African Journal of Pharmacy and Pharmacology Vol. 5(20), pp. 2193-2198, 29 November, 2011
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP10.196
ISSN 1996-0816 ©2011 Academic Journals

Full Length Research Paper

Effect of *Bidens bipircadta* L. on mouse uterine cervix cancer

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Accepted 31 October, 2011

Many evidences indicate that *Bidens bipircadta* L. has a strong pharmacologic function. However, hardly ever has it been reported whether *B. bipircadta* L. has an important action to the treatment of uterine cervix cancer. In the present study, the characteristics of *B. bipircadta* L. to anti-cancer were studied. The ethanolic extracts of *B. bipircadta* L. (BBE) consisted of flavonoids, saponin, polysaccharide, hydroxybenzene, protein, polypeptide, glycoside, organic acid, lignin, steride, triterpene, essential oil, anthraquinone, tannin and alkaloid. And the flavonoids content was 6.304% in *B. bipircadta* L. The animal models of transplanted tumors witnessed that BBE had a significant anti-uterine cervix cancer *in vivo*. The solid tumor growth of uterine cervix cancer (UCC) harboring mice was significantly inhibited by BBE. The tumor inhibition rates reached 45.52 and 33.97% in low-dosage (21.66 g/kg weight (wt)) and high-dosage (43.32 g/kg wt) BBE-treated mice. Then, the results showed that the apoptosis of U14 cells was induced by BBE. Finally, it was found that the Bax expression was up-regulated and the expressions of Bcl-2, mutant p53 (Mtp53) and PCNA were down-regulated in BBE-treated mice, which promoted the cell apoptosis. Overall, it was concluded that the proliferation of U14 cells was blocked by *B. bipircadta* L., which provides the rationale to develop *B. bipircadta* L. as natural drugs of anti-uterine cervix cancer.

Key words: *Bidens bipircadta* L., uterine cervix cancer, anticancer.

INTRODUCTION

Cell apoptosis is important in controlling tumors. When cell apoptosis is inhibited, cell proliferation is greatly promoted so that these physiologically not-required cells are difficultly removed by the organism (Sachs and Lotem, 1993). Certainly, if cell proliferation is inhibited, tumor development is blocked. Cell apoptosis is possibly controlled by genes. At present, it is investigated that many genes are related with cell apoptosis. Now, the B-cell lymphoma (Bcl) gene family is greatly studied because cell apoptosis is blocked by Bcl-2 and Bcl-xL genes, and is promoted by the Bax gene (Boise et al., 1993; Yin et al., 1997). In addition, cell apoptosis is promoted by wild-type protein 53 (Wtp53) but it is inhibited by mutant-type protein 53 (Mtp53). It was reported that cultivating leukemic cells were killed by monoclonal antibody of Mtp53 (Cheng, 1995). Except for the aforementioned genes, there are also other genes and factors like FAS, TGF-β and RB which are related with the cell apoptosis.

At present, anti-tumor substances are extracted from natural plants, which have been an important research in tumor treatment. It was reported that the cell apoptosis was induced by As_2O_3 (Chen et al., 1997; SU et al., 2000; Shen et al., 1999; Zhang et al., 1999; Zheng et al., 1999; Deng et al., 2000; Chen et al., 1997), elemene (Yang et al., 1996; Yuan et al., 1999), icarrin (Li et al., 1997), tanshinone IIA (Huang et al., 1998), triptollide (Yang et al., 1998), quercetin (Xiao et al., 1997; Wei et al., 1994), trichosanthin (Bi et al., 1998; Kong et al., 1998), rurcumin (Kuo et al., 1996; Xu et al., 1998; Chen et al., 1999; Jiang et al., 1996; Gautam et al., 1998) and taxol (Peng et al.,

