

# The Inhibitory Effect of Ritanserin (5HT2 Receptor Antagonist) on Growth and Angiogenesis in Model of HT29 Colon Cancer Cells Incubation in Nude Mice

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## Abstract

**Background:** Serotonin plays a proliferative role by stimulating the cAMP-dependent mitogen activated protein kinase pathway. Also, there is some evidence about the role of 5HT (5-Hydroxytryptamine) receptors in some cancers such as gastrointestinal cancers. Therefore, we have aimed to investigate the effect of 5HT2A receptor antagonist (ritanserin) on the growth of tumor and expression of apoptotic and angiogenesis factors as (Cyclooxygenase (COX2) and epidermal growth factor (EGFR)) in an in vivo model in nude mice.

**Method:** In this experimental study, ritanserin (5mg/kg, 0.05 cc, IP) was injected into mice for 21 days, and intradermal tumor induced by injecting 10,000,000 (HT29) suspension of colorectal cells into the flank muscle of nude mice and cisplatin used as positive control. Tumor size was examined macroscopically three times a week and angiogenic genes as EGFR and COX2 expressions were evaluated by real-time polymerase chain reaction ( $P < 0.05$ ) was considered statistically significant. Statistical analysis was performed using SPSS software version 22 with ANOVA test.  $P < 0.05$  was considered statistically significant.

**Results:** Tumor size in the ritanserin group significantly decreased as compared with the control group ( $P < 0.05$ ). Expression of COX2 was increased in the cisplatin group as compared with the control group ( $P < 0.05$ ). Also, ritanserin increased COX2 expression as compared with the control group ( $P < 0.05$ ), while this effect was more in the cisplatin-ritanserin group. Moreover, the expression of EGFR increased by ritanserin ( $P < 0.05$ ) and the combination of ritanserin and cisplatin shows a synergic effect.

**Conclusion:** Our study indicates that ritanserin, as a 5HT2A receptor antagonist, has an antitumor and antiangiogenic effect in the xenograft model in nude mice. The effect of ritanserin is partly due to the inhibition of COX2, as an inflammatory factor, and EGFR, as an angiogenic factor.

**Keywords:** Colon-specific antigen, Ritanserin, 5HT2A receptor, Cyclooxygenase 2 inhibitors, EGFR protein

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## Introduction

Cancer is one of the important diseases and still one of the leading causes of disease mortality in the world. Currently, one of the most common cancers of the gastrointestinal tract in Iran is colorectal cancer, as the third most common in Iranian men and fourth in women.<sup>1</sup> Invasion and metastasis of the biological branches of malignant tumors are a major cause of physical complications and cancer mortality. The process of forming new blood vessels, or angiogenesis, allows tumors to grow beyond 1-2 cubic millimeters. In general, in healthy and stable tissues, factors that inhibit angiogenesis predominate and in rapidly dividing tissues, molecules that stimulate the angiogenesis process predominate. Therefore, the inhibition of angiogenesis has been recognized as a contributing factor in the treatment of cancer.<sup>2,3</sup>

5-Hydroxytryptamine, or serotonin, is a neurotransmitter and a regulator of cell growth that strongly regulates the human body's functions as central or peripheral.<sup>1</sup> Serotonin is produced and stored in enterochromaffin cells of the gastrointestinal tract and its secretion is regulated by various stimuli which leads to increased uptake by circulating platelets and mast cells that provide a strong reservoir of serotonin.<sup>1</sup> Platelet activation has been associated with tumor progression and angiogenesis and in thrombotic environments tumors with platelet aggregation which often occur, lead to significant serotonin release which may be one of the mechanisms of tumor progression in angiogenesis.<sup>4</sup>

The serotonin 5-HT<sub>2</sub> receptor (5-HT<sub>2</sub>R) family consists three subtypes: 5HT<sub>2</sub>AR, 5HT<sub>2</sub>BR and 5HT<sub>2</sub>CR. 5-HT<sub>2</sub>AR is widely scattered in peripheral tissue, including the human gastrointestinal tract and acts as a mitogenic receptor in different cell types.<sup>4-7</sup>

In laboratory models, serotonin stimulates proliferation of endothelial cells by activating 5HT<sub>3</sub>, 5HT<sub>2</sub>, and 5HT<sub>1</sub> receptors. Also, it has shown that 5HT<sub>2</sub> receptor agonists could stimulate the proliferation of aortic smooth muscle cells and suggested role of monoamines (especially serotonin) in tumor behavior especially by affecting angiogenesis.<sup>4-6</sup>

The 5HT<sub>2</sub>A receptor signaling has been shown to have mitogenic effect through activating the phospholipase C- $\beta$  pathway and activating protein kinase C in rat glomerular frangial cells, and also this receptor has been described to be coupled with several mitogenic signal conduction pathways as pathways as extracellular signal-regulated kinase / mitogen - activated protein kinase (ERK/MAPK), Justine kinase/signal transducing avitvator transcription (JAK/STAT).<sup>7</sup> Also, according to some other studies, serotonin can play a proliferative role by stimulating the cAMP-dependent MAPK pathway.<sup>8,9</sup>

