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Research Article

Inhibition of Colon Cancer Cell Viability and Tumor Growth by Benzoximemethyl Amine Through Apoptosis

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Abstract

Background and Objective: Colon cancer is the 3rd most common cause of cancer deaths in the world, so, the present study was aimed to investigate the effect of benzoximemethyl amine on colon cancer cell viability *in vitro* and tumor development *in vivo*. **Materials and Methods:** Cell viability was determined by MTT assay and apoptosis by flow cytometry by using Hoechst 33342 staining. Transwell chamber and wound healing assays were used for assessment of cell migration and invasion, respectively. **Results:** Benzoximemethyl amine treatment of HCT15 and DLD-1 cells reduced viability at15 μM concentrations. Treatment of DLD-1 cells with benzoximemethyl amine markedly increased the levels of caspase-3/8/9, PPARγ activation and cleaved PARP. The expression of pIκBα, p50, p65 and iNOS was decreased markedly in DLD-1 cells on exposure to benzoximemethyl amine. Benzoximemethyl amine exposure suppressed DLD-1 cell migration and invasion potential. Exposure of DLD-1 cells to benzoximemethyl amine significantly decreased levels of MMP-2 and -9 expressions. Treatment of the mice with benzoximemethyl amine significantly decreased the tumor incidence and multiplicity. **Conclusion:** It can concluded that benzoximemethyl amine inhibited the colon cancer by leading to cell apoptosis and suppresses tumor metastasis. Benzoximemethyl amine may be developed as a potent drug candidate for the treatment of colon cancer.

Key words: Peroxisome proliferator, tumor metastasis, chemotherapy, benzoximemethyl amine, colon cancer, cell viability, tumor development

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Colon cancer is one of leading carcinoma and ranks, 3rd most common gastrointestinal cancer throughout the world[1]. In United States colorectal cancer is one of the main reason for cancer-related death[2]. There has been drastic increase in the detection of colon cancer cases because of the changes in food habits and lifestyle over past few years[1-3]. In most of the patients colon cancer is detected at the advanced or late stage because of the absence of early symptoms. In more than 32% patients colon cancer is detected at very late stage which is a major challenge to the available treatment strategy. Moreover, the rate of colon cancer recurrence post-surgery and its metastasis is very high[1-3]. The colon cancer treatment strategy available currently involves surgical intervention and subsequent adjuvant chemotherapy. The chemotherapy for colon cancer is limited by the development of drug resistance and adverse side effects[3].

Peroxisome proliferator-activated receptor γ (PPARγ) has been found in various types of cells and tissues such as liver, pancreas, kidney, adipose tissue and colon[4]. It regulates the level of inflammatory molecules in the intestines thereby playing an important role in inhibiting inflammation[5]. The PPARγ in association with its activators regulates inflammatory processes by the modulation of nuclear factor-κB (NF-κB) activation[5]. Activation of PPARγ by rosiglitazone during clinical trials successfully treated ulcerative colitis[6]. In addition, the intestinal inflammation inhibition in mice model by punicic acid is also associated with the activation[7] of PPARγ. Investigation of the mechanistic details revealed possibility of diverse mechanisms in the inflammatory gene expression inhibition[8] by PPARγ. Some of the possible mechanisms include interaction of PPARγ with NF-κB, MAPK activity modulation, co-activators competition, etc[4]. The present study was devised with an aim to evaluate the effect of benzoximemethyl amine on viability of colon cancer cells *in vitro* and on tumor development in mice model *in vivo*. The study demonstrated that benzoximemethyl amine suppresses HCT15 and DLD-1 cell viability through activation of apoptotic pathway. Moreover, the NF-κB activation was inhibited in DLD-1 cells and colon tumor development was suppressed in mice by benzoximemethyl amine.

MATERIALS AND METHODS

Study duration: The study was started in the month of April, 2016 and completed in February, 2019.

Cell culture: The HCT15 and DLD-1 cell lines were supplied by the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The cell cultures were carried out in RPMI-1640 medium, mixed with fetal bovine serum (10%). The media also contained penicillin (100 U mL^{-1}) and streptomycin (100 μg mL^{-1}). The cell culture was maintained for 24 h under an atmosphere of 5% CO_2 and 65% humidity at 37°C.

Cell viability assay: The HCT15 and DLD-1 cells were distributed at 2.5×10^6 mL^{-1} density in 96 well microtiter plates. The cells after culture for 24 h were incubated in medium containing 2, 4, 6, 8, 10, 12 and 15 μM concentrations of benzoximemethyl amine for 72 h. Then 10 μL of MTT solution was put in to each well and incubation was continued for 3 h more under standard conditions. Dimethyl sulfoxide (120 μL) was added to the wells for dissolving the solid formazan crystals formed. The cell viability was determined by recording absorbance in microplate reader at 572 nm. The measurements were made three times for each of the plate.

