Original Article

Banxia Xiexin decoction, a traditional Chinese medicine, alleviates colon cancer in nude mice

Shuai Yan[1#], Yinzi Yue[1#], Jinbang Wang[2], Wenting Li[3], Mingming Sun[1], Li Zeng[3], Xiaopeng Wang[1]

[1]Department of Anorectal Surgery, Suzhou Hospital of Traditional Chinese Medicine, Suzhou 215009, China; [2]Department of General surgery, Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou 450008, China; [3]First Clinical Medical School, Nanjing University of Chinese Medicine, Nanjing 210023, China

Contributions: (I) Conception and design: S Yan, L Zeng; (II) Administrative support: L Zeng, X Wang; (III) Provision of study materials or patients: W Li, M Sun; (IV) Collection and assembly of data: S Yan, J Wang, W Li; (V) Data analysis and interpretation: S Yan, Y Yue, M Sun; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Li Zeng. First Clinical Medical School, Nanjing University of Chinese Medicine, Nanjing 210023, China. Email: zengbingli@163.com; Xiaopeng Wang. Department of General surgery, Suzhou Hospital of Traditional Chinese Medicine, Suzhou 215009, China. Email: wxpeng2004@163.com.

Backgrounds: Banxia Xiexin decoction (BXD) is widely used in the treatment of acute and chronic gastritis, peptic ulcer, dyspepsia, gastrointestinal dysfunction, chronic hepatitis, oral ulcer and other diseases, but there are few reports on its treatment of colon cancer. The current study was designed to investigate the effect of BXD on colon cancer and its possible molecular mechanisms.

Methods: Fifty SPF BALB/c nude mice were selected to establish an animal model of colon cancer bearing nude mice. Establishment of nude mice intestinal cancer model by subcutaneous injection of intestinal cancer cells. Serum superoxide dismutase (SOD) and malondialdehyde (MDA) were detected by commercial kits. Pro-inflammatory cytokines in serum were detected. Western blotting was used to demonstrate the expression levels of related apoptosis proteins, inflammation and oxidative stress proteins.

Results: Our results showed that BXD could decrease SOD and increased MDA in nude mice bearing tumors. BXD increased pro-inflammatory cytokines in serum in nude mice bearing tumors. Western blotting revealed that the protein expressions of Bax, Caspase-3, Caspase-9 were increased by different concentrations of BXD, while Bcl-2 was decreased. BXD also decreased Nrf-2 and HO-1, and increased the levels of MAPK/NF-κB pathway in tumor tissue.

Conclusions: BXD has an obvious tumor inhibiting effect on SW 480 colon cancer transplanted tumor in nude mice via apoptotic pathway and MAPK/NF-κB pathway.

Keywords: Colon cancer; Banxia Xiexin decoction (BXD); nude mice

Submitted Jan 04, 2019. Accepted for publication Jun 24, 2019.
doi: 10.21037/atm.2019.07.26
View this article at: http://dx.doi.org/10.21037/atm.2019.07.26

Introduction

Colon cancer is one of the common malignant tumors of digestive tract. The incidence rate and mortality rate of colorectal cancer are the third and fourth respectively in the world. In recent years, the incidence rate and mortality rate of colorectal cancer have not increased significantly in the world. The incidence rate and mortality rate of colorectal cancer in developed countries, represented by the United States, have declined to varying degrees. However, the decline is mainly due to screening techniques and reduction of exposure to high-risk factors. Improvement of treatment methods also plays an important role in accelerating the reduction of the incidence rate of colorectal cancer (1). Colon cancer

has obvious regional distribution differences in the world. Generally speaking, the morbidity and mortality in developed areas are higher than those in developed areas. The incidence rate and mortality rate of colon cancer in China are still lower than those in developed countries and belong to low-incidence areas. However, the overall level of incidence rate and mortality rate are on the rise year by year. With the development of economy, changes in the national living environment, diet, lifestyle and social pressure are closely related to the occurrence of the disease (2). At present, the treatment of colon cancer in clinic mainly adopts surgery, radiotherapy and traditional Chinese medicine adjuvant chemotherapy. Because colon cancer is not easily detected in the early stage or diagnosed as multiple metastases in the late stage, there are not many cases treated by surgery, so chemotherapy is a common method. In recent years, the research on chemotherapy drugs for colon cancer has become a hot topic (3-5). Although the death rate has decreased in recent years due to the improvement of screening, diagnosis, prognosis and treatment methods, the currently available treatment methods include surgery, radiotherapy and chemotherapy, especially for patients with advanced colon cancer (6-8). Chemotherapy drugs have been widely used in patients with advanced colon cancer. However, many patients acquired resistance to chemotherapy drugs, resulting in treatment failure (9). Therefore, identifying new drugs with small side effects can improve the quality of life of colon cancer patients.

Traditional Chinese medicine is a suitable substitute for platinum drugs because they are more effective and have fewer side effects than synthetic drugs. Many traditional Chinese medicines are considered as promising drug sources for cancer prevention and treatment because they can attack many molecular targets. Banxia Xiexin decoction (BXD), the name of traditional Chinese medicine prescription. It is a reconciliation agent and has the effects of harmonizing liver and spleen, calming cold and heat, eliminating mass and resolving hard mass. Indication of intermingled cold and heat's new syndrome. Under the heart of the new, but full and painless, or vomiting, bowel sounds, greasy and yellowish tongue coating. BXD and has exhibited different pharmacological effects, including antioxidant, anti-inflammatory, anti-diabetes and anti-renal failure activities. Nevertheless, as far as we know, its potential role in apoptosis tumor suppression and the potential anti-cancer mechanism of colon cancer still need to be clarified.