Although Balakrishna P declared, in his review article, that serotonin acts as an oncogene, its role on tumor growth, which may be through 5-HT receptors expression in cancer tissues, is still complicated.<sup>9</sup> Another hypothesis is that the mitogenic effect of serotonin is dose-dependent, as in higher doses promote cell proliferation and at lower doses cause vasoconstriction on tumor vessels which leads to the inhibition of tumor growth. On the other hand, many studies have shown a decreased 5-hydroxytryptamine receptor 1B gene (HTR1B) expression in the lung, renal, osteosarcoma, and non-Hodgkin's lymphoma which suggests that serotonin may have tumor suppressor effect when interacts with 5-HTB receptor subtypes. However, some data suggest that 5-HT receptor expression is not only tissue-specific but also dysregulated in human cancers. As in human cholangiocarcinoma, cell lines 5-HT 1B, 1F, 2B, 3C, and 7 are downregulated where all the other subtypes are upregulated. Notably, 5-HT1B and 2B receptor subtypes are overexpressed in liver tumor cells.<sup>9</sup>

Many serotonin-targeting agents as selective serotonin reuptake inhibitors (SSRIs) are available for treating central nervous system disorders, and their use as anticancer agents is being evaluated. Although SSRIs enhance levels of serotonin in synaptic cleft and plasma, they do not have tumorigenesis effect. Instead, some SSRIs have shown cytotoxic effects in vitro at higher concentrations. Therefore, their use as anticancer drugs may be limited since concentrations used in patients treated with antidepressant doses of SSRIs are much lower.<sup>9</sup> Also, some other studies have

**Table 1.** Primer sequences used for quantitative PCR analysis

| Gene | Forward primer         | Reverse primer         | Size   |
|------|------------------------|------------------------|--------|
| EGFR | GGGATTCTTTACGCGCACTCCT | TCAGGCCAACGACCGCCAAA   | 198 bp |
| COX2 | GGCGCAGTTTATGTTGTCTGT  | CAAGACAGATCATAAGCGAGGA | 107 bp |

PCR: Polymerase chain reaction; EGFR: Epidermal growth factor; COX2: Cyclooxygenase 2

addressed the role of serotonin receptors in cancers like gastrointestinal, breast and bladder cancers. In most of these studies, 5HT receptors are reported to have attenuated tumor progression.<sup>1, 4, 7, 8, 10-12</sup>

Therefore, we aimed to investigate the effect of 5HT<sub>2A</sub> receptor antagonist (ritanserin) on the expression of apoptotic and angiogenic factors as (Cyclooxygenase (Cox2) and epidermal growth factor (EGFR)) in an in vivo model of colorectal cancer in nude mice in order to study tumor growth assay observationally in a macroscopic manner.

## Material and Method

This experimental study evaluated the apoptotic and antiangiogenic effects of 5HT<sub>2A</sub> receptor antagonist (ritanserin) in an in vivo model of antiangiogenic colorectal cancer. A total number of 16 nude mice (black-6) 3 weeks age, 20-25 gm weight, were provided from Pasteur Institute of Iran (Amol branch) and maintained in four groups of 4 in germ free conditions, 21 ± 2 temperature, 30% humidity and 12 hr light and dark cycle. Ritnserin was provided from Sigma-Aldrich co, (Germany), cisplatin vial (1mg/ml) from Sobhan Daroo co. (Iran), RPMI medium and MARTI Gel from Sigma (Germany) RNA extraction kit (Quiagene, Germany), cDNA synthetic kit (TAKARA, Japan), (Syber Green, Master mix, Arian Gene, Iran). Also, primer sequences were synthetic by Bioneer co (Germany). All procedures were confirmed by laboratory animal ethics committee of Mazandaran University of Medical Sciences by ethics number: IR.MAZUMS.REC.1398.1252.

Ritnserin was soluble in water with solvent (Ethanol 11.94 mg/ml.). The drugs were injected into mice for 21 days, 5 days before tumor induction and 16 days after tumor induction. Intradermal tumor was induced by injecting 10,000,000 (HT29) suspension of colorectal cells into the flank muscle of nude mice.

### Experimental groups

1-Four mice received ritanserin (5mg/kg, 0.05 cc, IP) once a day, for 21 days.<sup>13, 14</sup>

2-Four mice received cisplatin as an anticancer drug (10mg/kg, 0.05 cc, IP), once a day for 21 days.<sup>14</sup>

3-Four mice received a combination of ritanserin (2.5mg/kg) + cisplatin (5mg/kg) (0.05 cc, IP), once a day for 21 days.

4-Four mice as negative control, received Normal Slain 0.05%+ 10% DMSO solution (as drug vehicle) (0.05 cc, IP), once a day for 21 days.