Analysis of cell morphological by Hoechst 33342 staining: The DLD-1 cells were put in to the 12 well plates at 3×10^5 cells/well density and cultured for 24 h. The cells were then incubated in medium mixed with 10, 12 and 15 μM concentrations of benzoximemethyl amine for 72 h. Then cells were 3 times washed with PBS and subsequently fixed for 25 min with paraformaldehyde (4%) at room temperature. The cells were washed with PBS and then subjected to Hoechst 33342 staining for 20 min at 37°C. The fluorescence microscope (Olympus Corporation) was used for assessment of morphological changes in DLD-1 cells.

Western blot analysis: The DLD-1 cells after incubation for 72 h in medium containing 10, 12 and 15 μM concentrations of benzoximemethyl amine were harvested and lysed on treatment with lysis buffer [35 mMTris (pH 7.8), sodium chloride 125 mM, NP-40 0.6%, $NaVO_4$ 0.1 mM, aprotinin 2.4 μg mL^{-1}, leupeptin 2.4 μg mL^{-1} and $PhCH_2SO_2Cl$ (110 μg mL^{-1})]. The lysate was centrifuged to collect the supernatant in which concentration of proteins was determined by using Bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Inc.). The proteins were resolved by electrophoresis on SDS-PAGE (8-12%) and then transferred onto the PVDF membranes. The blocking of non-specific sites in the membranes was performed by treatment with 5%

non-fat milk. The protein was incubated for overnight with primary antibodies at 4°C. Then membranes were washed for 10 min with 4×TBS-T buffer and subsequently incubation were carried out with horseradish peroxidase-conjugated anti-rabbit secondary antibodies (Santa Cruz Biotechnology Inc., Santa Cruz, CA) for 3 h at room temperature. The complexes formed by antigen-antibody interaction were detected by the enhanced chemiluminescence (ECL) detection system (GE Healthcare Biosciences, Pittsburgh, PA).

Determination of cell motility: The DLD-1 cells at 2.5×10^6 cells/well density in 6 well plates were allowed to achieve 90-95% confluence. The tip of a 200 µL pipette was used to scratch through the cell monolayer. The loosely adhered and free floating cells were removed by rinsing the cells with phosphate-buffered saline. The cells were incubated with 10, 12 and 15 µM concentrations of benzoximemethyl amine for 72 h. The inverted microscope connected with a digital camera (Olympus) was used for capturing the images at ×250 magnification.

Migration assay: The modified 24 well Boyden chamber (8-10 µm-pore size) was used for determination of the effect of benzoximemethyl amine on migration potential. The DLD-1 cells were dispersed at 3×10^5 cell concentration in the upper compartment of the Transwell chamber in serum-free medium. The cell incubation with 10, 12 and 15 µM concentrations of benzoximemethyl amine or DMSO (control) was performed in lower chamber at 37°C for 72 h. The non-penetrated cells were cleaned by using cotton swabs and the penetrated cells fixed with methanol. The phase contrast microscope was used for examination of the methylene blue and eosin stained cells. The images were taken at ×250 magnification.

Animal study: A total of 50 male ICR mice aged 6-8 weeks and weighing 21-25 g were obtained from the Animal Laboratory of Shandong University (Jinan, China). The mice were put in the pathogen-free Animal Centre at room temperature (23±2°C) with a 12 h light/dark cycle under 50-70% humidity. The mice had free access to drinking water and a basal diet. The mice protocols were performed according to the Guide for the Care and Use of Laboratory Animals, Yantai Hospital of Traditional Chinese Medicine (Yantai, China). The approval for the study was obtained from Ethics Committee for Care and Use of laboratory animals Yantai Hospital. After acclimatization the mice were assigned into 5 groups: Normal control, untreated and three treatment groups (10, 15 and 20 mg kg^{-1} doses of benzoximemethyl amine). The colitis was induced in mice by the administration single 10 mg kg^{-1} dose of azoxymethane (AOM) through intraperitoneal route. Dextran Sulfate Sodium (DSS; 1.5%) was given to the mice 1 week after AOM injection for 7 days daily in drinking water. On the day 15th, mice in three treatment groups received 10, 15 and 20 mg kg^{-1} single doses of benzoximemethyl amine. The mice were sacrificed on the day 31st of AOM injection to determine the tumor multiplicity, incidence and tissue inflammation. The histopathological alteration in the colon tissues were examined by using hematoxylin and eosin (H and E) staining.