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1998; Chen et al., 1999, 1997; Scatena et al., 1998; Yeung et al., 1999) from natural plants. And these extracts nearly have no side effects to human bodies. Therefore, research about natural plants effect on cancers is more attended. The chemical constituents of *Bidens* L. include flavanoids, volatility substances, amino acids, etc. It was reported that the activity substances of the plant can inhibit cell growth, such as *Bidens bipinnata* strongly inhibiting the proliferation of HL-60 cell and V937 cell (Wang et al., 1997). In addition, the herbs like *Bidens pilosa* and *Bidens cernua* also have great anti-tumor activities (Ma et al., 2005). *Bidens bipircadta* L. is one of *Bidens* L. However, the effects of *B. bipircadta* L. on uterine cervix cancer is not reported at present. Therefore, it is necessary to study the action of *B. bipircadta* L. to uterine cervix cancer, which are important to develop *B. bipircadta* L. as new anti-tumor drugs.

MATERIALS AND METHODS

Antibodies and reagents

All chemical reagents were of analytical grade and were purchased from China unless otherwise specified. Rutin marks (Batch No. 050407) were from Yousi Biotechnology (Shanghai, China). Trypan blue was from Sigma. The monoclonal mouse anti-human p53, mouse anti-human Bcl-2, mouse anti-human Bax and mouse anti-human PCNA antibodies were from Zymed Company (American). Treptavidin-peroxidase (SP) and dimethylaminoazobenzene (DAB) kits were from Zhongshan Biotechnology (Beijing, China). CTX (Batch No. 011121) was purchased from Taisheng drugs manufacturers (Shanxi, china) and it makes 25 mg/L with normal sodium when used.

B. bipircadta L., U14 cells and animals

B. bipircadta L. was purchased from Minle drug store (Qinhuangdao, China). Mouse UCC U14 cell lines were from the Institute of Materia Medica of Chinese Academy of Medical Sciences. Kunming cleaning female mice, Lic No. SCXK (capital) 2004-0001, were from the Military Affairs Academy of Medical Sciences, 6 to 8 weeks of age and 18 to 22 g. The test temperature of mice is 22 to 25°C and the relative humidity is 40 to 60%.

BBE preparation

B. bipircadta L. was crushed into pieces and was soaked with 8 times of 90% volume ethyl alcohol and then, hotly recirculated 3 times, 2 h per time. After that, all BBE were merged and condensed for detecting the extract rate.

Qualitative experiments of chemical composition of *B. bipircadta* L.

According to "Natural Pharmaceutical Chemistry Experiment Technology" (Xie and Luo, 1993), the experiments were carried on systematically.

Flavonoids of *B. bipircadta* L. assaying

According to the standard curve of rutin drawing, the percent of flavonoids in *B. bipircadta* L. was detected. The concentration of BBE was 0.1 mg/ml. 0.3 ml of BBE solution was poured inside the measuring flask and 30% ethyl alcohol up to 5 ml was added. Then, 0.3 ml of 5% $NaNO_2$ was added. After the solution was placed 6 min, 0.3 ml of 10% $Al(NO_3)_3$ was added. After the solution was placed 6 min again, 4 ml of 1 mol/L NaOH was added and finally 30% ethyl alcohol up to the required scale division was adding. Next, the solution was placed 15 min, the sample absorbency was determined under 510 nm wavelength and the flavonoids content was counted according to standard curve of rutin.

Tumor inhibiting experiment

Animal modeling

U14 cells taken out from nitrogen canister were quickly put into 38 to 40°C water to defrost completely and then centrifuged at 1,000 rpm/min for 5 min. After the supernatant was removed, 1ml normal sodium was added. This process, from centrifugalization to adding normal sodium, was repeated 1 time. Then, it was dyed by the trypan blue, and the living cells were counted. When the living cell rate was more than 95%, the living cell number was regulated at 1×10^7 ml^{-1} and then the mouse was implanted 0.2 ml living cells in the abdominal cavity. After 7 to 10 days, U14 cells were put out and implanted to the mice of test group. Every mouse was implanted about 10^5 pfu of U14 cells and the implanted place was right forefoot oxter.