Tumor size was examined macroscopically three times a week (with caliper). After three weeks, the mice were anesthetized by ketamine /Xylesine (3/1) and killed by beheading. Then, the tumor tissue was removed and placed inside the RNA later and transferred to the freezer (at -70 °C) for gene expression study.

Tumor Volume = (Width<sup>2</sup>)×Length)×0.52<sup>15</sup>

This formula is extracted form following:<sup>15</sup>

$V = w^2.l/2$

The advantage of this model is that there is no need to approximate tumor height.<sup>15</sup>

### Real-time polymerase chain reaction (RT-PCR)

In this phase, the RT-PCR method was used to evaluate the angiogenic genes of EGFR and COX2. Quantitative RT-PCR was performed using specific primers for EGFR, COX2, and housekeeping-GAPDH genes with the quanti-fast sybr green PCR master mix and run on the gel electrophoresis.

The primers were designed with software based on the full murine EGFR, COX2, sequences and GAPDH as housekeeping gene based on NCBI Data base (Table1).

Quantitative RT-PCR reaction were performed in a 10 µL volume containing 5 µL of quantities SYBR green PCR master mix, 0.6 µL of forward and reverse primers, and 0.2 µL of first –strand cDNA according to the manufacturer's procedure. After an initial 15 min heating at 95 °C as an activation step, 36 cycles of denaturation at 95 °C for 15 sec and annealing and extension at 56 °C

**Table 2.** Measured volume of tumors in nude mice after 21 days of drug in four groups: ritanserine, cisplatin (Cis-Rit), control, 4 mice in each group, in three times (1398/6/25) (1398/6/30) (1398/7/5)

| Measurement of tumors 2019/9/16 |                    |                    |                    |                      |
|---------------------------------|--------------------|--------------------|--------------------|----------------------|
| Groups                          | Length             | Width              | Height             | Volume               |
| Group ritancerin                | 7mm                | 5 mm               | 4 mm               | 122 mm <sup>3</sup>  |
|                                 | 8 mm               | 6 mm               | 4 mm               | 192 mm <sup>3</sup>  |
|                                 | 12 mm              | 7 mm               | 6 mm               | 504 mm <sup>3</sup>  |
|                                 | 10 mm              | 8 mm               | 5 mm               | 400 mm <sup>3</sup>  |
| Group cisplatin                 | 5 mm               | 3 mm               | 2 mm               | 37 mm <sup>3</sup>   |
|                                 | 1 mm               | 1 mm               | 1 mm               | 0.5 mm <sup>3</sup>  |
|                                 | 2 mm               | 1 mm               | 1 mm               | 2 mm <sup>3</sup>    |
|                                 | 1 mm               | 1 mm               | 1 mm               | 0.5 mm <sup>3</sup>  |
| Group Cis-rit                   | 7 mm               | 6 mm               | 4 mm               | 147 mm <sup>3</sup>  |
|                                 | 2 mm               | 2 mm               | 1 mm               | 4 mm <sup>3</sup>    |
|                                 | 6 mm               | 3 mm               | 2mm                | 54 mm <sup>3</sup>   |
|                                 | 3mm                | 2 mm               | 2 mm               | 9 mm <sup>3</sup>    |
| Group control                   | 9 mm               | 7 mm               | 7 mm               | 283 mm <sup>3</sup>  |
|                                 | 11 mm              | 7 mm               | 5 mm               | 423 mm <sup>3</sup>  |
|                                 | 8 mm               | 6 mm               | 6 mm               | 192 mm <sup>3</sup>  |
| Measurement of tumors 2019/9/21 |                    |                    |                    |                      |