Statistical analysis: The values are presented as the mean±SD for 3 experiments performed independently. Evaluation of the statistical differences was performed by using Student's t-test and one-way analysis of variance (ANOVA). A $p<0.05$ was taken to represent statistically significant differences.

RESULTS

Cell viability: Changes in HCT15 and DLD-1 cell viability were analyzed at 72 h of exposure to 2, 4, 6, 8, 10, 12 and 15 µM concentrations of benzoximemethyl amine (Fig. 1a). Increase in the concentration of benzoximemethyl amine lead to a significant decrease in HCT15 and DLD-1 cell viability. The HCT15 and DLD-1 cell morphology showed significant changes at 72 h of exposure to 10, 12 and 15 µM concentrations of benzoximemethyl amine (Fig. 1b). The benzoximemethyl amine treated cells were rounded and segregated in comparison to the control cultures.

Effect of benzoximemethyl amine in DLD-1 cell apoptosis: Hoechst 33342 staining was used for determination of apoptotic features in DLD-1 cells on exposure to 10, 12 and 15 µM concentrations of benzoximemethyl amine (Fig. 2a). The percentage of cells with condensation of chromatin material and apoptotic bodies showed a significant increase with the enhancement of benzoximemethyl amine concentration from 10-15 µM (Fig. 2b).

Effect of benzoximemethyl amine on pro-apoptotic protein level of DLD-1 cells: Apoptosis induction by benzoximemethyl amine in DLD-1 cells was also investigated

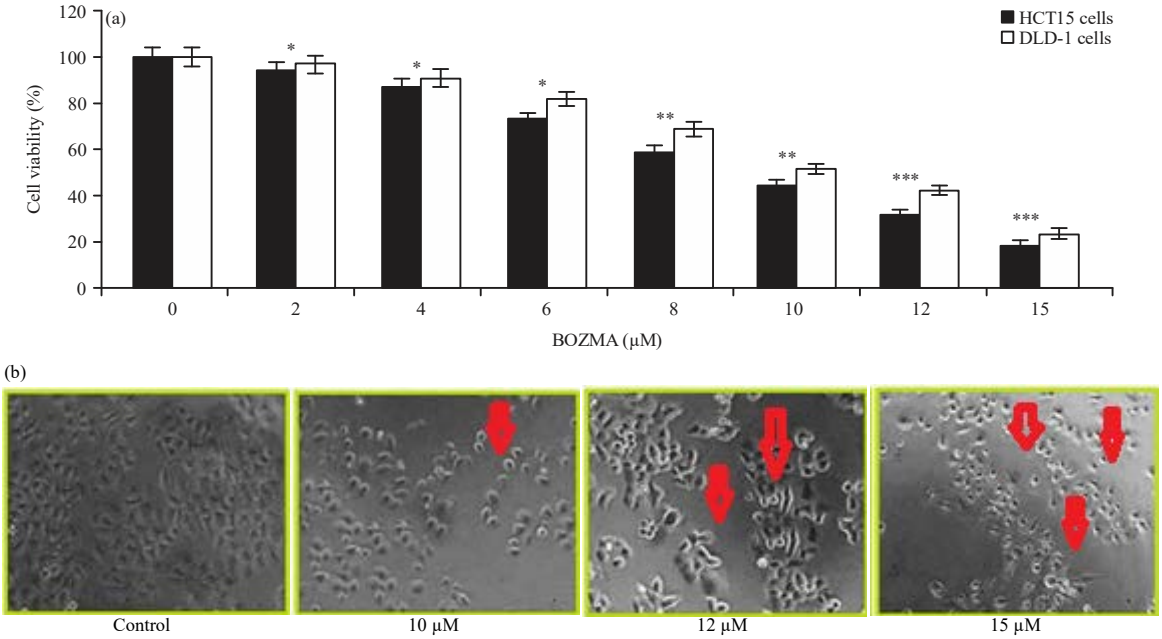

Fig. 1(a-b): Suppression of HCT15 and DLD-1 cell viability by benzoximemethyl amine, (a) Cells exposed to various concentrations of benzoximemethyl amine for 72 h were analyzed by MTT assay. The presented values are the Mean±SD, *p<0.05, **p<0.02 and ***p<0.01 vs. control cells and (b) Phase contrast microscopy was used for examination of changes in morphology of the cells by benzoximemethyl amine. Magnification x350

