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Indomethacin and juglone inhibit inflammatory molecules to induce apoptosis in colon cancer cells

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Abstract

Colorectal cancer (CRC) is the third most common fatal cancer. Indomethacin, a nonsteroidal anti-inflammatory drug, is known to reduce the occurrence of CRC. This study evaluated the potential anticolon cancer effects of juglone (5-hydroxy-1,4-naphthoquinone) in combination with indomethacin. Human colon adenocarcinoma cells (HT29) were subjected to treatment with indomethacin, juglone, and a combination of both. Morphological analysis, cell cycle regulation, and dual staining using acridine orange and ethidium bromide in control and treated cells revealed the apoptotic potential of these compounds. Bcl2 and inflammatory molecules (tumor necrosis factor-α, nuclear factor kappa B, and Cox-2) were found to be decreased with a concomitant increase in the expression of proapoptotic molecules (Bad, Bax, cytochrome c, and PUMA) as a result of the molecular regulation of Wnt, Notch, and peroxisome proliferator-activated receptor-γ signaling. Treatment with juglone was not as effective as with indomethacin; however, a combination of both was shown to be more effective, suggesting that juglone may be considered for therapeutic intervention of colon cancer.

KEYWORDS
apoptosis, HT29 cells, indomethacin, inflammation, juglone

1 | INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed and lethal form of cancer.[1] Risk factors include chronic infection, familial history of CRC, and westernized lifestyle. The estimated prevalence rate of CRC is higher in Indian population.[2] The equilibrium between proliferation, differentiation, and apoptosis maintains tissue homeostasis and the deregulation of these processes determines the development of CRC.[3] Wnt and Notch signaling pathways control cell proliferation, differentiation, and development; hence, perturbations in these signaling mechanisms act as a hallmark in a majority of cancers.[4,5] Peroxisome proliferator-activated receptor-γ (PPARγ) is a ligand-inducible transcription factor, belonging to the nuclear hormone receptor super family, which shows antitumorogenic activity.[6] Therefore, studies identifying the link between PPARγ, Wnt, and Notch signaling pathways may contribute to the development of new therapeutic targets against colon cancer.

Indomethacin, a nonsteroidal anti-inflammatory drug, is widely prescribed for fever, inflammation, and pain.[7,8] Clinical studies have demonstrated that indomethacin inhibits the proliferation of colon cancer cells in patients regularly administered this drug.[9,10] Regular intake of indomethacin may, however, result in side effects, especially, gastric ulceration[11] by decreasing blood flow into the mucosal regions and increasing gastric juice volume, leading to decrease in gastric pH.[12,13] A regimen using natural diet, rich in greens, fruits, and nuts potentially reduces the risk of cancer progression through their anti-inflammatory, antiproliferative, and immunomodulatory properties along with altered molecular signaling pathways.[14-16] Recently, compounds isolated from natural products were found to be beneficial in treating various metabolic diseases, including cancer.[17,18] Juglone (5-hydroxy-1,4-naphthoquinone), present in the roots, leaves, nut hulls, bark, and wood of the black walnuts, possesses antimicrobial, anti-inflammatory, immunomodulatory, diuretic, and laxative properties.[19] Juglone has been shown to inhibit ulcer formation in the gastric region.[20,21] Juglone also acts as a natural inhibitor of Pin1, which is elevated in cancerous conditions.[22] Recently, several approaches have been made to increase the efficacy of anticancer drugs. For example, methyl-β-cyclodextrin, a cholesterol-depleting agent has been shown to improve the efficacy of tamoxifen and doxorubicin in

several cancers.[23,24] The present study aims to investigate the synergistic effect of indomethacin and juglone in regulating Wnt/β-catenin, Notch, and PPARγ molecular signaling mechanisms in altered inflammatory cascades associated with colon adenocarcinoma, in vitro.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

Indomethacin (MP Biomedicals, LLC), juglone (Sigma-Aldrich), Dulbecco's modified Eagle medium (DMEM), and fetal bovine serum (FBS; HiMedia Laboratories Pvt Ltd, Mumbai, India), Antibodies against Bcl2, Bad, PUMA, PCNA, β-catenin, cyclin D1, Hes1, Bax, cytochrome c, tumor necrosis factor-α (TNF-α), Notch intracellular domain (NICD) and β-actin (Cell Signaling Technology). Nuclear factor kappa B (NFκB), Cox-2, and PPARγ antibodies were purchased from Cloud-Clone Corp (Katy, TX). All other chemicals, solvents, and reagents used were of maximum purity grade.

2.2 | Cell culture and drug preparation

Human adenocarcinoma cells (HT29) were obtained from National Center for Cell Science, Pune, India. The cells were maintained in DMEM, supplemented with 10% FBS, 1× antibiotic antimycotic solution at 37°C under 5% CO_2 and 95% humidity. Ten millimolar solution of indomethacin and 100 mM juglone stock solutions were prepared with dimethyl sulfoxide (DMSO) at 0.1% concentration and diluted using DMEM, as required.

2.3 | Cell viability assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay was performed to measure the effect of indomethacin and juglone on cell viability in HT29 cells.[25] Briefly, HT29 cells (1×10^3/well) were seeded in a 96-well plate. Upon reaching 70% confluence, the cells were incubated with various concentrations of indomethacin (100-1000 μM) and juglone (10-100 μM) for 24 and 48 hours. After incubation, the cells were treated with MTT (1 μg/ml/well) for 4 hours at 37°C. DMSO was added to dissolve formazan crystals and optical density was measured at 570 nm.