| Groups                          | Length             | Width              | Height             | Volume               |
| Group ritancerin                | 12 mm              | 9 mm               | 6 mm               | 774 mm <sup>3</sup>  |
|                                 | 9 mm               | 6 mm               | 5 mm               | 243 mm <sup>3</sup>  |
|                                 | 10 mm              | 6 mm               | 5 mm               | 300 mm <sup>3</sup>  |
|                                 | 10 mm              | 8 mm               | 6 mm               | 400 mm <sup>3</sup>  |
| Group cisplatin                 | 7 mm               | 5 mm               | 3 mm               | 122 mm <sup>3</sup>  |
|                                 | 1 mm               | 1 mm               | 1 mm               | 0.5 mm <sup>3</sup>  |
|                                 | 3 mm               | 2 mm               | 1 mm               | 9 mm <sup>3</sup>    |
|                                 | 2 mm               | 1 mm               | 1 mm               | 2 mm <sup>3</sup>    |
| Group Cis-rit                   | 3 mm               | 3 mm               | 3 mm               | 4.5 mm <sup>3</sup>  |
|                                 | 4 mm               | 4 mm               | 3 mm               | 32 mm <sup>3</sup>   |
|                                 | 10 mm              | 8 mm               | 6 mm               | 400 mm <sup>3</sup>  |
|                                 | 8 mm               | 5 mm               | 5 mm               | 160 mm <sup>3</sup>  |
| Group control                   | 9 mm               | 7 mm               | 7 mm               | 283 mm <sup>3</sup>  |
|                                 | 11 mm              | 7 mm               | 5 mm               | 4 mm <sup>3</sup>    |
|                                 | 8 mm               | 6 mm               | 6 mm               | 192 mm <sup>3</sup>  |
| Measurement of tumors 2019/9/27 |                    |                    |                    |                      |
| Groups                          | Length             | Width              | Height             | Volume               |
| Group ritancerin                | 12 mm <sup>3</sup> | 7 mm <sup>3</sup>  | 7 mm <sup>3</sup>  | 504 mm <sup>3</sup>  |
|                                 | 15 mm <sup>3</sup> | 9 mm <sup>3</sup>  | 7 mm <sup>3</sup>  | 1012 mm <sup>3</sup> |
|                                 | 11 mm <sup>3</sup> | 8 mm <sup>3</sup>  | 7 mm <sup>3</sup>  | 484 mm <sup>3</sup>  |
|                                 | 12 mm <sup>3</sup> | 12 mm <sup>3</sup> | 10 mm <sup>3</sup> | 864 mm <sup>3</sup>  |
| Group cisplatin                 | 1 mm <sup>3</sup>  | 1 mm <sup>3</sup>  | 1 mm <sup>3</sup>  | 0/50 mm <sup>3</sup> |
|                                 | 3 mm <sup>3</sup>  | 2 mm <sup>3</sup>  | 1 mm <sup>3</sup>  | 9 mm <sup>3</sup>    |
|                                 | 2 mm <sup>3</sup>  | 1 mm <sup>3</sup>  | 1 mm <sup>3</sup>  | 2 mm <sup>3</sup>    |
|                                 | 7 mm <sup>3</sup>  | 5 mm <sup>3</sup>  | 3 mm <sup>3</sup>  | 122 mm <sup>3</sup>  |
| Group Cis-rit                   | 4 mm <sup>3</sup>  | 4 mm <sup>3</sup>  | 3 mm <sup>3</sup>  | 32 mm <sup>3</sup>   |
|                                 | 11 mm <sup>3</sup> | 6 mm <sup>3</sup>  | 5 mm <sup>3</sup>  | 363 mm <sup>3</sup>  |
|                                 | 6 mm <sup>3</sup>  | 4 mm <sup>3</sup>  | 3 mm <sup>3</sup>  | 72 mm <sup>3</sup>   |
|                                 | 13 mm <sup>3</sup> | 9 mm <sup>3</sup>  | 6 mm <sup>3</sup>  | 760 mm <sup>3</sup>  |
| Group control                   | 8 mm <sup>3</sup>  | 6 mm <sup>3</sup>  | 4 mm <sup>3</sup>  | 192 mm <sup>3</sup>  |
|                                 | 9 mm <sup>3</sup>  | 6 mm <sup>3</sup>  | 4 mm <sup>3</sup>  | 243 mm <sup>3</sup>  |
|                                 | 7 mm <sup>3</sup>  | 6 mm <sup>3</sup>  | 5 mm <sup>3</sup>  | 147 mm <sup>3</sup>  |

