, exploring the intersection of neurotransmitters and cancer biology[3].

McCoy cells, a cell line originally derived from HeLa cells, have garnered attention in recent studies for their utility in diverse biological investigations[4]. Dopamine, primarily recognized for its role in neurotransmission and neuromodulation, has been implicated in various physiological processes. Recent findings suggest a nuanced relationship between dopamine and cancer, with investigations indicating the presence of dopamine receptors on certain cancer cells[5].

This revelation has sparked interest in understanding the potential anti-cancer properties of dopamine and its impact on cellular processes governing tumorigenesis Cancer, a complex and multifaceted disease, continues to challenge the boundaries of therapeutic interventions. While traditional approaches have made significant strides, emerging research now directs attention towards the intricate relationship between neurotransmitters and cancer cells.

This article delves into the evolving landscape of the anti-cancer activity of dopamine, with a focus on unraveling the molecular mechanisms underlying its potential as a novel therapeutic target[6]. Cancer remains a leading cause of mortality worldwide, necessitating continuous exploration of novel therapeutic avenues. Recent investigations have expanded beyond conventional approaches, shedding light on the role of neurotransmitters in cancer biology.

Dopamine, traditionally associated with the central nervous system, has emerged as a potential player in modulating cancer cell behavior[7]. Dopamine, a catecholamine neurotransmitter, is recognized for its pivotal role in neurological processes. However, recent studies have revealed the presence of dopamine receptors on various cancer cells, suggesting a broader impact beyond the nervous system. The dopaminergic system's involvement in cancer biology prompts a re-evaluation of its potential as a therapeutic target. Understanding the anti-cancer activity of dopamine requires a nuanced exploration of the molecular mechanisms involved.

Dopamine receptors, including D1-like and D2-like receptors, are expressed differentially across various cancer types. Activation or inhibition of these receptors influences downstream signaling pathways, impacting cellular processes such as proliferation, apoptosis, and angiogenesis[8]. McCoy cells, derived from HeLa cells, offer a valuable experimental platform for investigating the interplay between dopamine and cancer. Their unique characteristics make them suitable for in vitro studies, enabling researchers to decipher the specific interactions and responses within a controlled environment . In vitro study exploring the anti-cancer effects of dopamine on McCoy cells have shown promising results. Dopamine's ability to modulate cell cycle progression, induce apoptosis, and inhibit angiogenesis suggests a multifaceted impact on cancer cell behavior[9]. These findings lay the groundwork for further investigations into the potential therapeutic applications of dopamine. Transitioning from in vitro to in vivo studies is crucial for validating the translational potential of dopamine as an anti-cancer agent.

Animal models can provide insights into the systemic effects, bioavailability, and overall safety profile of dopamine-based interventions[10]. Moreover, these studies help bridge the gap between laboratory discoveries and potential clinical applications. The clinical implications of dopamine's anti-cancer activity are a subject of growing interest[11]. While challenges such as drug delivery and specificity need to be addressed, the prospect of incorporating dopamine-based therapies into existing treatment regimens offers a novel approach to enhance therapeutic efficacy and minimize side effects[12].

Despite the promising findings, several challenges lie ahead. Clarifying the context-specific effects of dopamine on different cancer types, optimizing drug delivery strategies, and addressing potential side effects are crucial considerations. Future research should focus on refining our understanding of dopamine's anti-cancer mechanisms and advancing towards clinical trials[13].

MATERIALS AND METHODS

Cell viability Assay – MTT assay

The MTT assay was employed to assess the viability of McCOY cells, obtained from the National Centre for Cell Sciences in Pune, India, upon exposure to dopamine treatment. To initiate the experiment, cells were cultured at a density of 10^3 cells per well in DMEM (containing 10% FBS and 1% pen-strep) and were incubated at 37°C with 5% CO2 for 24 hours to promote cell adherence[14]. Prior to commencing the experiment, the cells were allowed to reach 80% confluence. For the treatment groups, dopamine at concentrations of 0, 50, 100, 250, and 500 µg/ml was added in DMSO, with a maximum DMSO concentration of 0.1%. After 48 hours of treatment, the cell viability was assessed and compared to the untreated control cells[15]. The IC50 value was determined using the probit method. Furthermore, an in vitro protein denaturation assay was conducted using a previously established method[16].