2.4 | Cell treatments

HT29 cells were grown to confluence and transferred to 6- or 24-well plates and treated with indomethacin (150 μM) or juglone (75 μM) or combination of both.

2.5 | Morphological analysis

Morphological changes were observed using an inverted microscope (Carl Zeiss). HT29 cells were seeded on 1 cm^2 sterile glass slides (1×10^5/well). After the treatment period, the cells were prepared for scanning electron microscopic (SEM) imaging as described previously.[26]

2.6 | Acridine orange-ethidium bromide dual staining

HT29 cells, after treatment with indomethacin or juglone or combination of both, were stained with a combination of acridine orange and ethidium bromide (AO/EtBr) (100 μg/mL). Cells with disrupted nuclei and

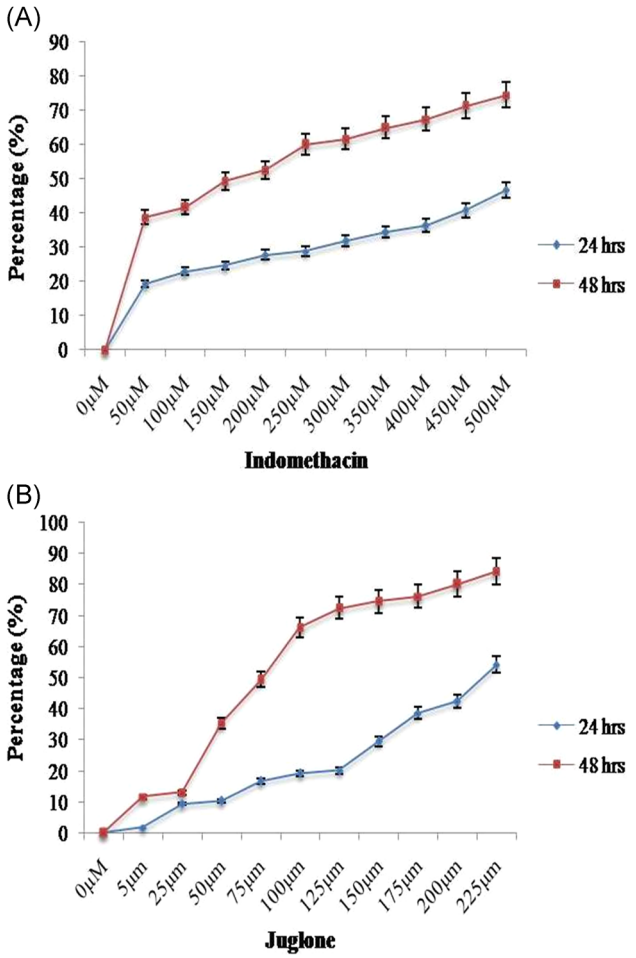


FIGURE 1 The cytotoxic effect of (A) indomethacin and (B) juglone on HT29 cells was analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. A, HT29 cells were treated with indomethacin at different concentrations (50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 μM) for 24 and 48 hours, the estimated IC_{50} value of indomethacin was found to be 150 μM after 48 hours. B, HT29 cells were exposed to different concentrations (5, 25, 50, 75, 100, 125,150, 175, 200, and 225 μM) of juglone for 24 and 48 hours, the IC_{50} value of juglone was found to be 75 μM after 48 hours

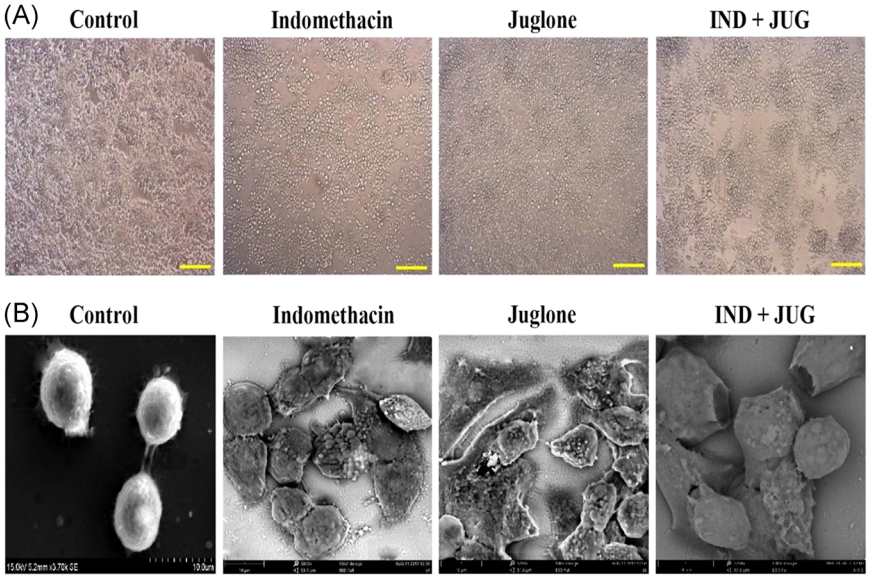


FIGURE 2 A, Morphological analysis using inverted microscope (×10, scale bar = 100 μm) and (B) scanning electron microscopic image (scale bar = 10 μm) of HT29 cells with IC_{50} concentrations of indomethacin and juglone treatment. A, Control cells were observed with monolayer formation. Cytotoxicity was observed in indomethacin, juglone, and IND + JUG group. B, Control cells showed cell to cell interaction and indomethacin, juglone, and IND + JUG groups indicate apoptotic features

condensed chromatin were taken as indicative of apoptotic cells. Cells were viewed using a fluorescence microscope (Carl Zeiss).[27]