Red arrows show apoptotic changes

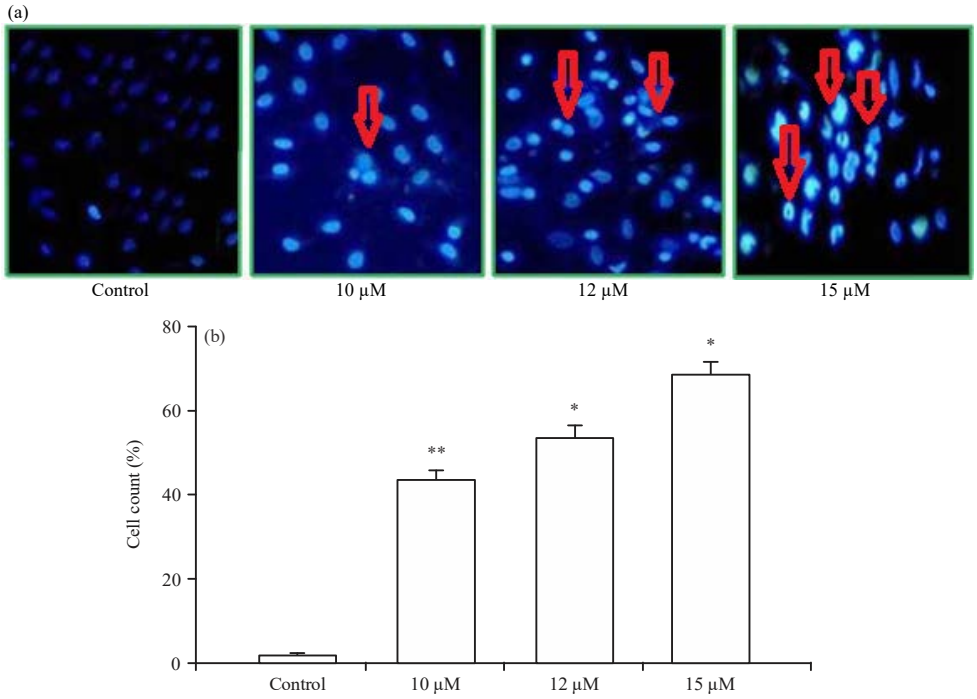

Fig. 2(a-b): (a) Apoptosis induction by benzoximemethyl amine in DLD-1 cells. At 72 h of exposure to benzoximemethyl amine and (b) Fluorescent microscope was used to determine apoptotic features in Hoechst 33342 stained cells and results were quantified. Magnification x250, *p<0.05 and **p<0.02

Red arrows show apoptotic cells

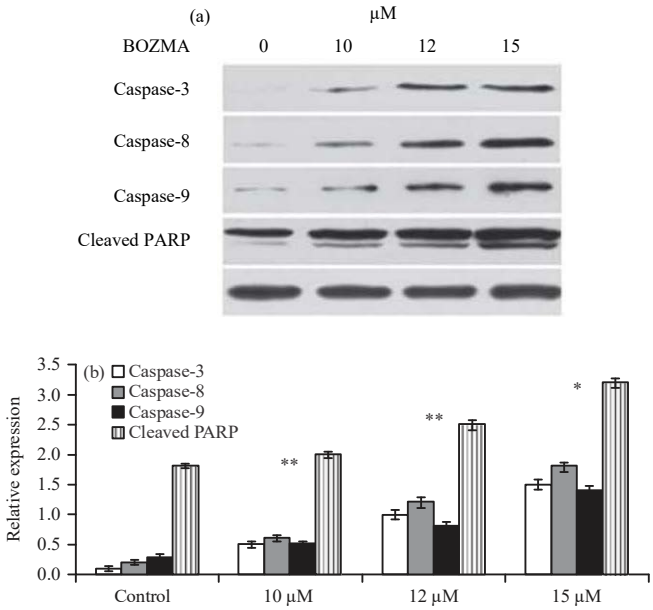

Fig. 3: Effect of benzoximemethyl amine on pro-apoptotic proteins, (a) DLD-1 cells treated with benzoximemethyl amine for 72 h were assessed by western blotting and (b) Expression of proteins was compared to β-actin level (internal loading control), *p<0.05 and **p<0.02

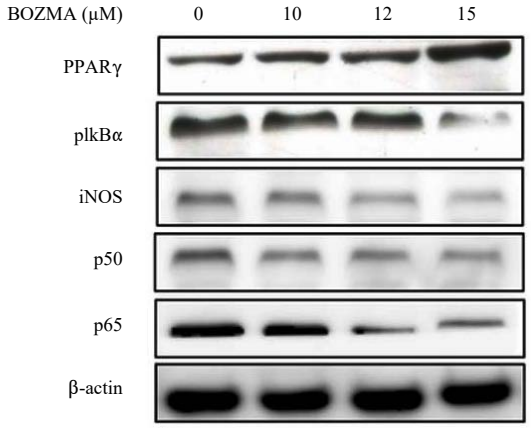

Fig. 4: Benzoximemethyl amine inhibits NF-κB activation in DLD-1 cells. The cells exposed to benzoximemethyl amine for 72 h were analyzed for activation of NF-κB by western blotting assay

by the assessment of pro-apoptotic protein expression (Fig. 3). The DLD-1 cells on treatment with 10, 12 and 15 μM concentrations of benzoximemethyl amine showed a marked enhancement in the level of caspase-3, -8 and -9. The level of cleaved PARP was also increased markedly by benzoximemethyl amine treatment in DLD-1 cells.