RT- PCR reaction

Following exposure to dopamine for 48 hours, McCOY cells were subjected to TRIzol reagent (TaKaRa Bio, Dalian, China) to facilitate the isolation of total RNA. Subsequently, cDNA was synthesized through reverse transcription using the Prime Script RT Reagent Kit. Amplification of the target genes was carried out using the Mx3005P Real-Time PCR System (Agilent, CA, USA), with adherence to the kit's provided guidelines for the Q-PCR experiment. To quantify the relative mRNA expression levels of each gene, normalization was performed against the GAPDH RNA using the 2CT method. The primers utilized in this study were sourced from Invitrogen, based in Shanghai, China [17].

Molecular Docking Analysis

The structure of dopamine (Pubchem with CID 681) and NF- McCoy (PDB: 5T8O) were retrieved. Schrodinger software suite was used to prepare the epik states and to optimize the ligand. The docking was carried out using the extra precision method (XP) and Glide score was calculated (kcal/mol) [18].

Statistical Analysis

The significant value was confirmed using One Way/Two Way ANOVA (Bonferroni post hoc test or Newmann Keuls post hoc test).

RESULTS AND DISCUSSION

One of the primary factors in the development of cancer is persistent inflammation. Its involvement in the development and spread of colon malignancies has been thoroughly investigated. Dopamine inhibited inflammatory effects on McCOY cells. On McCOY cells, the dopamine decreased the expression of inflammatory genes such TNF-α, NF-β, and p38 but enhanced the expression of Bax.

Effect of Dopamine on cell viability

The MTT assay was used to assess the cytotoxic effects of dopamine. The experiment employed a variety of concentrations, including 0, 50, 100, 250, and 500 g/ml. In contrast to controls, the findings demonstrated that dopamine exhibited dose-dependent cytotoxicity with McCOY cells (Figure 1). The IC_{50} values for doxorubicin and dopamine were 129.411.56 and 2503.1, respectively. Pro- and anti-inflammatory

genes were expressed at concentrations of 50 and 100 g/ml, respectively.

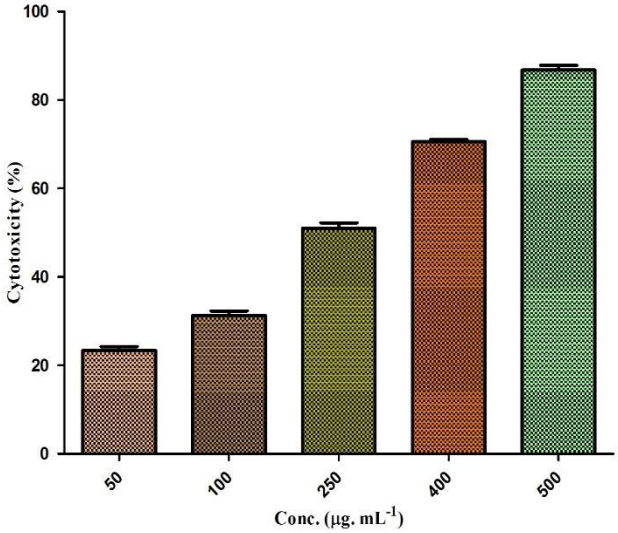
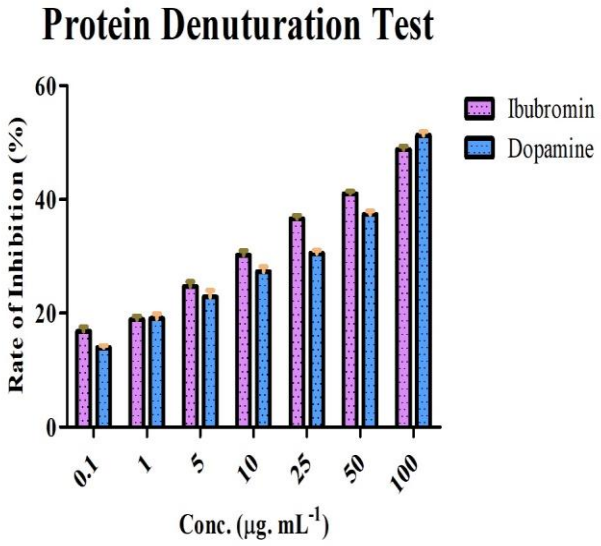