2.7 | Cell cycle analysis

Control and treated cells were harvested and prepared as single cell suspensions in phosphate-buffered saline, fixed with 70% ice-cold ethanol and stored at 4°C for 24 hours. After fixing, the cells were stained with propidium iodide (0.05 mg/mL), as described earlier[28] and analyzed using a FACSCalibur flow cytometer (Becton Dickinson).

2.8 | Reverse transcriptase polymerase chain reaction

Total RNA was extracted from HT29 cells and cDNA was synthesized, by subsequent RNA reverse transcription, as per the manufacturer's instructions (TaqMan Reverse Transcription Reagent; Applied Biosystems). Polymerase chain reaction (PCR) ready mix (Invitrogen) was used to amplify the cDNA. Primer sequences and amplification conditions used are shown in Table S1.

2.9 | Western blot analysis

Thirty to forty micrograms of protein samples isolated from control and experimental cells were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membrane. The membranes were blocked with 3% BSA, washed, and incubated with respective primary antibodies (Bcl2, Bad, Bax, cytochrome c, PUMA, PCNA, TNF-α, β-catenin, cyclin D1, NICD, Hes1, β-actin at a dilution of 1:2000 and NFκB, Cox-2, PPARγ at 1:500 dilution), overnight. The membranes were washed and incubated with horseradish peroxidase-coupled secondary antibody for 2 hours at room temperature, and washed three times. The specific reactive proteins were captured using enhanced chemiluminescent kit (Amersham, UK).

2.10 | Statistical analysis

All the data were analyzed with SPSS version 16 software. Hypothesis testing methods included one-way analysis of variance, followed by least significant difference test. $p < .05$ was considered to be statistically significant.

3 | RESULTS

3.1 | Cytotoxic effect of indomethacin and juglone in HT29 cells

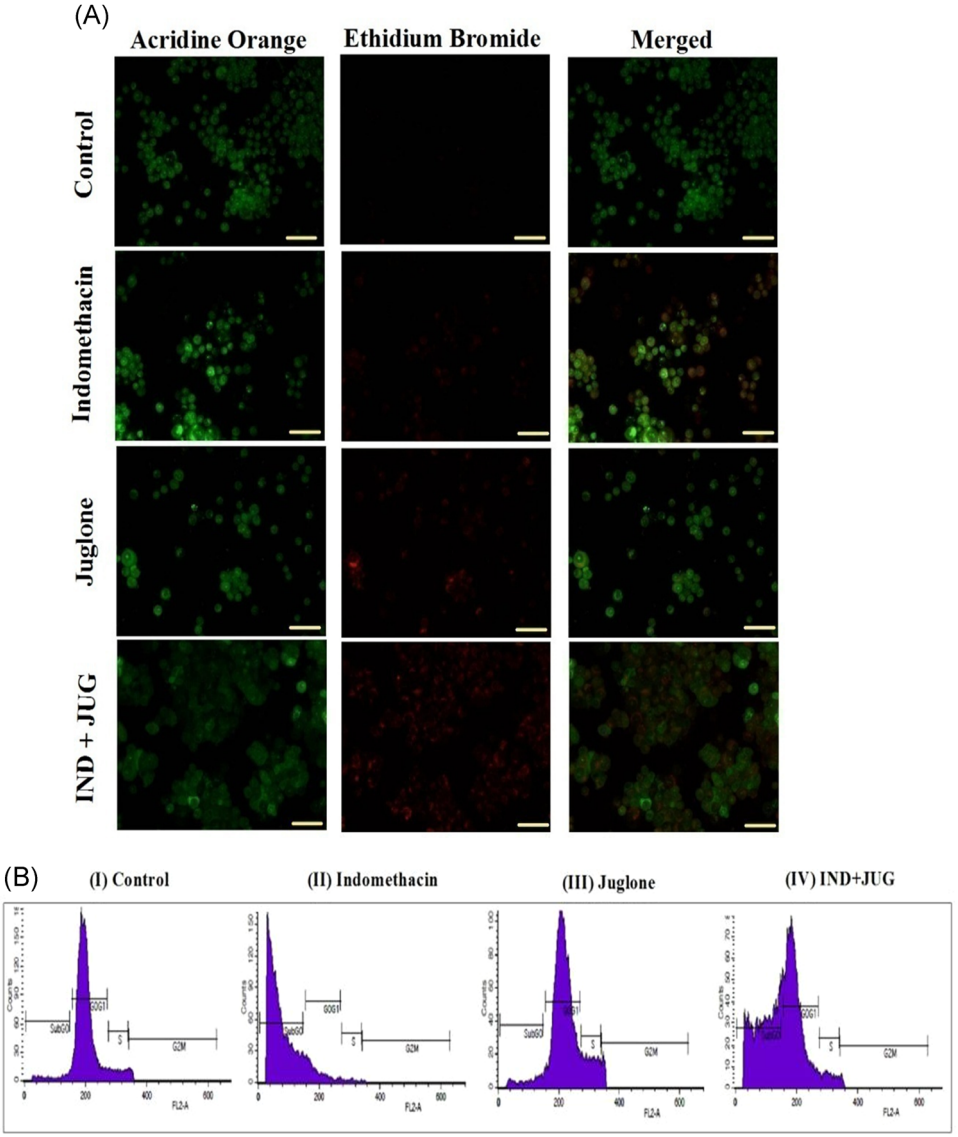
The cytotoxic effect of indomethacin and juglone in HT29 cells was examined in a dose- and time-dependent manner. The estimated IC_{50} value of indomethacin was found to be 150 μM (Figure 1A) and 75 μM for juglone for 48 hours (Figure 1B).