for 1 min were carried out. Then, mRNA expression was assayed by comparing the test with the standard curve of the specific target and housekeeping gene in each PCR run. Also, a melting curve was designed

to show the confidence of production.

#### Statistical analysis

For statistical analysis, we used Friedman and One-way ANOVA tests accompanied with post

TUKEY-TEST. Also, 23 SPSS software was used for these tests.

## Results

### Tumor growth study

The results of growth tumors measurement in three times points are shown in table 1 and figures 1 and 2.

The statistical analysis of tumor growth by Friedman test showed that:

1- In the control group, there was no significant difference in tumor volumes across the three time points ( $P = 0.166$ ).

2- In the ritanserine-cisplatin (Rit-Cis) group, there was a significant difference in tumor volumes across the three time points ( $P = 0.018$ ), particularly between the first and third measurements ( $P = 0.014$ ).

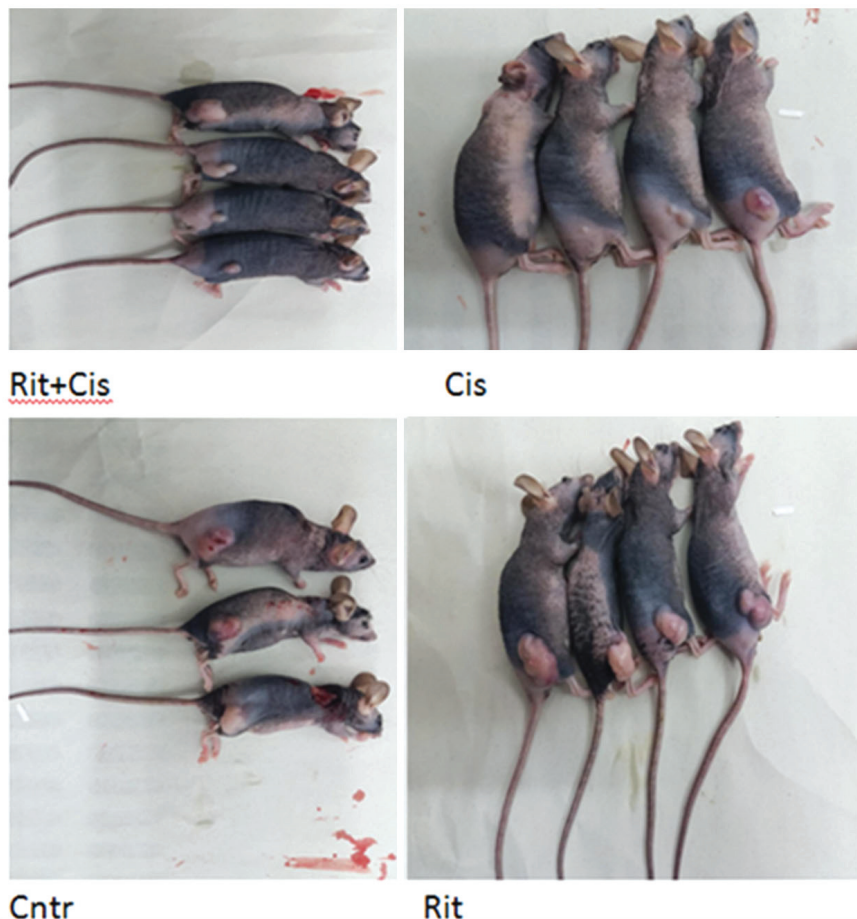
3- In the cisplatin group, there was no significant

difference in tumor volumes across the three time points ( $P = 0.333$ ) and tumor volume remained almost constant, indicating the arresting effect of cisplatin as a positive control on tumor growth.

4- In the ritanserine group, there was a significant difference between the first and third time points ( $P = 0.009$ ), but no significant difference was found between the second and third time points ( $P = 0.064$ ), indicating that the effect of ritanserine on tumor growth diminished over time. The effect of ritanserine on tumors growth is attenuating after the second day of treatment, when it showed an attenuation in tumor growth.

### RT-PCR results

The melting curve analysis for the designed primers showed that each primer pair performed as expected, without any non-specific peaks or secondary structures. Results of melting curve for the designed primers showed that each pair of



**Figure 1.** This figure shows the nude mice in four groups, Cisplatin (Cis), Rit (ritanserine) Rit+Cis and control groups caliper. Tumor sizes ( $\text{mm}^3$ ) were measured using a caliper following drug administration.

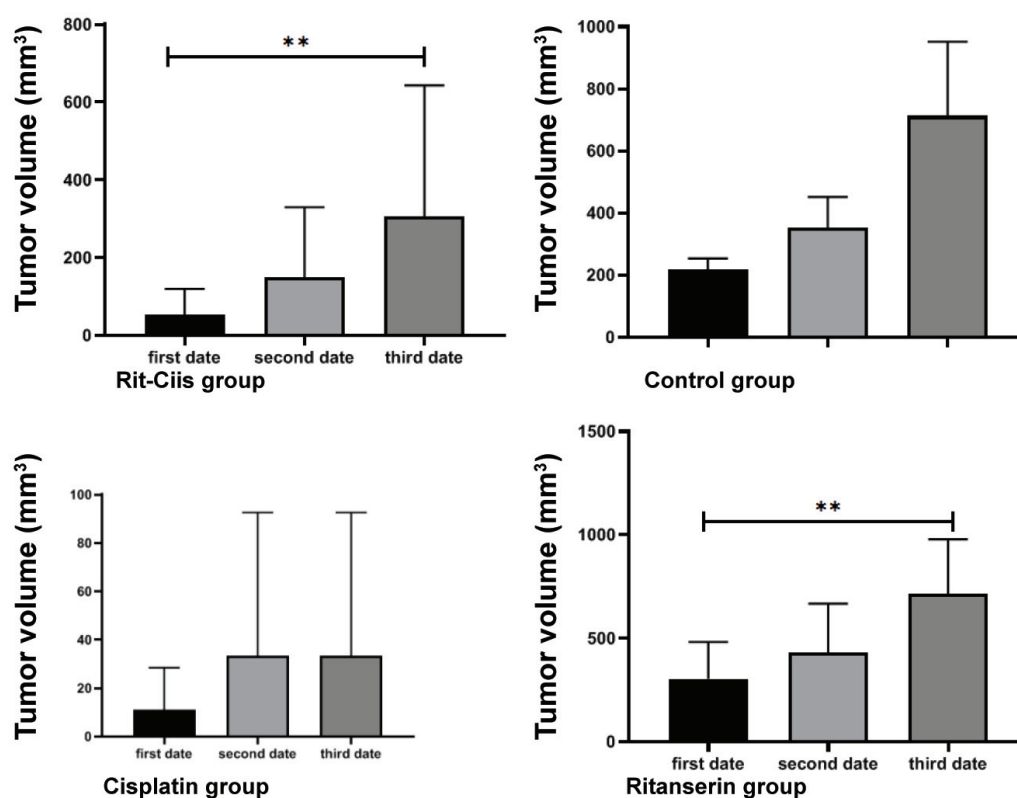
primers acts as especial shape and did not have any non-special patches and secondary structures (Figure 3).