Effect of benzoximemethyl amine on NF-κB activity: The NF-κB activation by benzoximemethyl amine in DLD-1 cells was examined on exposure to 10, 12 and 15 μM concentrations (Fig. 4). Western blotting showed that benzoximemethyl amine exposure of DLD-1 cells for 72 h caused increase in PPARγ activation. The expression of pl κBα, p50, p65 and iNOS was suppressed markedly in DLD-1 cells on exposure to benzoximemethyl amine.

Suppression of DLD-1 cell metastasis by benzoximemethyl amine: Wound-healing assay of DLD-1 cells at 72 h of exposure to benzoximemethyl amine revealed a marked decrease in migration potential (Fig. 5a). Increase in the concentration of benzoximemethyl amine from 10-15 μM caused a marked suppression of migration potential in DLD-1 cells. Benzoximemethyl amine treatment of DLD-1 cells also lead to a marked decrease in invasion potential in comparison to the control cells (Fig. 5b). The invasion of cells was decreased by benzoximemethyl amine at 72 h in concentration dependent manner.

Suppression of matrix metalloproteinases by benzoximemethyl amine in DLD-1 cells: The level of MMP-2 and 9 in DLD-1 cells on treatment with benzoximemethyl amine was determined by using western blotting (Fig. 6). Benzoximemethyl amine treatment of DLD-1 cells lead to a significant suppression in the levels of MMP-2 and 9 in concentration based manner. The expression of MMP-2 and 9 in DLD-1 cells was reduced to the minimum level on treatment with 15 μM concentration.

Benzoximemethyl amine inhibits tumor growth in mice: The effect of benzoximemethyl amine on colon cancer tumor development was investigated in the mice model. Treatment of the mice with 10, 15 and 20 mg kg^{-1} doses of benzoximemethyl amine significantly decrease the tumor incidence and multiplicity in comparison to the untreated group (Fig. 7a, b). The results from H and E staining showed that benzoximemethyl amine treatment of the mice suffering from colon cancer significantly inhibited inflammation and inflammatory cell accumulation in comparison to the control group (Fig. 7a). The tumor multiplicity in the untreated group was 3.17±0.87. Treatment of the mice suffering from colon cancer with 10, 15 and 20 mg kg^{-1} doses of benzoximemethyl amine reduced tumor multiplicity to 1.98±0.56, 1.07±0.32 and 0.34±0.12, respectively (Fig. 7b).

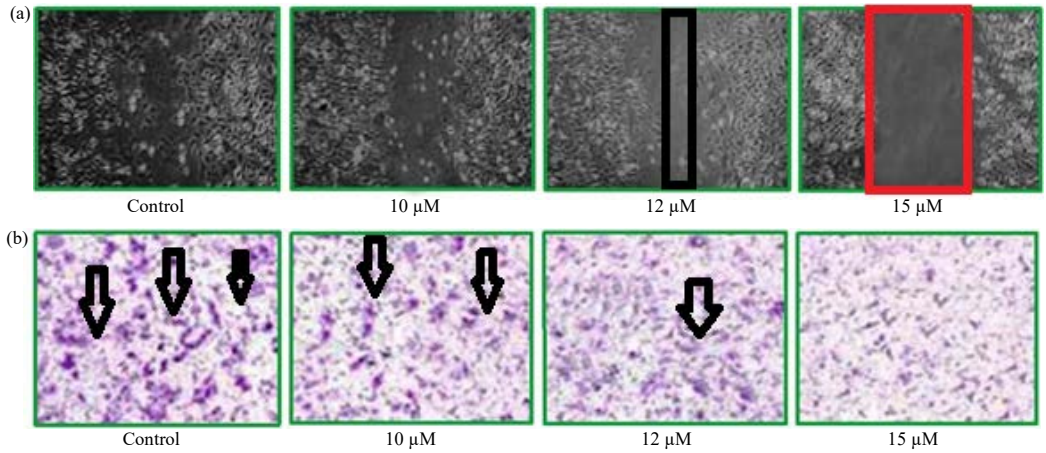

Fig. 5(a-b): Benzoximemethyl amine inhibits metastasis of DLD-1 cells, (a) The benzoximemethyl amine treated cells were subjected to wound healing assay for determination of cell migration and (b) The DLD-1 cell invasion at 72 h of benzoximemethyl amine treatment was determined by Trans-well chamber assay. Magnification x250. Black arrows show cell invasion