Effect of BBE on tumor

After U14 cells were implanted for 24 h, the mice were divided into 4 groups randomly, which were 10 mice in every group. The groups comprised a negative control group and a positive control group, and 2 BBE test groups. Every mouse of negative control group was given the distilled water. Every mouse of positive group was given 0.2 ml of cyclophosphamide (CTX) per 10 g weight. Two BBE test groups were a low-dosage BBE group and a high-dosage BBE group. According to human therapeutic dose (35 g/60 kg per day), the therapeutic dose of 20 g weight mouse was 0.14 g *B. bipircadta* L. per day. According to the 3 and 6 times therapeutic dose of *B. bipircadta* L. to each mouse, every mouse with 20 g weight of low-dosage BBE test group was given BBE 0.42 g per day and every mouse with 20 g weight of high-dosage BBE test group was given BBE 0.84 g per day. Drugs were given 1 time per day and continued to 12 days. After discontinuation, mice were killed to observe and weigh tumors for counting the inhibition rate of tumor.

Flow cytometry assay

New tumor tissues were sheared to cause the froths and then digested with 0.25% trypsin. After centrifugalization, the supernatant was removed. Then, the precipitation was washed 2 times with phosphate-buffered saline (PBS), centrifuged at 800 rpm/min for 5min and fixed with 2 ml precooling ethyl alcohol overnight at 4°C. Next, PBS was added to remove ethyl alcohol. And after centrifugalization the precipitation was added 100 µl PBS to make cells suspension and then RNAase and Propidium Iodide (PI) were added to dye for 30 min. After that, the cell density was adjusted to 10^6 ml^{-1} for assaying in flow cytometry.

Immunity histochemistry

According to SP kit descriptions, the paraffin sections were deparaffinaged and then dipped into gradient ethyl alcohol. After the

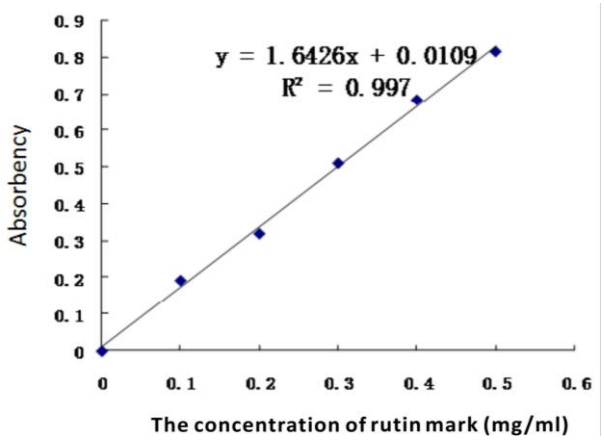

Figure 1. According to the known data of rutin marks (data no showed), the regression curve was drawn. The equation, Y = 0.0164x + 0.0109, was obtained, and "Y" means absorbency and "x" represents concentration. The regression coefficient was 0.997.