3.2 | Morphological analysis of indomethacin and juglone in HT29 cells

The morphological appearance of HT29 cells is shown in Figure 2A. Control cells were undifferentiated, epithelial, significantly proliferating, and formed a monolayer (Figure 2A). Indomethacin and juglone cotreated group showed a remarkably reduced number of cells when compared with treatment with single drug alone. SEM imaging of the treated groups portrayed multiple membrane blebs, flattened cells, and enlargement of the perinuclear space and the formation of apoptotic bodies when compared to control. Cells treated with both the drugs exhibited more apoptotic changes when compared with single drug treatment (Figure 2B).

3.3 | AO/EtBr staining

HT29 cells of control and treated groups were stained with AO/EtBr. Green fluorescence cells are indicative of live cells. A significant number of cells treated with indomethacin and juglone projected reddish orange color, indicative of apoptosis. The indomethacin or juglone treated groups also showed apoptotic cells; however, the intensity was more in the indomethacin group compared with the juglone-treated group (Figure 3A).



Marker	(I)	(II)	(III)	(IV)
All	75.87	32.82	65.93	64.95
Sub G0	2.8	27.87	3.42	27.72
G0 G1	65.28	3.73	50.89	31.02
S	5.44	0.43	8.81	2.69
G2M	1.27	0.05	1.77	0.39

FIGURE 3 A, Fluorescence images of HT29 cells stained with acridine orange/ethidium bromide (AO/EtBr) after being exposed to indomethacin and juglone for 48 hours, ×10 (100 μm). B, Flow cytometric analysis of HT29 cells treated with indomethacin, juglone, and IND + JUG using propidium iodide staining to analyse cell cycle progression. I, Control; II, indomethacin; III, juglone; and IV, IND + JUG