#### *EGFR and COX2 expression results*

The effect of ritanserin on the expression of inflammatory (COX2) and angiogenesis (EGFR) factors in tumors isolated from nude mice by RT-PCR have shown that COX2 expression levels in the cisplatin group increased (51/63) compared with the control group. This increase may represent a resistance response to the inhibitory effect of cisplatin as an antitumor drug. Additionally, ritanserin treatment further increased COX2 expression in the cisplatin group compared with the baseline level (1/00) (Figure 4a). The Rit-Cis group showed a reduction in expression (70/71) indicating a possible synergistic effect between cisplatin and ritanserin. The level of the expression of COX2 gene in cisplatin group has been increased

(51/63) compared with control group which indicated that cisplatin as antitumor drug could have inhibition effects on COX2 gene expression; this increase is a feedback effect in response to cisplatin inhibitory effect. Also, ritanserin could have increased this expression (11/31) compared with control (1/00) (Figure 4a) and this effect for Rit-Cis group was more pronounced (70/71) indicating a synergistic effect between cisplatin and ritanserin; however, according to relative expression curve (Figure 4c) this effect for both ritanserin and Rit-Cis was not significant compared with control group through one way ANOVA analysis.

Also, figures 4b and 4d show how the expression of EGFR as an angiogenic factor, and cisplatin as antitumor drug could have increased in negative feedback (22.7) and ritanserin alone could have increased this expression (6.23). A combination of Rit-Cis had synergistic effect (36.28) compared



**Figure 2.** Statistical analysis of tumor growth in nude mice in three time periods by Friedman test A: In the control group, there is no significant difference between the increases in tumor volume in three time periods. B: In the Rit-cis group, there was a significant difference between the three time periods between the first and third dates ( $P = 0.018$ ). C: In the cisplatin group, there was no significant difference as tumor volume between the three dates ( $P = 0.333$ ). D: In the ritanserin group, there was a significant difference between the first and third time in terms of tumor volume ( $P = 0.009$ ). But there was no significant difference between the second and third times ( $P = 0.064$ ).

Rit-Cis: Ritanserin - Cisplatin

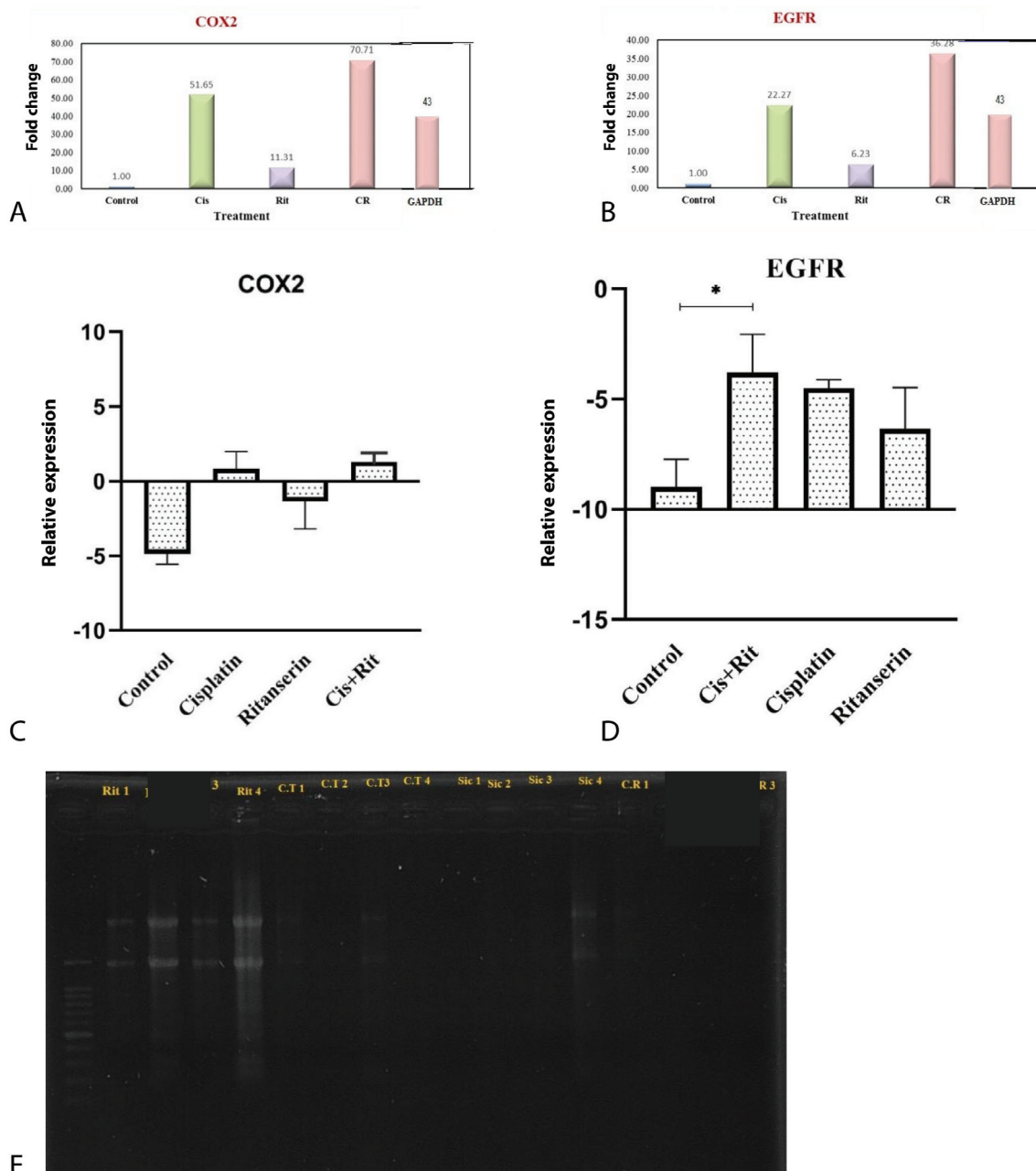
with control group (1) and figure 4d indicated synergic effect between cisplatin and ritanserin ( $P < 0.05$ ).