Methods

Reagents

The commercial malondialdehyde (MDA) and superoxide dismutase (SOD) kits were purchased by Jiancheng Bioengineering Institute (Nanjing, China). Enzyme-linked immunosorbent assay (ELISA) tests for the detection of IL-6, IL-1β and TNF-α were produced by Nanjing KeyGEN Biotech. CO., Ltd. (Nanjing, China). All the antibodies were provided by Cell Signaling Technology (Danvers, USA).

BXD decoction

The herbal of BXD were prepared for crude plant medicines from Nantong Tong Ren Tang pharmacy. Each herb was authenticated by the herbal medicinal botanist, Professor Qinan Wu, at the Department of pharmacy of Nanjing University of Chinese Medicine. According to the standard process10, herbal medicines of BXD with the above ratio are used to produce BXD freeze-dried powder. According to the standard of 1 g/mL (w/v), distilled water is stored at 4 ℃ before being dissolved for use.

High-performance liquid chromatography (HPLC) analysis of BXD decoction

HPLC was performed using Agilent 1200 HPLC with G1321A FD, and Eclipse AAA column (4.6×150 mm, 5 μm), and a column temperature of 40 ℃. Mobile phase A (formic acid: water =1:1,000) and mobile phase B (acetonitrile). The gradient elution procedure was 0–13 min, B (0–63%), 0–8 min, B (63%), 9–10 min, B (100%), 11 min, B (0–100%), and 12–13 min, B (0%). The flow rate was 0.4 mL min^{-1}.

Animals

BALB/c nude mice (8 weeks, 20–22 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) were housed in an air-conditioned room at 23±2 ℃ with a 12 h light/dark cycle. Water and food were provided *ad libitum*.

All animal care and experimental procedures were approved by the Ethics Committee for Animals Experiments of the Nanjing University of Chinese Medicine, Nanjing, China (approved document NUCTM-2004); all experiments were performed in accordance with Guidelines for Laboratory Animal Welfare, Ethics Committee for

Animals Experiments of the Nanjing University of Chinese Medicine, Nanjing, China (decision No. NUCTM-KO-2012-009) who was adopted in complete accordance with the current National Guidelines for Animal Welfare of China (No. GB/T 35892-2018).

Cell line and cell culture

Colon cancer SW480 cells (SW480) were purchased from the American Type Culture Collection. The cell lines were cultured in MEM medium supplemented with 10% fetal bovine serum. All cultures were maintained in an incubator at 37 ℃ with 5% CO_2 in a humidified atmosphere.

Animal modeling and group administration

SW480 suspension (3×10^6 cells per mouse, 0.2 mL) was injected subcutaneously into the left armpit of nude mice and 40 mice were inoculated. Nude mice inoculated with SW480 all showed subcutaneous nodules with a diameter of about 5 mm after 7 days, and the transplanted tumor model was established. On the 8th day after inoculation, 40 nude mice that had established subcutaneous transplanted tumor models were randomly divided into 4 groups (n=10): model (M) group, BXD groups (BXD, 3 g/kg, 6 g/kg), group, 5-Fu group (20 mg/kg), intraperitoneal injection of the corresponding drug, 0.2 mL/mouse. the M group was given the same amount of normal saline, once every other day, 10 times in a row.

Observation index

The body weight, the maximum diameter (a) and the minimum diameter (b) of the tumor were measured once every other day, and the tumor volume was calculated according to the formula: $v = ab^2/2$ (mm^3).

Evaluation of SOD and MDA in serum

The levels of SOD and MDA in serum were also determined using the commercial kits on the basis of the manufacturer's instruction (Jiancheng Bioengineering Institute, Nanjing, China).

Determination of inflammatory cytokines in serum

The levels of IL-6, IL-1β and TNF-α in serum were measured by ELISA kits according to the manufacturer's instructions.

Histological examination of tumor tissue

The tumor tissue was collected and fixed in 10% (v/v) neutral buffered formalin solution. Embedded with paraffin wax and cut into 4 μm thick. The tissue was stained with H&E and observed by optical microscope (Nikon, Tokyo, Japan).

TUNEL method was used to detect apoptosis

Dewaxing tumor section slices to water, incubating proteinase K at room temperature for 30 min, washing with PBS, dripping and add TUNEL reaction mixed solution, incubate for 2 h at 37 ℃, rinse thoroughly, and add conversion agent. POD was incubated at 37 ℃ for 30 min. After washing, DAB was used for color development. The intestinal apoptosis in each group was detected under microscope.

Western blot

The tumor tissue was homogenized and lysed in a RIPA buffer (Beyotime, Nanjing, China). Then, the tissues were centrifugation at 12,000 rpm for 20 min and collected the supernatant. The concentration of the protein was determined by a bicinchoninic acid (BCA) protein assay (Beyotime, Nanjing, China). The samples were separated by 10% sodium dodecyl sulphate polyacrylamide gels and then transferred onto nitrocellulose membranes. The membranes were blocked with 5% skim milk in for 2 h and incubated with specific antibodies at 4 ℃ for a whole night. After washing the membranes for 4 times on the second day, the blots were incubated with horseradish peroxidase-conjugated second antibody for 2 h. The content of the protein was showed by a gel imaging system (Tanon Science & Technology Co., Ltd., China).