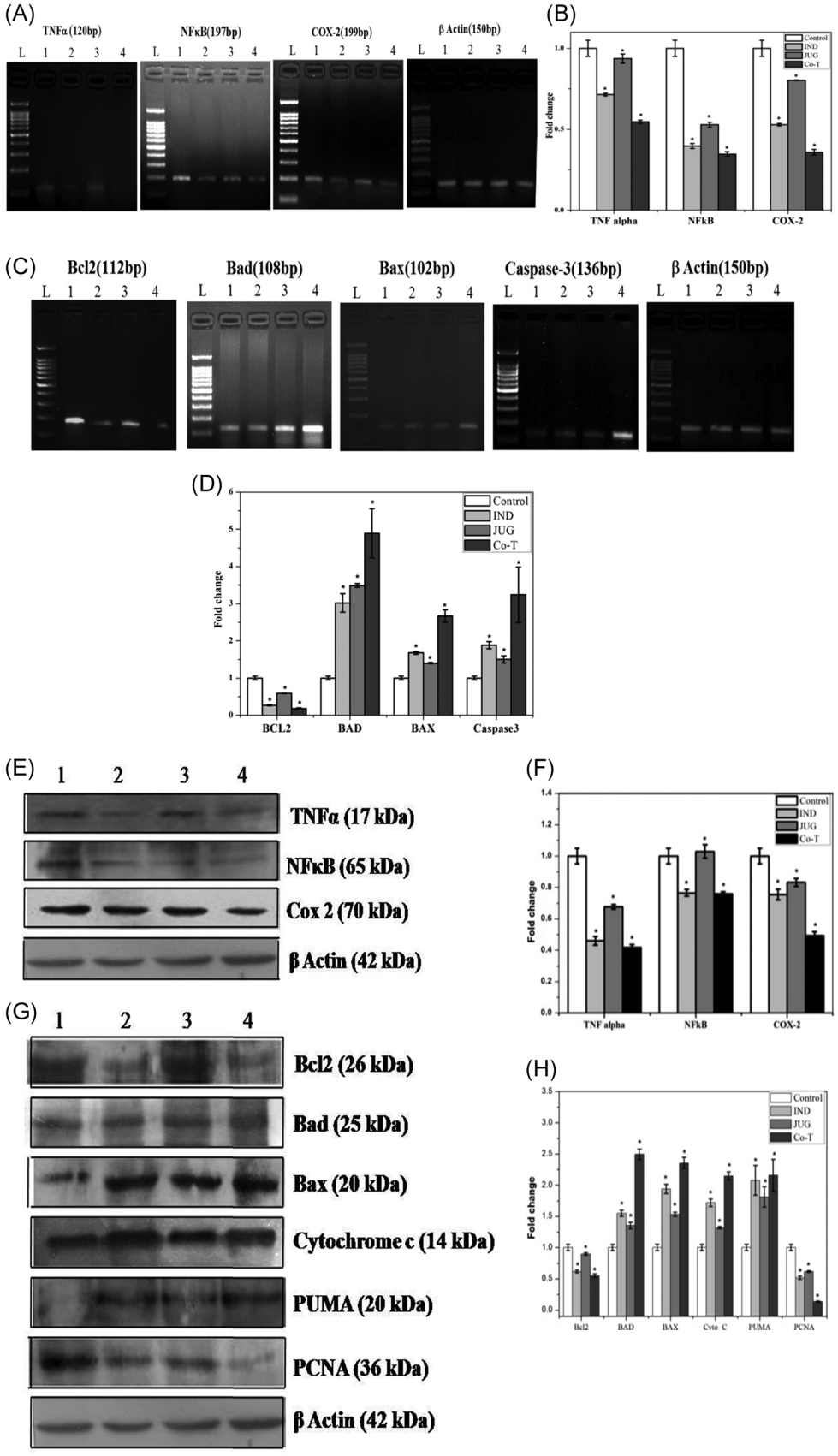


FIGURE 4 Continued.

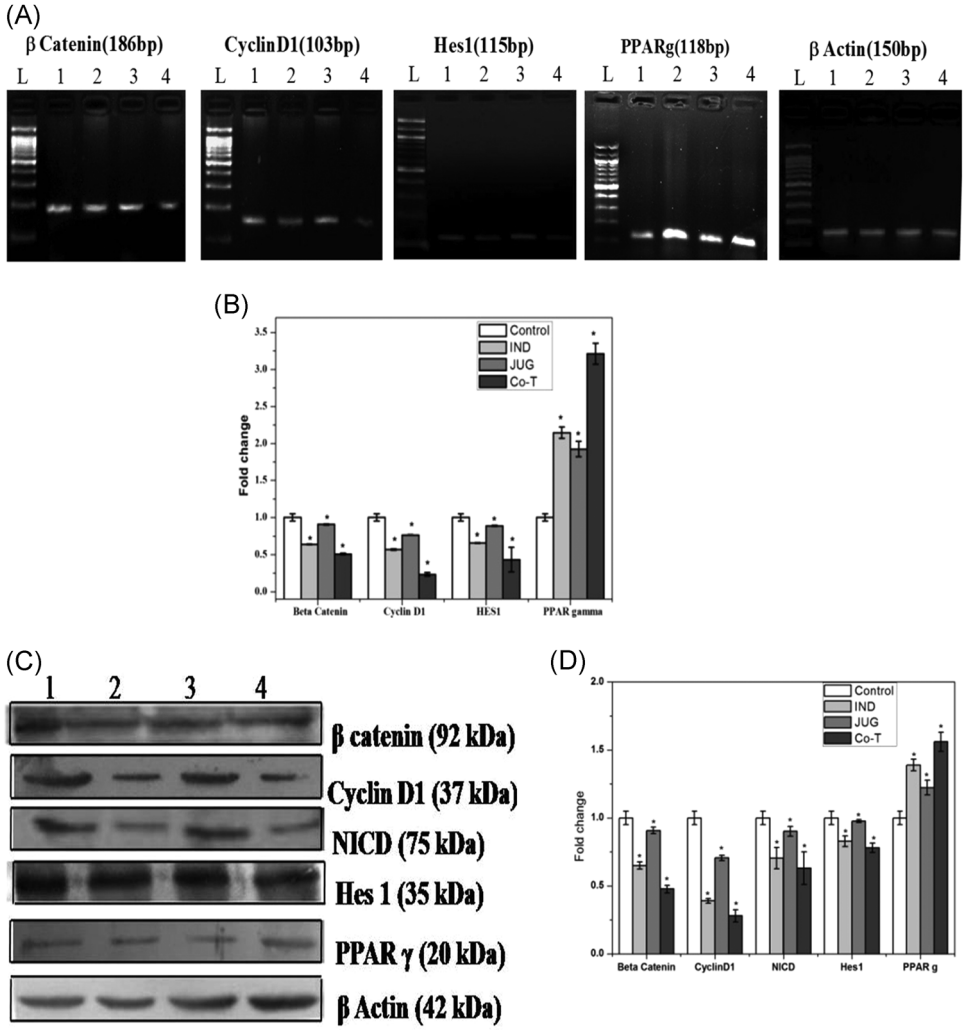


FIGURE 5 Effect of indomethacin, juglone, and IND + JUG on β-catenin, cyclin D1, Hes1, Notch intracellular domain (NICD), and peroxisome proliferator-activated receptor-γ (PPARγ). A, Reverse transcriptase polymerase chain reaction (RT-PCR) analysis of β-catenin, cyclin D1, Hes1, PPARγ, and β-actin as internal control. L-100bp Ladder, 1-control, 2-indomethacin treated, 3-juglone treated, and 4-indomethacin + juglone. C, Western blot analysis of β-catenin, cyclin D1, NICD, Hes1, PPARγ expression, and β-actin served as internal control. 1-control, 2-indomethacin treated, 3-juglone treated, and 4-indomethacin + juglone. B, D, Densitometric analysis of RT-PCR and Western blot analysis were normalized with β-actin. Hypothesis testing method included one-way analysis of variance. Statistical significance at $p < .05$. Comparisons are made with control group and drug treated groups