Figure 1: Effect of Doxorubicin, Dopamine on McCOY cells. Different concentration (50-500µM) of respective drugs treated with McCOY cells for MTT assay. X axis represented the log concentration (µg/ ml) and Y axis represented cytotoxicity (%). (Two Way Anova – Bonferroni post hoc test) with p value if p < 0.05 ---*, p< 0.005 ..., p< 0.001-----***)**

Dopamine inhibited Bax

On dopamine-treated McCOY cells, the BCl-2 gene's mRNA expression was assessed by qPCR. Two concentrations, 50 and 100 g/ ml, were selected for the study based on the IC50. At 100 g/ml, dopamine reduces the expression of BCl-2 in McCOY cells. The BCl-2 decline in 100 g/ ml was larger than in 50 g/ ml. As a result, dopamine treatment causes a dose-dependent decrease in BCl-2 expression. As a result, the improved gene expression was dose-dependent (Figure 2).



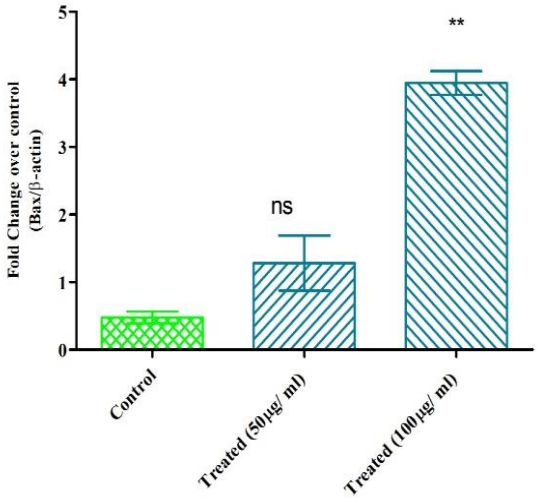


Figure 2: Dopamine inhibited the expression of BCl- 2 mRNA on McCOY. Drug concentration (log) is shown on the X axis, while fold change over control is shown on the Y axis., and yellow represented control. Bax expression in higher concentration 100µM. (One Way Anova –Newmann Keuls post hoc test) with p value p< 0.005 ..., ns- non- significant)**

Dopamine decreased TNF-α mRNA expression in McCOY cancer cells

Dopamine dose-dependently decreased the mRNA expression of TNF- in McCOY cells. 50 and 100 g/ ml of each concentration were employed in the investigation. When exposed to a dose of 100 g/ml, the cancer cells' TNF- levels were lower than they were when exposed to a dose of 50 g/ml[19]. The findings demonstrated that, in comparison to control, the mRNA expression of TNF- substantially decreased (by a factor of about four). Additionally, at a dose of 50 M relative to control, NF-B mRNA expression was significantly reduced. As a result, in comparison to control cells, the changed gene expression in the treated cells was dose-dependent (Figure 3).