Statistical analysis

Data are expressed as means ± SDs of at least three separate experiments. Statistical comparisons between experimental groups were performed by ANOVA with Tukey multiple comparison test. A value of $P<0.05$ was considered statistically significant.

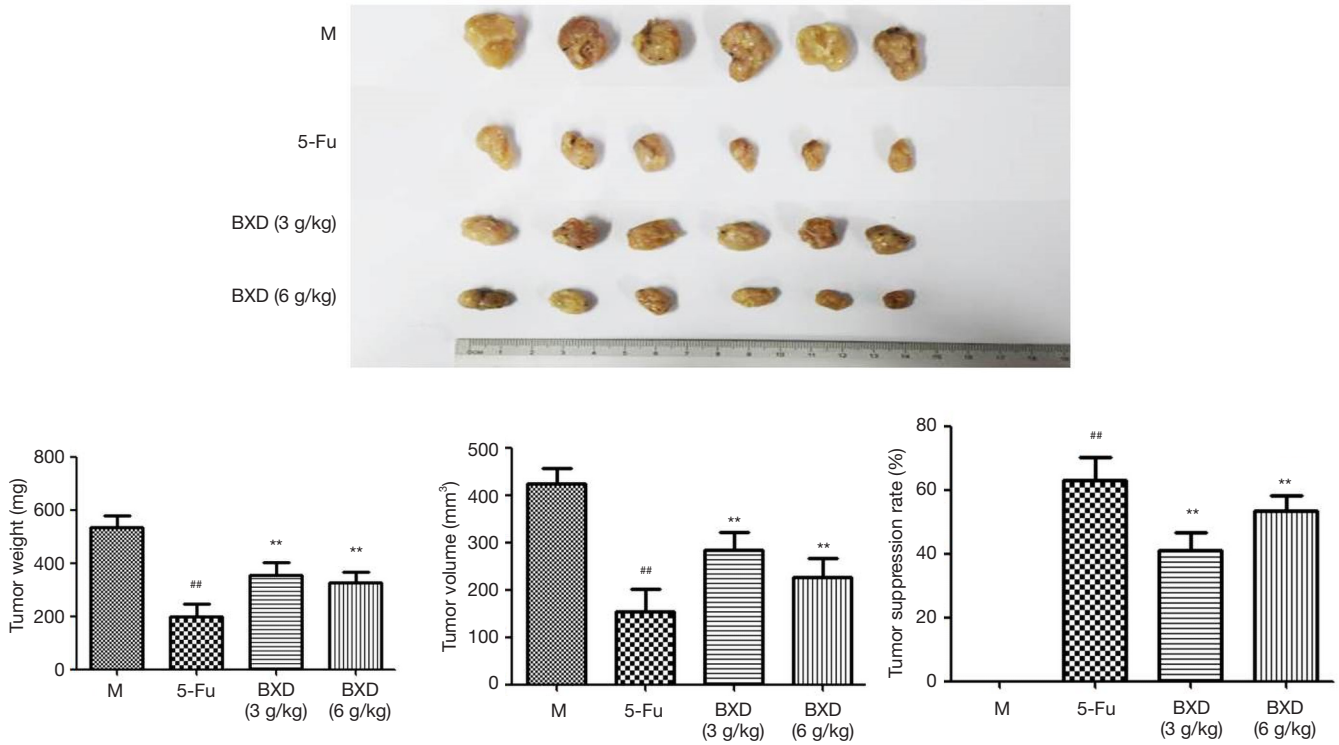

Figure 1 Effect of BXD on tumor weight and tumor volume. The data are expressed as mean values ± SDs. ##, P<0.01; **, P<0.01 (compared with M group). M, model; BXD, Banxia Xiexin decoction.

Results

Effect of BXD on tumor weight and tumor volume

The tumor weight and tumor volume in BXD were significantly lower than those in the M group. with the increase of dosage, the tumor weight in each dosage group of BXD decreased significantly (*Figure 1*).

HPLC analysis of BXD

As data shown in the *Figure 2*, four compounds baicalin and berberine have were identified, and the contents of baicalin and berberine were 0.145 and 0.481 μg/mg.

Effect of BXD on SOD and MDA in serum

As illustrated in *Figure 3*, compare with M group, BXD also decreased SOD and increased MDA in nude mice bearing tumor.

Effect of BXD on pro-inflammatory cytokines in serum

As illustrated in *Figure 4*, compare with M group, BXD increased pro-inflammatory cytokines in serum in nude mice bearing tumor. This result shows that BXD can improve inflammatory reaction in nude mice bearing tumor.

Effect of BXD on apoptotic protein in tumor tissue

As illustrated in *Figure 5*, compare with M group, western blotting revealed that the protein expressions of Bax, Caspase-3, Caspase-9 were increased by different concentrations of BXD, while Bcl-2 were decreased.

Effect of BXD on inflammation protein in tumor tissue

As illustrated in *Figure 6*, compare with M group, western blotting revealed that the protein expressions of p-JNK, p-ERK, p-p38 and p-P65 were increased by different concentrations of BXD.