3.4 | Cell cycle analysis of indomethacin and juglone treated HT29 cells

In the control group, 2.80% of cells were in sub G0 phase, 65.28% of cells in G0/G1 phase, while 5.44% and 1.27% of cells were in S and G2/M phase. HT29 cells treated with indomethacin alone showed 27.87% of cells in sub G0 phase, 3.73% of cells in G0/G1 phase, 0.43% and 0.05% in S and G2/M phase, respectively. Cells exposed to juglone showed 3.42%, 50.89%, 8.81% and 1.77% in sub G0 phase, G0/G1 phase, S phase, and G2/M phase, respectively. Cells treated with indomethacin and juglone revealed 27.72% of cells in sub G0 phase, 31.02% in G0/G1

FIGURE 4 Effect of indomethacin, juglone, and IND + JUG on inflammatory molecules (tumor necrosis factor-α [TNF-α], nuclear factor kappa B [NFκB], and Cox-2), apoptotic molecules (Bcl2, Bad, Bax, cytochrome c, PUMA, and caspase-3), and proliferative marker PCNA. A, Reverse transcriptase polymerase chain reaction (RT-PCR) analysis of TNF-α, NFκB, Cox-2, and β-actin as internal control. C, RT-PCR analysis of apoptotic molecules: Bcl2, Bad, Bax, caspase-3, and internal control β-actin. L-100bp Ladder, 1-control, 2-indomethacin treated, 3-juglone treated and 4-indomethacin + juglone. Western blot analysis of (E) TNF-α, NFκB, Cox-2, and β-actin as internal control. G, Bcl2, Bad, Bax, cytochrome c, PUMA, PCNA, and β-actin as internal control. 1-control, 2-indomethacin treated, 3-juglone treated, and 4-indomethacin + juglone. B, D, F, H, Densitometric analysis of RT-PCR and Western blot analysis were normalized with β-actin. Hypothesis testing method included one-way analysis of variance. Statistical significance at $p < .05$. Comparisons were made with control group and drug treated groups

phase, while 2.69% in S phase, and 0.39% in G2M phase (Figure 3B).

3.5 | Indomethacin and juglone reduced the expression of inflammatory molecules and induced apoptosis

Inflammatory cytokines such as TNF-α, NFκB, and Cox-2 were evaluated in HT29-treated cells. These inflammatory cytokines were found to be reduced in the cotreated cells when compared with indomethacin- or juglone-treated cells. However, indomethacin-treated cells revealed reduced inflammatory response as compared to those treated with juglone (Figures 4A and 4E). Proapoptotic molecules such as Bad, Bax, caspase-3, cytochrome c, and PUMA were found to be significantly upregulated in the cotreated group and cells treated with indomethacin demonstrated better upregulation than the juglone treated cells, where juglone treatment also revealed, increased expression as compared to control. The expression of antiapoptotic molecule Bcl2 was the opposite when compared with proapoptotic molecules. The protein expression of PCNA was significantly reduced in treatment groups as compared with control (Figures 4C and 4G). Both the gene and protein expression of β-catenin, NICD, cyclin D1, and Hes1 revealed downregulated expression in indomethacin, juglone, and cotreated cells when compared with control. PPARγ was increased in combination of indomethacin and juglone treated cells when compared with indomethacin or juglone treatment; the control group showed decreased expression. PPARγ was intensely expressed in the indomethacin group compared with the juglone group (Figures 5A and 5C).

4 | DISCUSSION

The initiation and progression of CRC are associated with inflammation, alteration of signaling pathways, and subsequent inhibition of apoptosis.[29] Inflammatory cytokines promote growth in cancer cells and support their survival by interacting with signaling molecules.[30,31] Indomethacin and juglone effectively target the inflammatory molecules and induce apoptosis in various cancer cells.[32,33] Therefore, the aim of the study was to elucidate the roles of Wnt, Notch, and PPARγ pathways in colon carcinogenesis. In this study, treatment with indomethacin and juglone inhibits inflammation and cancer cell proliferation and induces apoptosis in HT29 cells. Indomethacin has previously been shown to inhibit cancer cell proliferation.[34] However, it causes side effects like gastric ulcer.