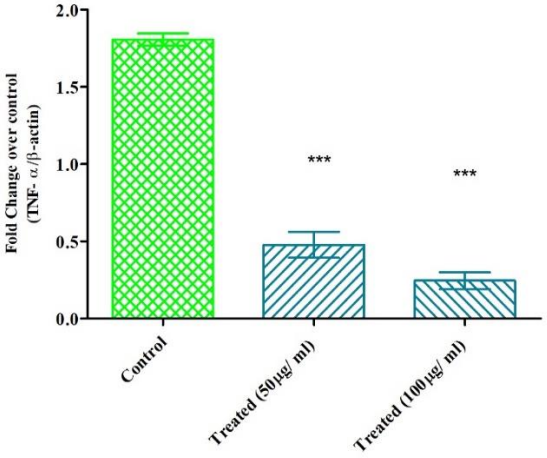


Figure 3: Dopamine reduced the expression of TNF-α mRNA on McCOY cells. The fold change over control is displayed on the Y axis, while drug concentration (log) is displayed on the X axis. TNF-α expression was found with a statistically significant reduction at a concentration of 100 µg/ ml compared to the control

Dopamine inhibited NF-κB mRNA expression on the McCOY cells

The ratio of the NF-κB in McCOY cells treated with dopamine was analyzed using qPCR. The expression was dose- dependent manner. At 50 µg/ ml, dopamine showed reduced NF-κB expression significantly, compared with the control (Figure. 4). Our results indicated that dopamine decreased the inflammation by attenuating the pro-inflammatory markers such as NF-κB and BCl-2. Meanwhile, it increased expression of TGF- α on dose- dependent manner.

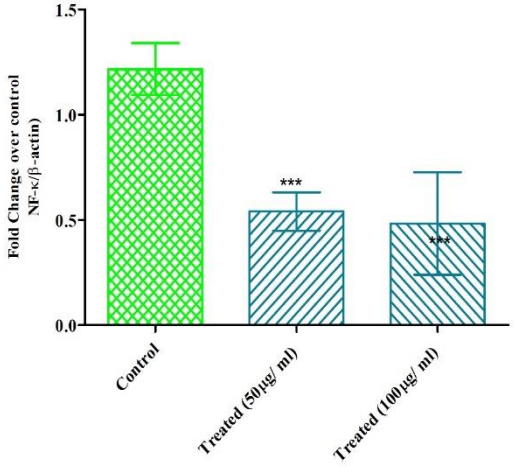


Figure 4: Effect of dopamine the expression of NF-κB mRNA on McCOY cells. On the Y axis is the fold change compared to the control, and on the X axis is the drug concentration (log)

Molecular Docking

The protein structure (PDB: 5T8O), which contains the structure of murine NF-kappaB and kinase-bound imidazolbenzoxepin molecule, was downloaded from the PDB databank (Figure. 5). After the protein structure was refined by the protein wizard, the binding site detector was utilized to identify the protein's binding pockets.

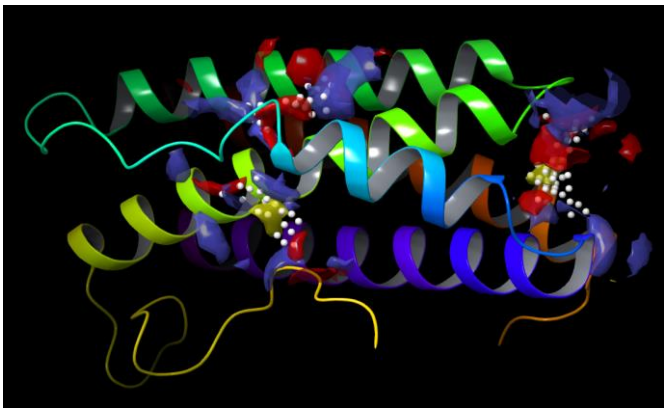


Figure 5: Structure of NF- κB, (PDB – 5T80). It was docked with dopamine for analysing its protein – ligand binding properties

The confirmation and orientation of the ligand- inhibitor complex at the active or docking site is an important concept for drug discovery. In the present study, Schrodinger docking software was used and the results were represented in Figure. 7 and 8.