Effect of BXD on oxidative stress protein in tumor tissue

As illustrated in *Figure 7*, compare with M group, western blotting revealed that the protein expressions of Nrf-2 and

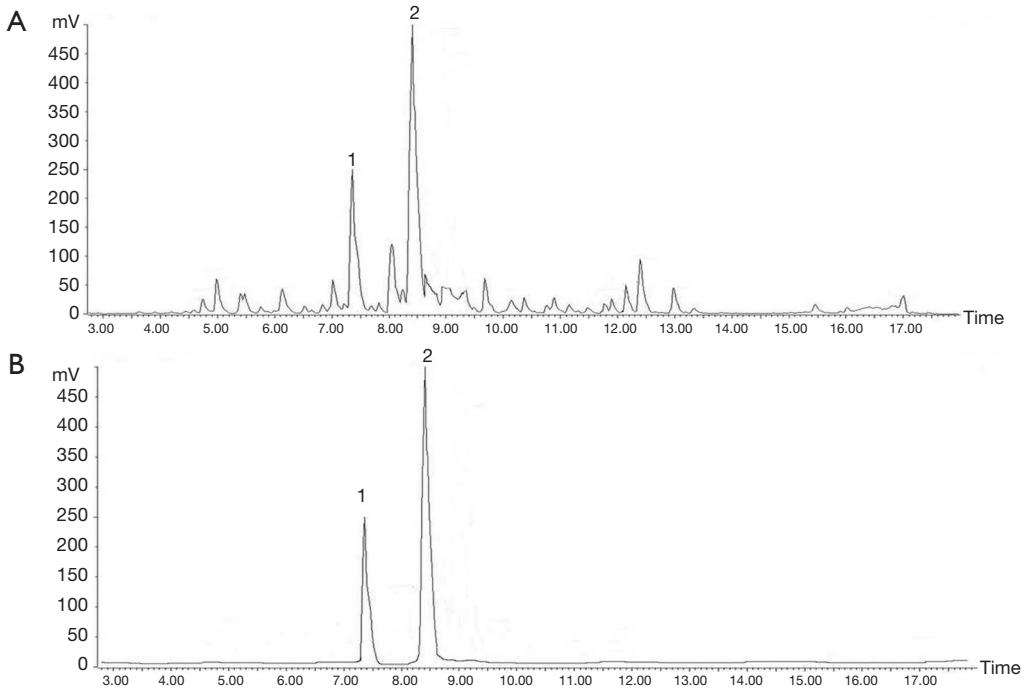

Figure 2 HPLC analysis of BXD. (A) HPLC of BXD sample; (B) HPLC of standards. 1, baicalin; 2, berberine. HPLC, high-performance liquid chromatography; BXD, Banxia Xiexin decoction.

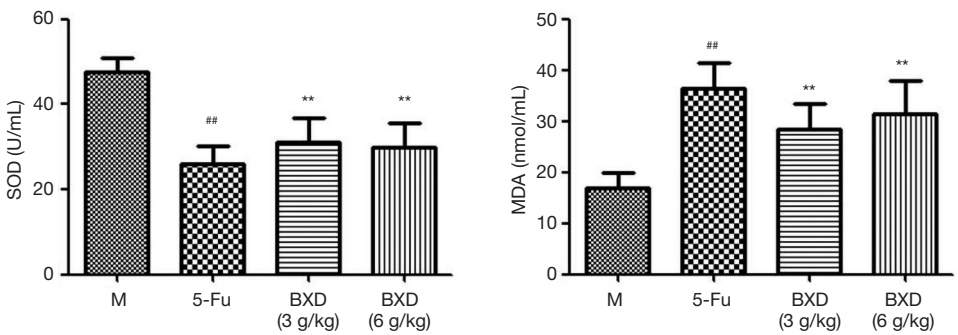

Figure 3 Effect of BXD on SOD and MDA in serum. The data are expressed as mean values ± SDs. ##, P<0.01; **, P<0.01 (compared with M group). SOD, serum superoxide dismutase; M, model; BXD, Banxia Xiexin decoction; MDA, malondialdehyde.

HO-1 were decreased by different concentrations of BXD.

Effect of BXD on pathological changes in tumor tissue

As illustrated in *Figure 8*, the pathological slices showed that the increase of the dosage of BXD could result in inflammatory cell infiltration, nucleus contraction, lysis, decreased blood vessel count, fibrous connective tissue hyperplasia, and the morphological changes of tumor bed necrosis. In the 5-Fu group, there were a large number of vacuoles, nucleus contraction and lysis, more necrotic tissue and inflammatory cell infiltration in the tumor tissue, bleeding foci and obvious fibrosis formation. The pathological nucleus division was seen in the M group, and the cells were separated into different sizes of cancer nests, and the outer layers of the tissues showed more neovascularization.