Inflammation plays a critical role in cancer initiation.[35] It stimulates mediators such as, NFκB, and TNF-α, which activate multiple downstream proteins, like interleukins and Cox-2, which lead to cancer cell survival through the activation of antiapoptotic Bcl2 family proteins.[36] Overexpression of Bcl2 causes the loss of proapoptotic molecules Bax, Bad, cytochrome c, and PUMA in cancer.[37] The ratio of antiapoptotic and proapoptotic molecules is dependent on the apoptosis-inducing effect of drugs. Cells treated with drugs (indomethacin, juglone, and combination of indomethacin and juglone) not only exhibited decreased expression of antiapoptotic Bcl2, but also showed an increased expression of proapoptotic proteins, which is in agreement with previous reports.[38-41] The results obtained in this study show that indomethacin, juglone, and cotreatment of these two drugs, exhibits their action, presumably, by downregulating the inflammatory mediators, inhibiting the activation of antiapoptotic molecules and increasing the levels of proapoptotic molecules to induce apoptosis in HT29 cells. Previous reports have shown that the activated inflammatory cytokine NFκB may trigger the Wnt/β-catenin pathway, which could interact with the Notch/NICD signaling pathway to mediate cancer initiation and progression.[42,43] Activation of the Wnt signaling pathway leads to aberrant translocation of β-catenin and initiating tumor progression through the upregulation of its target genes such as cyclin D1, c-jun, c-Myc, and fra-1.[44] The activation of the Notch/NICD signaling pathway causes the accumulation, transcription, and overexpression of its target gene Hes1, which plays a key role in tumorogenesis by maintaining stemness and also involved in cell proliferation, differentiation, and apoptosis.[45] It has been previously reported that the overexpression of Notch signaling alone does not contribute to cancer initiation; however, cross-talk between the Wnt and Notch pathways is known to increase the risk of cancer initiation[42,46]; while, their interaction with PPARγ decreases the risk of oncogenic upregulation upon its activation by an external drug as a ligand. Ligand activated PPARγ downregulates the transcription process of Wnt, Notch, and their target genes to lead the cells to apoptosis.[47,48]

The results of this study indicate that indomethacin effectively activates PPARγ as compared with juglone alone, but a combination of the two was also found to be effective in activating PPARγ. The upregulation of PPARγ was accompanied with a concomitant decrease in the expression of inflammatory cytokines, thereby inhibiting cancer survival and progression in HT29 cells. The combination of juglone and indomethacin exhibited better efficacy in a mouse model of DMH/DSS-induced colon carcinogenesis through regulation of β-catenin and Notch pathways (data not shown). To conclude, juglone, in combination with indomethacin, induces apoptosis in HT29 cells through modulation of Wnt, Notch, and PPARγ pathways and can be considered as a therapeutic strategy for the treatment of colorectal carcinogenesis.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