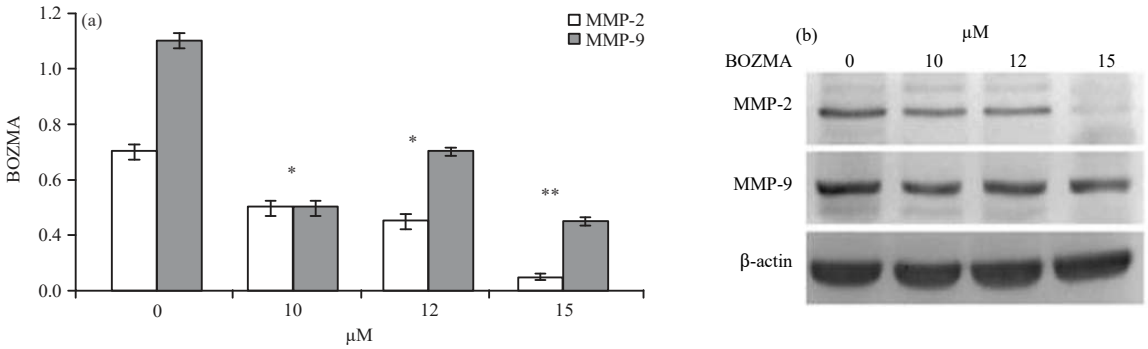

Fig. 6(a-b): (a) Effect of benzoximemethyl amine on MMP-2 and 9 in DLD-1 cells and (b) At 72 h of benzoximemethyl amine exposure the MMP-2 and 9 levels in DLD-1 cells were determined by western blotting assay, *p<0.05 and **p<0.02

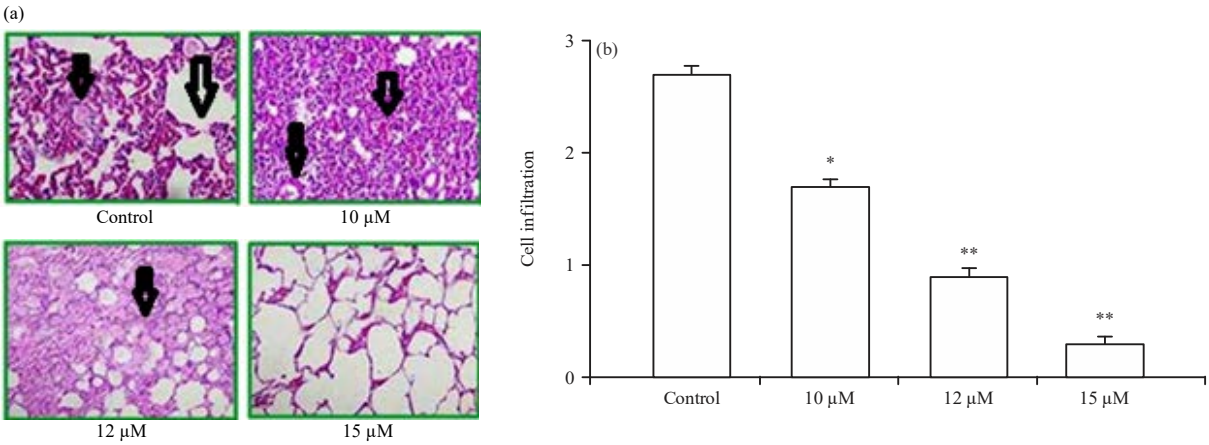

Fig. 7(a-b): Inhibition of colon tumor growth in mice by benzoximemethyl amine. The mice model of colon cancer was treated with 10, 15 and 20 mg kg^{-1} doses of benzoximemethyl amine. The mice were sacrificed on the day 31st of AOM injection to determine (a) Histopathological changes and (b) Tumor incidence, *p<0.05 and **p<0.02
Black arrows indicated cell infiltration