TUNEL staining results

The nucleus of TUNEL positive cells is brown, scattered

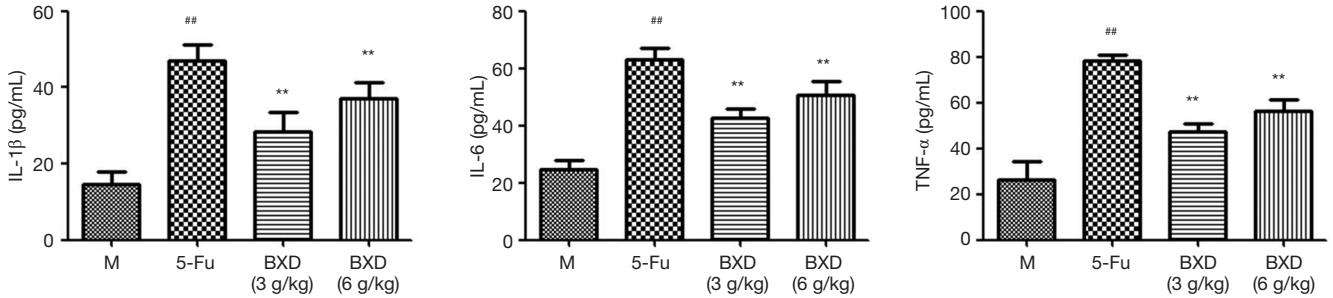

Figure 4 Effect of BXD on pro-inflammatory cytokines in serum. The data are expressed as mean values ± SDs. ##, P<0.01; **, P<0.01 (compared with M group). M, model; BXD, Banxia Xiexin decoction.

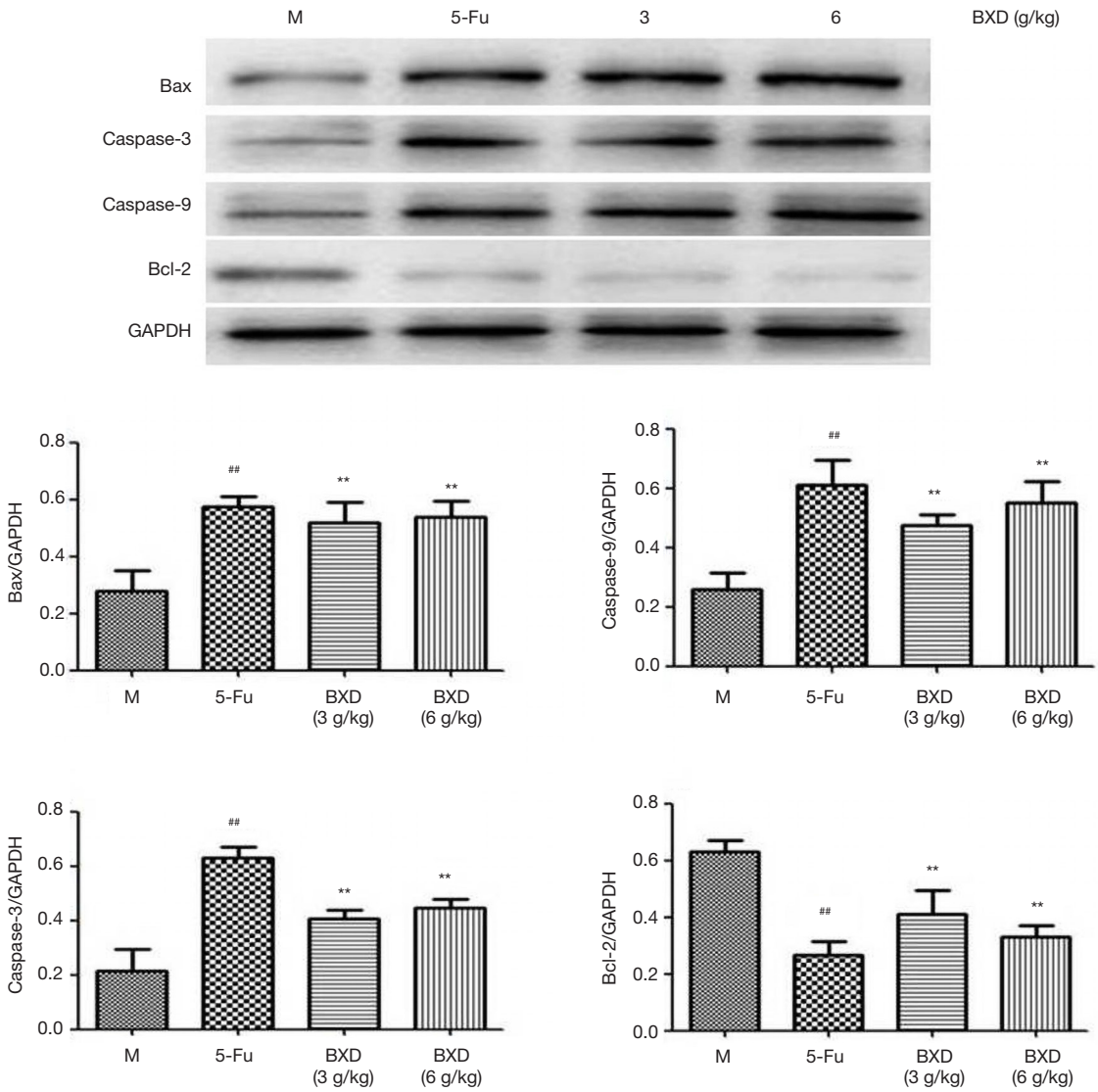

Figure 5 Effect of BXD on apoptotic protein in tumor tissue. The data are expressed as mean values ± SDs. ##, P<0.01; **, P<0.01 (compared with M group). M, model; BXD, Banxia Xiexin decoction.