Row	In	Title	Stars	Ionization Penalty	State Penalty	Tot Q	flags	docking score	XP GScore	glide gscore	gl
		glide-dock_XP_dop_1_pv (6)									
1		sitemap_1_protein	☆☆☆								
2		dopamine	☆☆☆	00	0.0068	0.0000	1	0	-4.729	-4.729	-4.729
3		dopamine	☆☆☆	00	0.0068	0.0000	1	0	-4.350	-4.350	-4.350
4		dopamine	☆☆☆	00	0.0068	0.0000	1	0	-4.120	-4.120	-4.120
5		dopamine	☆☆☆	00	0.0068	0.0000	1	0	-4.016	-4.016	-4.016
6		dopamine	☆☆☆	00	0.0068	0.0000	1	0	-3.346	-3.346	-3.346
		glide-dock_XP_dop_2_pv (6)									
7		sitemap_1_protein	☆☆☆								
8		dopamine	☆☆☆	00	0.0068	0.0000	1	0	-3.854	-3.854	-3.854
9		dopamine	☆☆☆	00	0.0068	0.0000	1	0	-3.727	-3.727	-3.727
10		dopamine	☆☆☆	00	0.0068	0.0000	1	0	-2.793	-2.793	-2.793
11		dopamine	☆☆☆	00	0.0068	0.0000	1	0	-2.729	-2.729	-2.729
12		dopamine	☆☆☆	00	0.0068	0.0000	1	0	-1.277	-1.277	-1.277
	1	glide-dock_XP_dop_3_pv (6)									
13		sitemap_1_protein	☆☆☆								
14		dopamine	☆☆☆	00	0.0068	0.0000	1	0	-5.381	-5.381	-5.381
15		dopamine	☆☆☆	00	0.0068	0.0000	1	0	-5.378	-5.378	-5.378
16		dopamine	☆☆☆	00	0.0068	0.0000	1	0	-5.121	-5.121	-5.121
17		dopamine	☆☆☆	00	0.0068	0.0000	1	0	-4.620	-4.620	-4.620
18		dopamine	☆☆☆	00	0.0068	0.0000	1	0	-4.447	-4.447	-4.447