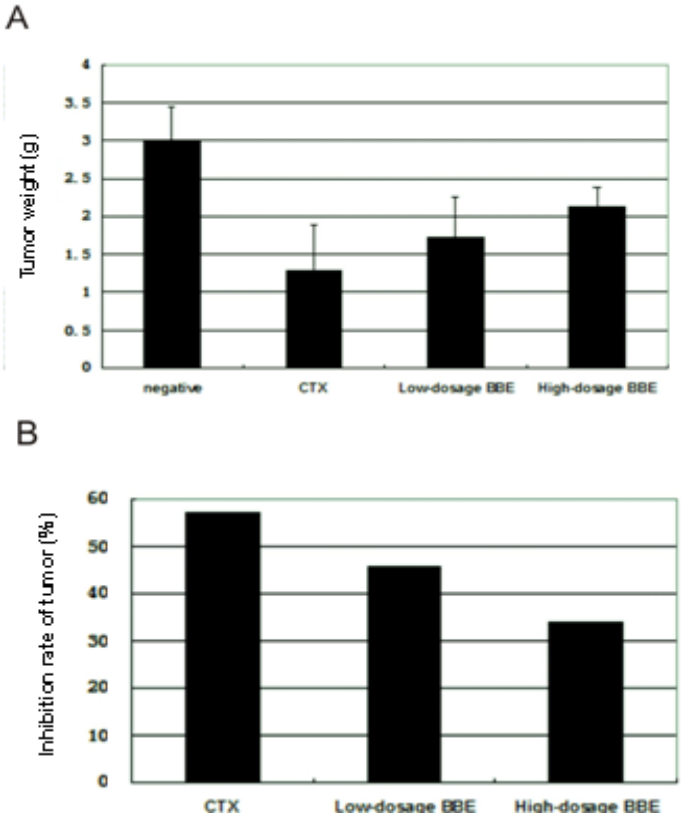

Figure 2. Effect of BBE on the solid tumor harboring mice. (A) The tumors weights were 3.01 g of negative control group, 1.29 g of CTX, 1.73 g of low-dosage BBE and 2.12 g of high-dosage BBE, respectively. Data are means ± SD of three independent experiments. (B) The tumor inhibition rate is the percentage that the negative-group tumor weight which minuses the weight of test-group tumor divides the weight of negative-group tumor (×100). It is examined that the tumor inhibition rates were 57.14% of CTX, 45.52% of low-dosage BBE and 33.97% of high-dosage BBE, respectively.

antigens were fixed, the sections were washed and then incubated with 3% peroxydase at room temperature. The sections were then washed with PBS and added antibody I. After incubated overnight at 4°C, the sections were washed with PBS and added antibody II, they were incubated at room temperature for 30 min, and the sections were washed with PBS and were dyed with DAB and campeachy and finally observed under the microscope.

Western blotting

The proteins of tumor tissues were separated by electrophoresis through 10 to 12% polyacrylamide SDS-containing gels. Prestained protein markers (Sigma) were used for molecular weight standards. Proteins were electrophoretically transferred onto nitrocellulose membranes (Beyotime, China). Blots were blocked with 5% skim milk in 0.05% Tween-TBS (blocking buffer) for 30 min.

Membranes were probed with monoclonal mouse anti-human p53, mouse anti-human Bcl-2, mouse anti-human Bax and mouse anti-human proliferating cell nuclear antigen (PCNA) in blocking buffer overnight at 4°C. The primary antibodies were detected with a polyclonal goat anti-mouse Ig coupled to horseradish peroxidase (HRP), followed by enhanced chemiluminescence, with SuperSignal ECL Western Blotting Detection Reagents (Beyotime, China) and light detection with Fuji Photo Film (Tokyo, Japan).

RESULTS

BBE qualitation and detection of flavonoids content

386.8 g BBE was obtained from 5 kg *B. bipircadta* L. and the extract rate was 7.715%. The chemical constituents of *B. bipircadta* L. mainly contained flavonoids, saponin, polysaccharide, hydroxybenzene, protein, polypeptide, glycoside, organic acid, lignin, steride, triterpene, essential oil, anthraquinone, tannin and alkaloid.

To determine the flavonoids content of BBE, the standard curve of rutin was firstly drawn (Figure 1). According to the curve, the regression equation, namely Y = 1.6426x + 0.0109, was obtained. The absorbency value of BBE was 0.413 and led into the aforementioned equation. It is obtained that the flavonoids content was 0.817 mg in 1 mg BBE. According to the extract rate, it was counted that the flavonoids content in *B. bipircadta* L. was 6.304%.

Effect of BBE on the proliferation of tumor cells

In BBE-treated mice, the proliferation of tumor cells was obviously inhibited. Tumor weighs in the other 3 groups clearly dropped when compared with negative control group (Figure 2A). The tumor inhibition rates in low-dosage BBE group, high-dosage BBE group and CTX control group were 45.52, 33.97 and 57.14%, respectively (Figure 2B). The difference between negative control group and other 3 groups were significant ($P < 0.01$). However, the difference between BBE and CTX was quiet, which indicated that BBE and CTX possibly have the same effect on tumor inhibition.