DISCUSSION

The study demonstrated that benzoximemethyl amine suppresses HCT15 and DLD-1 cell viability through activation of apoptotic pathway. Moreover, the NF-κB activation was inhibited in DLD-1 cells and colon tumor development was suppressed in mice by benzoximemethyl amine. Apoptosis is one of the vital cellular processes that controls cell count and proliferation in order to regulate homeostasis in the body of multicellular organisms[9]. Induction of apoptosis in carcinoma cells by chemotherapeutic agents is an attractive strategy used for the treatment of various types of cancers[10,11]. It is hypothesized that ability of the cancer cells to undergo apoptosis contributed significantly to the resistance towards treatments strategies[11,12]. Therefore, compounds which activate apoptosis act as promising candidates for the treatment of cancer[12]. In the present study benzoximemethyl amine treatment significantly inhibited the viability of two tested colon cancer cell lines (HCT15 and DLD-1). These findings suggested that benzoximemethyl amine may act as chemotherapeutic agent for the colon cancer. There is enough evidence that apoptosis induction by most of the anticancer drugs is associated with the disruption of mitochondrial membrane potential and activation of caspase pathway[13-15]. The mitochondrial membrane potential disruption is taken to be the indication of mitochondria damage and initiation of apoptosis activation. This is generally followed by the mitochondrial efflux of cytochrome c and activation[16-19] of the caspase-3/8/9. In the present study benzoximemethyl amine treatment of DLD-1 cells lead to a marked increase in cytochrome c and level of caspase-3/8/9. These results suggested that benzoximemethyl amine treatment caused apoptosis of DLD-1 cells through caspase dependent pathway.

PPARγ agonists are linked with the regulation of cancer cell proliferation and apoptosis and therefore act as promising candidates for tumor treatment[20]. The PPARs are known to inhibit inflammation by targeting the related transcriptional pathways such as; NF-κB signalling. The production of several inflammatory mediators like iNOS, COX-2 and TNF-α is facilitated by the transcriptional factor[21], NF-κB. It was reported that NF-κB is activated in the colon cancer cells and its inhibition leads to suppression of colon tumor growth[22]. In the present study treatment of DLD-1 cells with benzoximemethyl amine markedly reduced the production of inflammatory molecules iNOS, COX-2 and TNF-α. Thus benzoximemethyl amine caused inhibition of NF-κB activation in DLD-1 cells in concentration based manner. Suppression of MMP-2 and MMP-9 by anticancer molecules leads to the inhibition of carcinoma cell migration and invasion[23]. In the present study, level of MMP-2 and 9 molecules in DLD-1 cells was suppressed on treatment with benzoximemethyl amine. The potential of the colon cancer cells to undergo metastasis was markedly inhibited by benzoximemethyl amine treatment. Therefore, benzoximemethyl amine exhibits inhibitory effect on carcinoma cell metastasis by inhibition of MMP-2 and 9 expression.

CONCLUSION AND RECOMMENDATION

Current study demonstrated that benzoximemethyl amine inhibits colon cancer by leading to cell apoptosis and suppresses tumor metastasis by increasing the levels of caspase-3/8/9, PPARγ activation and cleaved PARP and decreasing the expression of pI κBα, p50, p65 and iNOS. So, the Benzoximemethyl amine may be developed as a potent drug candidate for the treatment of colon cancer.

SIGNIFICANCE STATEMENT

This is the first study to demonstrate that benzoximemethyl amine exhibits inhibitory effect on colon cancer cell viability *in vitro* and tumor development *in vivo*. The benzoximemethyl amine therefore can be beneficial for the treatment of colon cancer. This study will assist researchers and clinicians to develop benzoximemethyl amine as the novel and efficient treatment strategy either alone or in combination therapy for the colon carcinoma treatment.

REFERENCES

1. Fang, X.L., 2009. Study the curative effect of advanced stage colon carcinoma treated by L-OHP combined with 5-FU/CF. Chin. J. Clin. Ration. Drug Use, 2: 8-9, (In Chinese).
2. Tenesa, A. and M.G. Dunlop, 2009. New insights into the aetiology of colorectal cancer from genome-wide association studies. Nat. Rev. Genet., 10: 353-358.
3. Sileri, P., S. D'Ugo, D. Benavoli, V.M. Stolfi, G. Palmieri, A. Mele and A.L. Gaspari, 2009. Metachronous splenic metastasis from colonic carcinoma five years after surgery: A case report and literature review. South. Med. J., 102: 733-735.
4. Dubuquoy, L., C. Rousseaux, X. Thuru, L. Peyrin-Biroulet and O. Romano *et al*., 2006. PPARγ as a new therapeutic target in inflammatory bowel diseases. Gut, 55: 1341-1349.