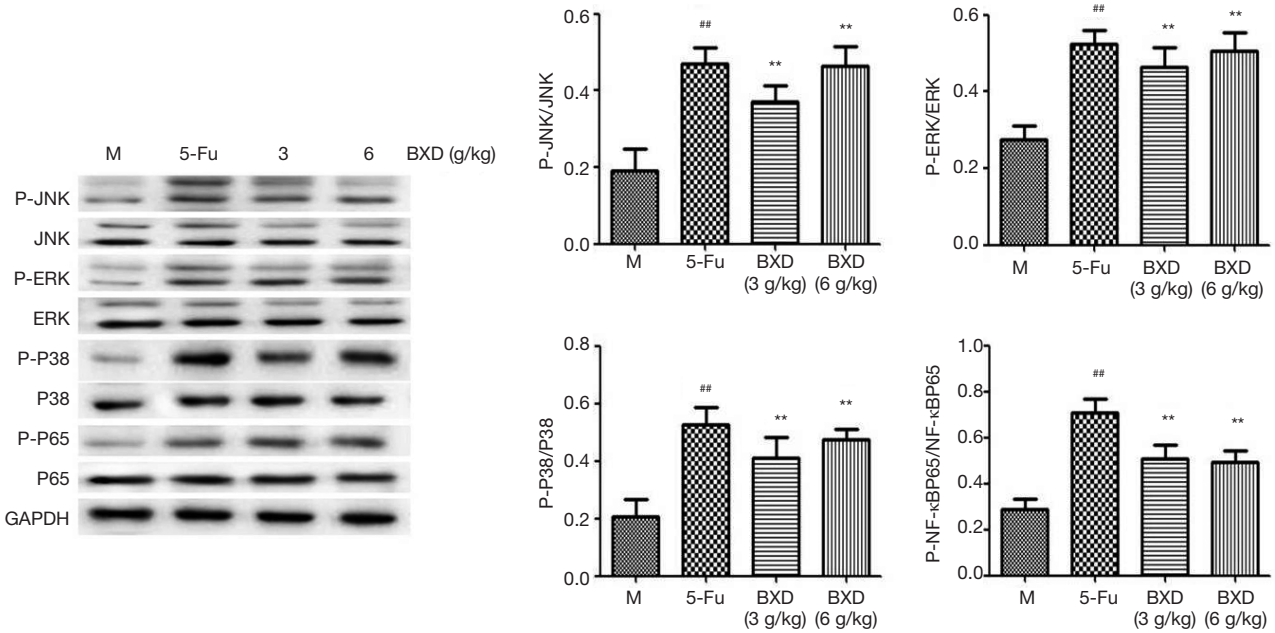

Figure 6 Effect of BXD on inflammation protein in tumor tissue. The data are expressed as mean values ± SDs. ##, P<0.01; (compared with M group); **, P<0.01 (compared with M group). M, model; BXD, Banxia Xiexin decoction.

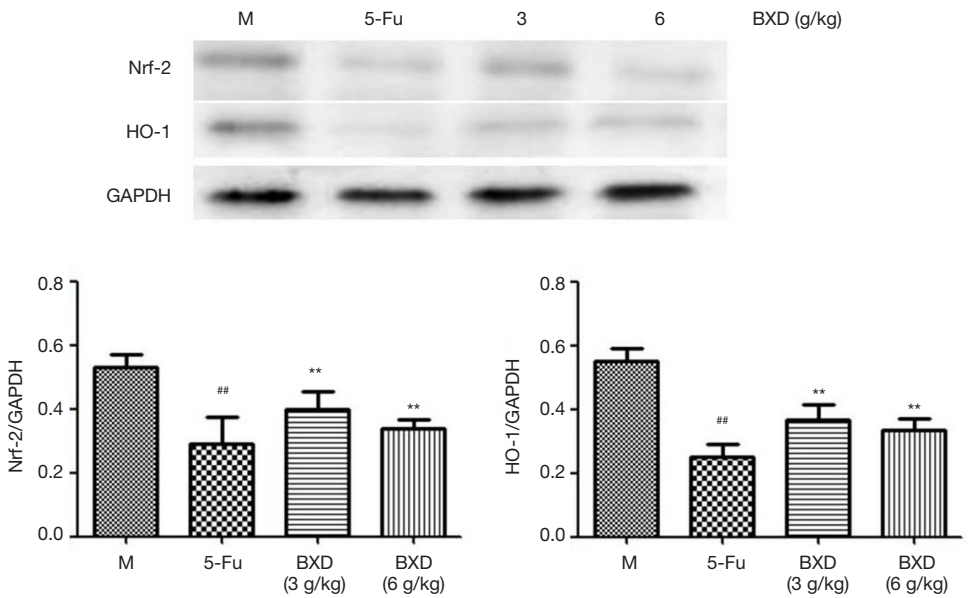

Figure 7 Effect of BXD on oxidative stress protein in tumor tissue. The data are expressed as mean values ± SDs. ##, P<0.01; **, P<0.01 (compared with M group). M, model; BXD, Banxia Xiexin decoction.

in single distribution, with colored nucleus and non-colored cytoplasm. The nucleus of apoptotic cells is reduced in volume or shrinks, with irregular shape, some are round, some are irregular and some are vacuolar. Compared with the M group, TUNEL positive cells were increased in BXD group and 5-Fu group (*Figure 9*).