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REFERENCES

[1] M. Arnold, M. S. Sierra, M. Laversanne, I. Soerjomataram, A. Jemal, F. Bray, *Gut* **2017**, *66*, 683.
[2] P. S. Patil, A. Saklani, P. Gambhire, S. Mehta, R. Engineer, A. De'Souza, S. Chopra, M. Bal, *Indian J. Surg. Oncol.* **2017**, *8*, 484.
[3] R. A. Gupta, R. N. DuBois, *Nat. Rev. Cancer* **2001**, *1*, 11.
[4] M. Caspi, A. Zilberberg, H. Eldar-Finkelman, R. Rosin-Arbesfeld, *Oncogene* **2008**, *27*, 3546.
[5] A. Kazanjian, N. F. Shroyer, *Curr. Colorectal Cancer Rep.* **2011**, *7*, 121.
[6] T. H. Chang, E. Szabo, *Cancer Res.* **2000**, *60*, 1129.
[7] D. Zhou, I. Papayannis, G. G. Mackenzie, N. Alston, N. Ouyang, L. Huang, T. Nie, C. C. Wong, B. Rigas, *Carcinogenesis* **2013**, *34*, 943.
[8] S. Nalamachu, R. Wortmann, *Postgrad. Med.* **2014**, *126*, 92.
[9] G. Zhang, C. Tu, G. Zhang, G. Zhou, W. Zheng, *Leuk. Res.* **2000**, *24*, 385.
[10] Y. C. Guo, C. M. Chang, W. L. Hsu, S. J. Chiu, Y. T. Tsai, Y. H. Chou, M. F. Hou, J. Y. Wang, M. H. Lee, K. L. Tsai, W. C. Chang, *Molecules* **2013**, *18*, 6584.
[11] P. Guha, A. Dey, A. Chatterjee, S. Chattopadhyay, S. Bandyopadhyay, *Br. J. Pharmacol.* **2010**, *159*, 726.
[12] Y. K. Shim, N. Kim, *Korean J. Gastroenterol.* **2016**, *67*, 300.
[13] S. Sabiu, T. Garuba, T. O. Sunmonu, A. O. Sulyman, N. O. Ismail, *Pharm. Biol.* **2016**, *54*, 180.
[14] N. Muhammad, R. Steele, S. T. Isbell, N. Philips, R. B. Ray, *Oncotarget* **2017**, *8*, 66226.
[15] S. Bhattacharya, N. Muhammad, R. Steele, J. Kornbluth, R. B. Ray, *Cancer Prev. Res.* **2017**, *10*, 337.
[16] S. Bhattacharya, N. Muhammad, R. Steele, G. Peng, R. B. Ray, *Oncotarget* **2016**, *7*, 33202.
[17] A. Sharma, N. Singh, *Int. J. Recent Sci. Res.* **2015**, *6*, 4825.
[18] J. Benites, H. Toledo, F. Salas, A. Guerrero, D. Rios, J. A. Valderrama, P. B. Calderon, *Oxid. Med. Cell Longev.* **2018**, *2018*, 1618051.
[19] K. B. Aithal, S. Kumar, B. N. Rao, N. Udupa, S. B. S. Rao, *Integr. Cancer Ther.* **2012**, *11*, 68.
[20] N. Mohammad, S. V. Singh, P. Malvi, B. Chaube, D. Athavale, M. Vanuopadath, S. S. Nair, B. Nair, M. K. Bhat, *Sci. Rep.* **2015**, *5*, 11853.
[21] N. Mohammad, P. Malvi, A. Meena, S. Singh, B. Chaube, G. Vannuruswamy, M. J. Kulkarni, M. Bhat, *Mol. Cancer* **2014**, *13*, 204.
[22] J. F. Morton, *Folk remedies of the low country*, E. A. Seemann Pub., Miami, FL **1974**.
[23] S. Arora, K. Kaur, S. Kaur, *Teratog., Carcinog., Mutagen.* **2003**, *23*, 295.
[24] E. Campaner, A. Rustighi, A. Zannini, A. Cristiani, S. Piazza, Y. Ciani, O. Kalid, G. Golan, E. Baloglu, S. Shacham, B. Valsasina, *Nat. Commun.* **2017**, *8*, 15772.
[25] S. P. C. Cole, *Cancer Chemother. Pharmacol.* **1986**, *17*, 259.
[26] X. Yan, H. Luo, X. Zhou, B. Zhu, Y. Wang, X. Bian, *Oncol. Rep.* **2013**, *30*, 2733.
[27] H. J. Zhou, J. L. Zhang, A. Li, Z. Wang, X. E. Lou, *Cancer Chemother. Pharmacol.* **2010**, *66*, 21.
[28] R. Solmi, M. Lauriola, M. Francesconi, D. Martini, M. Voltattorni, C. Ceccarelli, G. Ugolini, G. Rosati, S. Zanotti, I. Montroni, G. Mattei, *BMC Cancer* **2008**, *8*, 227.
[29] P. Allavena, C. Garlanda, M. G. Borrello, A. Sica, A. Mantovani, *Curr. Opin. Genet. Dev.* **2008**, *18*, 3.
[30] X. Li, S. Jiang, R. I. Tapping, *Cytokine* **2010**, *49*, 1.
[31] A. G. Long, E. T. Lundsmith, K. E. Hamilton, *Curr. Colorectal Cancer Rep.* **2017**, *13*, 341.
[32] J. H. Paik, J. H. Ju, J. Y. Lee, M. D. Boudreau, D. H. Hwang, *J. Biol. Chem.* **2000**, *275*, 28173.
[33] X. Peng, Y. Nie, J. Wu, Q. Huang, Y. Cheng, *Biochem. Biophys. Res. Commun.* **2015**, *462*, 245.
[34] A. Amanullah, R. Mishra, A. Upadhyay, P. P. Reddy, R. Das, A. Mishra, *J. Cell. Physiol.* **2018**, *233*, 1685.
[35] S. I. Grivennikov, *Semin. Immunopathol.* **2013**, *35*, 229.
[36] J. M. Schuetz, A. Grundy, D. G. Lee, A. S. Lai, L. C. Kobayashi, H. Richardson, J. Long, W. Zheng, K. J. Aronson, J. J. Spinelli, A. R. Brooks-Wilson, *PLoS One* **2019**, *14*, e0209010.
[37] S. Zaman, R. Wang, V. Gandhi, *Leuk. Lymphoma* **2014**, *55*, 1980.
[38] Y. M. Ko, T. Y. Wu, Y. C. Wu, F. R. Chang, J. Y. Guh, L. Y. Chuang, *J. Ethnopharmacol.* **2011**, *137*, 1283.
[39] Y. Zhu, S. Bu, *Evidence-Based Complementary Altern. Med.* **2017**, *2017*, 5787218.
[40] M. Moradzadeh, A. Tabarraei, H. R. Sadeghnia, A. Ghorbani, A. Mohamadkhani, S. Erfanian, A. Sahebkar, *J. Cell. Biochem.* **2018**, *119*, 2288.
[41] Y. B. Ji, G. S. Xin, Z. Y. Qu, X. Zou, M. Yu, *Genet. Mol. Res.* **2016**, *15*.
[42] N. Gopalakrishnan, M. Saravanakumar, P. Madankumar, M. Thiyagu, H. Devaraj, *Mol. Cell. Biochem.* **2014**, *396*, 281.
[43] X. Zhang, T. Chen, J. Zhang, Q. Mao, S. Li, W. Xiong, Y. Qiu, Q. Xie, J. Ge, *Cancer Sci.* **2012**, *103*, 181.
[44] S. B. Raja, V. Rajendiran, N. K. Kasinathan, P. Amrithalakshmi, S. Venkatabalasubramanian, M. R. Murali, H. Devaraj, S. N. Devaraj, *Food Chem. Toxicol.* **2017**, *106*, 92.
[45] Z. Zhang, R. Yan, Q. Zhang, J. Li, X. Kang, H. Wang, L. Huan, L. Zhang, F. Li, S. Yang, J. Zhang, *Brain Res.* **2014**, *1583*, 65.
[46] S. K. Kay, H. A. Harrington, S. Shepherd, K. Brennan, T. Dale, J. M. Osborne, D. J. Gavaghan, H. M. Byrne, *PLoS Comput. Biol.* **2017**, *13*, e1005400.
[47] E. A. Jansson, A. Are, G. Greicius, I. C. Kuo, D. Kelly, V. Arulampalam, S. Pettersson, *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 1460.
[48] L. Sabatino, M. Pancione, C. Votino, T. Colangelo, A. Lupo, E. Novellino, A. Lavecchia, V. Colantuoni, *World J. Gastroenterol.* **2014**, *20*, 7137.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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