5. Bassaganya-Riera, J., K. Reynolds, S. Martino-Catt, Y. Cui and L. Hennighausen *et al*., 2004. Activation of PPAR γ and δ by conjugated linoleic acid mediates protection from experimental inflammatory bowel disease. Gastroenterology, 127: 777-791.
6. Liang, H.L. and Q. Ouyang, 2006. [A clinical trial of rosiglitazone and 5-aminosalicylate combination for ulcerative colitis]. Zhonghua Nei Ke Za Zhi, 45: 548-551, (In Chinese).
7. Bassaganya-Riera, J., M. DiGuardo, M. Climent, C. Vives and A. Carbo *et al*., 2011. Activation of PPARγ and δ by dietary punicic acid ameliorates intestinal inflammation in mice. Br. J. Nutr., 106: 878-886.
8. Ricote, M. and C.K. Glass, 2007. PPARs and molecular mechanisms of transrepression. Biochim. Biophys. Acta (BBA): Mol. Cell Biol. Lipids, 1771: 926-935.
9. Trump, B.E., I.K. Berezesky, S.H. Chang and P.C. Phelps, 1997. The pathways of cell death: Oncosis, apoptosis and necrosis. Toxicol. Pathol., 25: 82-88.
10. Thompson, C.B., 1995. Apoptosis in the pathogenesis and treatment of disease. Science, 267: 1456-1462.
11. Smets, L.A., 1994. Programmed cell death (apoptosis) and response to anti-cancer drugs. Anti-Cancer Drugs, 5: 3-9.
12. Gottesman, M.M., 2002. Mechanisms of cancer drug resistance. Annu. Rev. Med., 53: 615-627.
13. Ling, Y.H., L. Liebes, Y. Zou and R. Perez-Soler, 2003. Reactive oxygen species generation and mitochondrial dysfunction in the apoptotic response to Bortezomib, a novel proteasome inhibitor, in human H460 non-small cell lung cancer cells. J. Biol. Chem., 278: 33714-33723.
14. Ryan, L., Y.C. O'Callaghan and N.M. O'Brien, 2005. The role of the mitochondria in apoptosis induced by 7β-hydroxycholesterol and cholesterol-5β,6β-epoxide. Br. J. Nutr., 94: 519-525.
15. Tang, L. and Y. Zhang, 2005. Mitochondria are the primary target in isothiocyanate-induced apoptosis in human bladder cancer cells. Mol. Cancer Ther., 4: 1250-1259.
16. Doi, S., H. Soda, M. Oka, J. Tsurutani and T. Kitazaki *et al*., 2003. The histone deacetylase inhibitor FR901228 induces caspase-dependent apoptosis via the mitochondrial pathway in small cell lung cancer cells. Mol. Cancer Ther., 3: 1397-1402.
17. Ogbourne, S.M., A. Suhrbier, B. Jones, S.J. Cozzi and G.M. Boyle *et al*., 2004. Antitumor activity of 3-ingenyl angelate: Plasma membrane and mitochondrial disruption and necrotic cell death. Cancer Res., 64: 2833-2839.
18. Rotem, R., A. Heyfets, O. Fingrut, D. Blickstein, M. Shaklai and E. Flescher, 2005. Jasmonates: Novel anticancer agents acting directly and selectively on human cancer cell mitochondria. Cancer Res., 65: 1984-1993.
19. Wu, C.C., M.L. Chan, W.Y. Chen, C.Y. Tsai, F.R. Chang and Y.C. Wu, 2005. Pristimerin induces caspase-dependent apoptosis in MDA-MB-231 cells via direct effects on mitochondria. Mol. Cancer Ther., 4: 1277-1285.
20. Yamaguchi, K., M. Cekanova, M.F. McEntee, J.H. Yoon and S.M. Fischer *et al*., 2008. Peroxisome proliferator-activated receptor ligand MCC-555 suppresses intestinal polyps in $Apc^{Min/+}$ mice via extracellular signal-regulated kinase and peroxisome proliferator-activated receptor-dependent pathways. Mol. Cancer Therapeut., 7: 2779-2787.
21. Surh, Y.J., 2008. NF-κB and Nrf2 as potential chemopreventive targets of some anti-inflammatory and antioxidative phytonutrients with anti-inflammatory and antioxidative activities. Asia Pac. J. Clin. Nutr., 17: 269-272.
22. Rajakangas, J., M. Misikangas, E. Paivarinta and M. Mutanen, 2008. Chemoprevention by white currant is mediated by the reduction of nuclear β-catenin and NF-κB levels in Min mice adenomas. Eur. J. Nutr., 47: 115-122.
23. Chiu, Y.W., T.H. Lin, W.S. Huang, C.Y. Teng and Y.S. Liou *et al*., 2011. Baicalein inhibits the migration and invasive properties of human hepatoma cells. Toxicol. Applied Pharmacol., 255: 316-326.