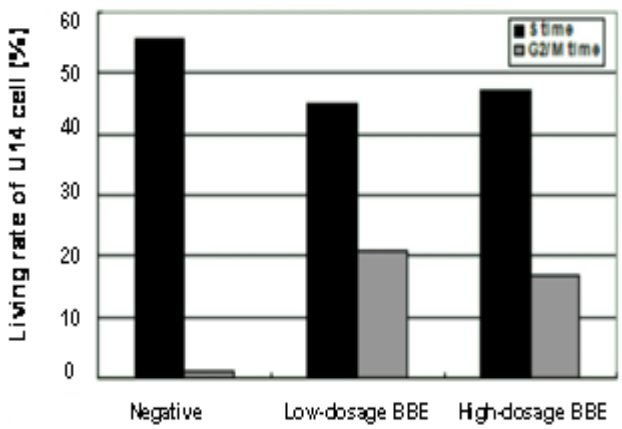

Figure 3. Living rate of U14 cells was detected by flow cytometry. In the negative group, the living rate of U14 cells was 55.5% in S time and 1.04% in G2/M time, respectively. Living rates of U14 cells of high-dosage and low-dosage BBE were 46.9 and 44.8% in S time and then 16.7 and 21% in G2/M time, respectively.

U14 cells apoptosis by BBE

Whether the U14 cell cycle is affected by BBE was investigated through the analysis of the flow cytometry. BBE-treated U14 cells, G2/M-time cells were increased and S-time cells were reduced. The living rates of U14 cells of BBE-treated mice were more than the one of the negative control group in G2/M time cells but just opposite in S time cells, which showed that the U14 cells in BBE-treated mice were blocked in G2/M time (Figure 3). Therefore, it was concluded that the apoptosis of U14 cells was developed.

Effect of BBE on the protein expressions

Bcl family genes were examined by immunoblotting assay. The Bax expression was investigated. There was a large area where Bax proteins were dyed buffy in BBE-treated mice (Figure 4A) and the protein expression of Bax in BBE-treated mice increased when compared with the negative control group (Figure 4C). The expression rate of Bax in BBE-treated mice was improved (Figure 4B). In addition, the protein expression of Bcl-2 decreased when compared with negative control group (Figure 4C). The rates of Bcl-2 expression were about 41.50% of low-dosage BBE and 55.73% of high-dosage BBE when compared with negative control group, respectively (Figure 4B). We thought that the Bcl-2 expression of BBE-treated mice were obviously lower than the ones of negative control group. The discussed results showed that Bcl family genes were affected by BBE and the proliferation of U14 cells was inhibited.

Then, P53 and PCNA were detected. The results indicated that the expressions of Mtp53 and PCNA in