Figure 6: Binding Efficiency of Dopamine with NF- κB

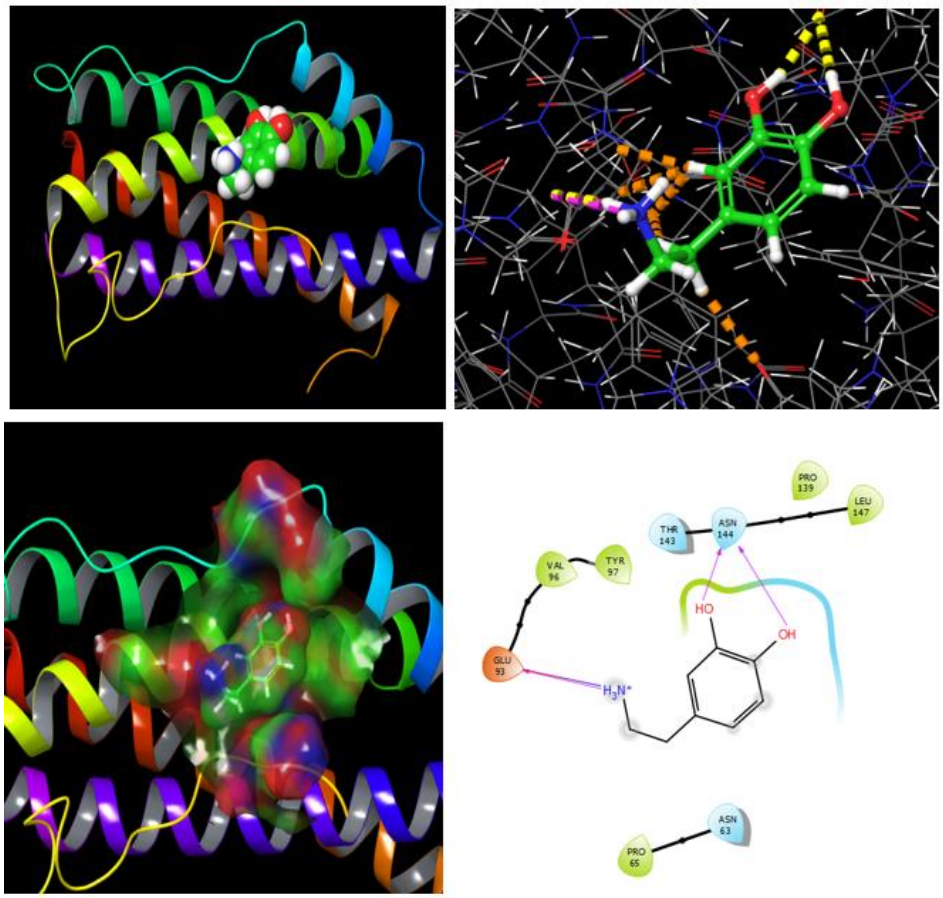


Figure 7: Molecular Binding of Dopamine with NF- κB. Structural conformity of protein – ligand binding (a). The binding of dopamine lead to electrostatic cloud formation (b). Ligand view inside the protein (c). Formation of hydrogen bond (d)

The NF-κB-dopamine complex's expected binding free energy was discovered to be -2.456 kcal/mol (Figure. 6). The pose with highest docking energy represented in Figure. 7. Dopamine bonded with NF- κB with two intramolecular hydron bonding (Figure.7a). Using the LigPlot+ program and the BIOVIA DS Visualizer, we also investigated the presence of intermolecular interactions in protein-ligand complexes in this study (Figure. 7 b-c). Dopamine made two hydrogen bonds with NF- κB through two amino acids, aspartic acid (ASP55) and alanine (ALA54) respectively.

DISCUSSION

The dopamine pathway has lately come to light as a possible target in anticancer medicines, drawing on decades' worth of evidence linking dopamine effect and cancer. Repurposing dopaminergic medications has considerable benefit to patients[20]. The exercise is one the easiest way for gaining dopamine at tissue level. Despite this, a thorough investigation into the potential uses of this therapy approach in cancer is warranted given the abundance of evidence connecting DA to cancer and non-tumor cells in the tumor microenvironment.

Summarizing the prior empirical research on the reciprocal relationship between dopamine, we found strong evidence for strong anti-cancer activity of dopamine through the regulation of NF- κB in McCOY, colon cancer cells[21]. Dopamine decreased pro inflammatory markers compared to control. The relationship between an increase in extracellular dopamine and NF-κB activation and inflammation reveals certain intracellular targets that may be exploited to discover novel targets in colon cancer cells for effective treatment strategies. Our results concordant with the previous studies[22]. Prolong inflammation stimulating the immune cells to secret a lot of inflammatory cytokines (such IL-1, IL-6, and TNF- α) [23]. Previously, we showed that dopamine substantiated BCl-2 in lung cancer, A549 cells[24].

Dopamine had shown a strong inhibition on inflammation on cancer sites. Different biochemical mechanism had been reported previously. It enhanced the therapeutic efficacy of the pancreatic cancer. It deprived the cAMP and inhibited the PKA/p38 signaling by activating the DRD4 receptor. DRD4 is one of the dopamine family receptors (DRD1- DRD5). Thus, it inhibited the activation of tumor associated macrophages[25]. Dopamine inhibit NF-κB by activating DRD2, DRD3, DRD4 and DRD5 receptors. DRD2 activation led to gastric cancer reduction in previous study[26]. In contrast, Kline et al., (2018) showed that knockout of DRD2 in colon cancer did not alter properties and had an independent mechanism[27].

CONCLUSION

In light of our research outcomes, it is increasingly evident that dopamine serves as an endogenous bioactive compound with notable anti-inflammatory properties[28]. These properties hold the promise of making dopamine a valuable candidate for the treatment not only of malignant colon cancer but also for addressing various pathological conditions triggered by inflammation mediated through the NF-κB signaling pathway[29]. This potential therapeutic application of dopamine highlights its multifaceted role in modulating inflammation-related disorders, expanding its significance in the realm of medical research and treatment development[30].

Competing interests: The authors declare no competing interests.

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