Discussion

Colon cancer is one of the most common digestive diseases

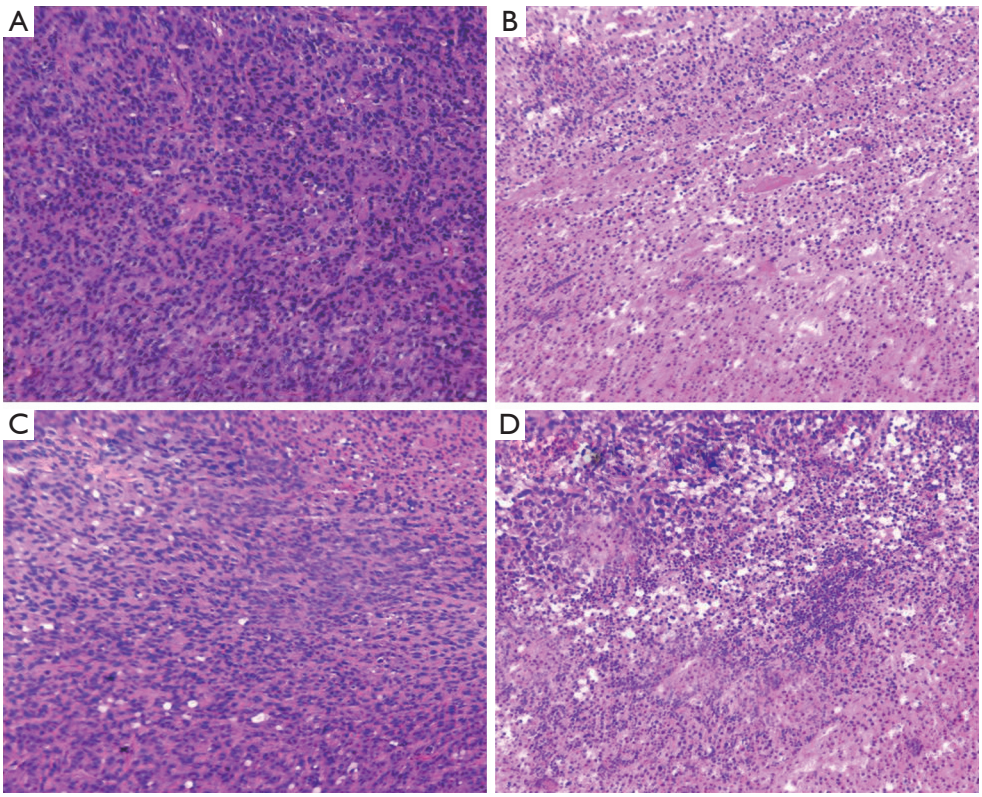

Figure 8 Effect of BXD on pathological changes in tumor tissue (×200), hematoxylin-eosin staining. (A) Model group; (B) 5-Fu; (C) BXD (3 g/kg); (D)BXD (6 g/kg). BXD, Banxia Xiexin decoction.

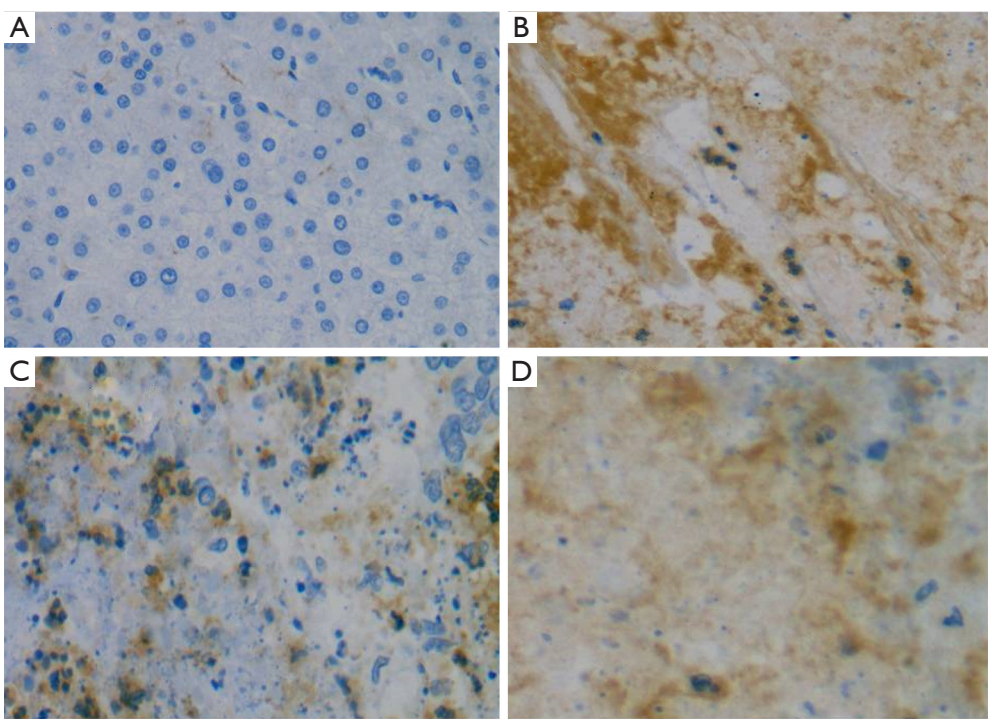

Figure 9 TUNEL staining results (×200). (A) Model group; (B) 5-Fu; (C) BXD (3 g/kg); (D)BXD (6 g/kg).