## Discussion

Our study shows that ritanserin, as 5HT<sub>2A</sub> receptor antagonist, could have retarded size of tumor macroscopically in a xenograft model of HT29-induced colon cancer. This effect may be

mediated through the inhibition of EGFR and COX2 genes which are involved in angiogenesis and inflammation.

5-HT receptors are found in both the central and peripheral nervous systems, in intestinal enterochromaffin cells and blood platelets. They play key roles in various physiological processes, important for a variety of physiological functions, including platelet aggregation, smooth muscle



**Figure 3.** Results of RT-PCR for expression assay of angiogenesis factor genes in tumors isolated from nude \*: Significant,  $P < 0.05$  (relative expression = fold changes) (a-d, COX2, and EGFR expression curves) (e-Gel electrophoresis picture).

RT-PCR: Real time polymerase chain reaction; EGFR: Epidermal growth factor; COX2: Cyclooxygenase; Cis: Cisplatin; Rit: Ritanserin

contraction, appetite, cognition, perception, mood, and other central nervous system functions.<sup>1</sup> These diverse physiological functions are mediated by multiple 5-HT receptor classes and subtype, encoded by distinct genes ranging from 5-HT1-5-HT7.<sup>1-3</sup>

The 5HT2A receptor, which is present in membranes of platelets and vascular smooth muscle, is known to play an important role in platelet aggregation and vasodilation.<sup>7</sup> It has also been suggested that the mitogenic activity of 5-HT may result from the stimulation of phospholipase C- $\beta$  and protein kinase C. The stimulation of 5-HT receptors also promotes the MAPK and accordingly ERK signaling pathways. This signaling cascade leads to increased cell division, a common response to extracellular stimuli such as growth factor, growth factors, hormones, or neurotransmitters in mammalian cells, ultimately resulting in transcription factor phosphorylation and cell proliferation.<sup>7, 8</sup>

Although the mitogenic effects of 5-HT receptors have been investigated in breast, prostate and bladder cancer,<sup>7, 8</sup> their role in colorectal cancer has been clearly established, but the evidences whether these receptors play a significant role in colorectal cancer is not clear enough.<sup>7-9</sup>

Interestingly, some studies have shown that the regular use of a high intake of SSRIs is associated with a reduced risk of colorectal cancer. Also, in an in vitro study, it has shown that fluoxetine could reduce the growth of COLO320 DM colon cancer cells.<sup>11</sup>

We have previously found a mitogenic effect for 5HT1BR, 5HT3AR, and 5-HT3BR in colorectal cancer cells and tissues.<sup>1, 11</sup> Also, in an in vitro study we have investigated the effect of ritanserin, a selective 5HT2AR antagonist, alone or in combination with curcumin on colorectal cancer cell lines.<sup>8</sup> In this study,<sup>8</sup> ritanserin alone and in combination with curcumin reduced the cell viability of colorectal cancer cell lines and suggested a constitutive activation of 5HT2AR in those cells in consistent with the immunoreactivity of 5HT2AR both in colorectal tumor biopsies and cancer cells in-vitro.<sup>7</sup>

Accordingly, it is well known that cytoplasmic distribution of 5-HT2A receptor justify its inter-

nalization from membrane and subsequent initialization through some proliferative signaling pathways such as MAPK/ERK1/2 and JAK/STAT.<sup>16</sup>