in China. Various inflammatory stimulus factors can collect a large number of macrophages to gather in intestinal submucosa, and activate macrophages to secrete various pro-inflammatory factors. Inflammatory factors further induce apoptosis and necrosis of intestinal epithelial cells, which is one of the main mechanisms of colon cancer. In recent years, great progress has been made in the treatment of colon cancer in China, but the problem of multidrug resistance is becoming more and more serious. The combination of traditional Chinese and western medicine has opened up a new way for the treatment of colon cancer. BXD comes from Zhang Zhongjing's Treatise on Febrile Diseases, Differentiation of Taiyang Disease and Pulse Syndrome and Treatment of Middle energizer: "However, for those with fullness but no pain, this is the new type, and Bupleurum root is not suitable for it, so BXD is appropriate." BXD is a representative prescription for the treatment of "hypochondrium fullness". Modern research reports that this recipe is widely used in the treatment of acute and chronic gastritis, peptic ulcer, dyspepsia, gastrointestinal dysfunction, chronic hepatitis, oral ulcer and other diseases.

Colon cancer is a common digestive tract tumor, ranking 4th in the incidence of malignant tumors in urban areas of China. Even in some first-tier cities, the incidence rate is in the second place, and the mortality rate is increasing year by year (10). As one of the important components of comprehensive treatment, traditional Chinese medicine plays an indispensable role in prolonging the life of patients and improving the quality of life in adjuvant surgery, radiotherapy and chemotherapy for colon cancer (11,12). In recent years, the multi-target therapy of Chinese medicine for anti-cancer has attracted more and more attention. However, the complex composition, the incomplete research on the structural basis of anti-cancer components and the anti-cancer structure-activity mechanism have limited the further development and application of Chinese medicine. Therefore, it is necessary to search for Chinese herbal medicines with good anti-cancer activity and to study the structure and mechanism of their effective components in depth to provide more information for the treatment of clinical tumors (13). Traditional Chinese medicine is a suitable substitute for chemotherapy drugs because they are more effective and have fewer side effects than synthetic drugs. Many traditional Chinese medicines are considered as promising sources of drugs for the prevention and treatment of cancer because of their multi-target characteristics. BXD, the name of traditional Chinese medicine prescription. It is a reconciliation agent and has the effects of harmonizing liver and spleen, calming cold and heat, eliminating mass and resolving hard mass. Indication of intermingled cold and heat's new syndrome. Under the heart of the new, but full and painless, or vomiting, bowel sounds, greasy and yellowish tongue coating. BXD and has exhibited different pharmacological effects, including antioxidant, anti-inflammatory, anti-diabetes and anti-renal failure activities. In this study, we found that BXD showed an inhibitory effect on the progression of colon cancer, which shows that BXD inhibit colon cancer. This may be a potential treatment strategy for patients in future.

Apoptosis usually occurs in a steady state in normal cells and tissues or as a defense mechanism to eliminate defects or unwanted cells (14). It is reported that it is a necessary supplement to inhibit cell proliferation and plays an important role in the development and regulation of the immune system, the removal of damaged cells and the destruction of apoptosis. In this study, compare with M group, western blotting revealed that the protein expressions of Bax, Caspase-3, Caspase-9 were increased by different concentrations of BXD, while Bcl-2 were decreased.

Disease development and progress, including cancer, that play an important role in cell apoptosis and cell cycle when cell signaling pathways are deregulated. Members of the MAPK/NF-kB pathway are considered to be important participants in coping with cell stress in cancer. Therefore, these genes can be used as therapeutic targets. Cell stress, such as DNA damage, ribosome and endoplasmic reticulum stress, stimulates MAPK/NF-kB pathway activity while strictly maintaining a low level under normal conditions. Here, we find that MAPK/NF-kB is up-regulated in colon cancer treated with BXD, which is helpful to promote apoptosis. This is consistent with HE staining in nude mice bearing tumor.

Two of the most important members associated with oxidative stress include Nrf-2 and HO-1. Understatement of HO-1 andNrf-2 proto-oncogene damages the therapeutic effect of existing cancer treatment schemes by inducing apoptosis of tumor cells. In the present study, the finding from western blot analyses clearly revealed that the protein expressions of Nrf-2 and HO-1 were decreased by different concentrations of BXD. This is consistent with the results of MDA and SOD staining in nude mice bearing tumor.

Conclusions

In short, our results show that BXD target apoptosis and causing death of colon cancer cells. BXD also regulation of oxidative stress and inflammation in nude mice bearing tumor. Therefore, BXD can be used as a kind of anti-tumor drug to develop and treat colon cancer.

Acknowledgments

Funding: This work was supported by Jiangsu Province Youth Medical Talent Project (No. QNRC2016255). The authors gratefully acknowledge financial support from project funded by the National Natural Science Foundation of China (No. 81804058).

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All animal care and experimental procedures were approved by the Ethics Committee for Animals Experiments of the Nanjing University of Chinese Medicine, Nanjing, China (approved document NUCTM-2004).

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Cite this article as: Yan S, Yue Y, Wang J, Li W, Sun M, Zeng L, Wang X. Banxia Xiexin decoction, a traditional Chinese medicine, alleviates colon cancer in nude mice. Ann Transl Med 2019;7(16):375. doi: 10.21037/atm.2019.07.26