Also, we have previously shown that 5HT1B receptors are fully expressed in HT29 cell line and tumor tissues<sup>1</sup> and that serotonin and 5HT1B receptor agonist increased the proliferation of tumor cells but 5HT1B receptor antagonist had antiproliferative and apoptotic effects on HT-29 cells. These findings provide some evidence for the potential role of the 5HT1B receptor in colorectal cancer and in previous studies role of 5HT3, 5HT4 and 5HT1 receptors in colorectal cancer has shown too, in which we have found that 5HT1B and 5HT3 receptors are well expressed in colorectal cancer cells.<sup>1, 10, 11</sup>

Another study showed that serotonin enhances colorectal cancer cell lines growth in a dose dependent manner. This effect of serotonin is imitated by DOI (5HT2A receptor agonist) and inhibited by ritanserin (5HT2A receptor antagonist) in vitro suggesting that this effect is receptor dependent through 5HT2AR.<sup>8</sup>

Accordingly, the mitogenic effect of serotonin through 5-HT2AR on cancer cells has demonstrated by several studies as it has increased as a dose-dependent manner HT1376 bladder cancer cell growth<sup>12</sup> and raised human breast and prostate cancer cell proliferation in vitro.<sup>7</sup> Some reports also shown that 5-HT2AR may be implied in the autocrine loops of growth factors contributing to cell growth in some aggressive tumors.<sup>7</sup> In human placental choriocarcinoma cell lines, by activating the 5-HT2AR, cell growth has been increased by activating cell cycle progression.<sup>17, 18</sup>

Ritanserin, a specific 5HT2AR antagonist, inhibited significantly the cell viability and enhanced apoptosis in an in vitro model of colorectal cancer cell lines in a dose dependent manner.<sup>8</sup> Interestingly, ritanserin, in combination with curcumin, induced two-fold enhancement of cancer cell growth reduction and cell death.<sup>8</sup>

Ritanserin is a reversible serotonin receptor antagonist specific for the 5HT2A subunit. It acts as an active site inhibitor of diacylglycerol kinase alpha (DGKA).<sup>9, 18</sup> The inhibition of DGKA can directly reduce the viability of cancer cells, inhibit

angiogenesis and in particular can increase T cell activation and increase cancer immunity, and ritanserin is a specific inhibitor of DGKA.<sup>9</sup>

Diacylglycerol (DG) kinase (DGK) phosphorylates DG to generate phosphatidic acid (PA), both of which act as key signaling molecules. DGK $\alpha$  is expressed highly in many refractory cancer cells, as melanoma, hepatocellular carcinoma, and glioblastoma cells, reduces apoptosis, and enforce proliferation. In cancer cells, PA produced by DGK $\alpha$  has an important role in proliferation/antiapoptosis. In addition to cancer cells, DGK $\alpha$  is highly plentiful in T cells and makes a nonresponsive state (energy), playing the main mechanism by which advanced cancers avoid immune action. In T cells, DGK $\alpha$  makes energy through DG consumption. Thus, a DGK $\alpha$ -specific inhibitor is expected to be a dual effective anticancer effect that inhibits cancer cell proliferation and simultaneously increases T cell function. Also, the inhibition of DGK $\alpha$  enhances the anticancer effects of programmed cell death-1/programmed cell death ligand 1 blockade synergistically. Thus, DGK $\alpha$  inhibition provides promising new goals of treatment for refractory cancers.<sup>9</sup>

Our recent study, in parallel with previous studies especially those that have shown the role of the 5-HT<sub>2A</sub> receptor in colon cancer, has demonstrated that ritanserin, a 5-HT<sub>2A</sub> receptor antagonist, exhibits antitumor and antiangiogenic effects in a xenograft model using nude mice. Some of these effects may be mediated through the inhibition of COX2, an inflammatory factor, and EGFR, an angiogenic factor. Further studies are certainly required to investigate these mechanisms in more details. Our study provides additional evidence supporting the investigation of angiogenic markers and further aspects of the anticancer effects of ritanserin in clinical trials and with considering more angiogenic markers to investigate more aspects of anticancer effect of ritanserin. The main limitations of the present study were the number of nude mice in each group due to their high cost, and the high costs of antibodies for immunohistochemistry.

## Conclusion

The present study shows the effect of ritanserin, as 5HT<sub>2A</sub> receptor antagonist, in xenograft model of colon cancer and its effect on angiogenic factors, COX2 and EGFR and can be a new insight for the role of 5HT<sub>2A</sub> receptor in colon cancer.

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## Authors' Contribution

F.A.: data collection, R.A.: study design, supervision, writing and drafting the article, E.F.: supervision of study, editing the article. H.G.: RT-PCR analysis and preparing graphs.

All authors read and approved the final manuscript version and agreed with all parts of the work in ensuring that any queries about the accuracy or integrity of any component of the work are appropriately investigated and handled.

## Conflict of Interest

None declared.

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