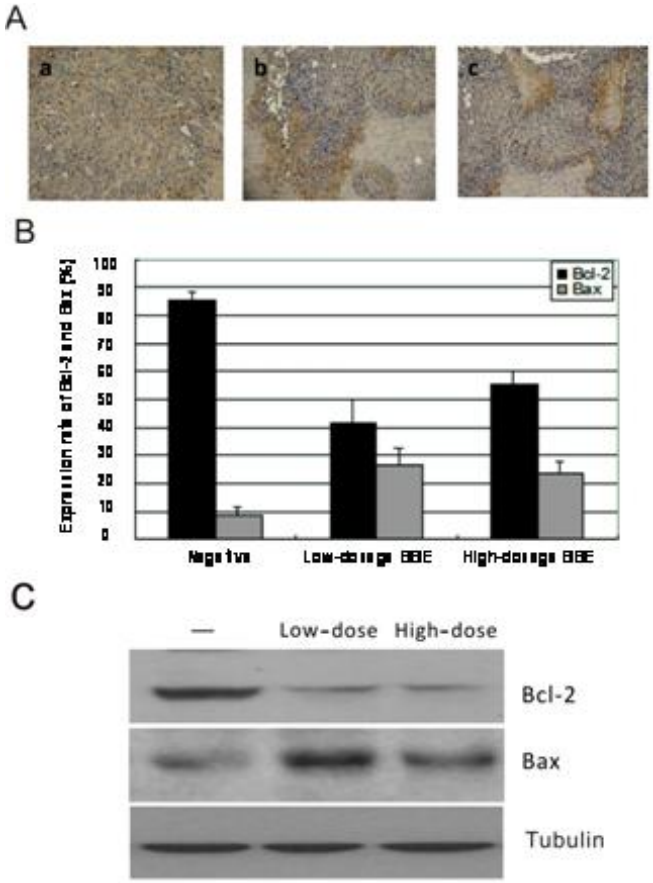

Figure 4. Expression of Bcl-2 and Bax in tumor cells. (A) In the immunohistochemistry assay, Bax were dyed buffy. There was large area where Bax were dyed buffy in low-dosage BBE test group (b) and high-dosage BBE test group (c) when compared with negative group (a). (B) Expression rate of Bcl-2 and Bax in the tumor were investigated (×100). In negative group, the expression rates of Bcl-2 and Bax were 85.73 and 8.73%. But their expression rates were 41.50 and 26.5% of low-dosage BBE and 55.73 and 23.73% of high-dosage BBE. Data are means ± SD of three independent experiments. (C) After the proteins of tumor tissues were prepared, Bcl-2 and Bax were analyzed by western blot. Data are representative of three independent experiments.

BBE-treated cells dropped and remarkable discrepancy was found when compared with negative control group (Figure 5A and B), which led to the apoptosis of U14 cells.

In summary, we concluded that the expressions of Bcl-2, Mtp53 and PCNA were inhibited, but Bax expression was promoted by BBE.

DISCUSSION

At present, the method of animal tumor transplanted *in vivo* was often used in studying cancers. Through the investigation to animal states and weights and death rates, whether the drugs inhibits tumor growth was

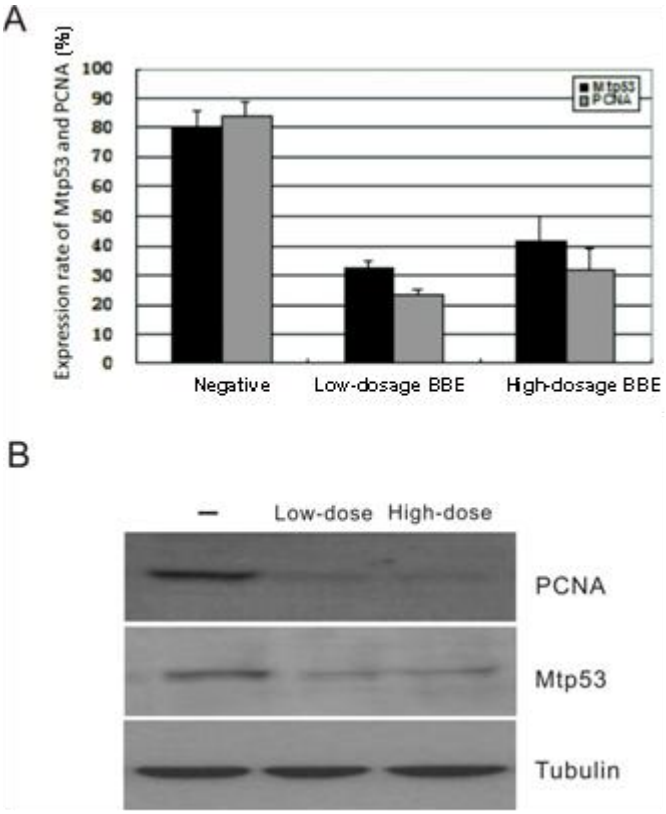

Figure 5. Expression of Mtp53 and PCNA in tumor cells. (A) Expression rates of Mtp53 and PCNA in tumor were investigated through the immunohistochemistry. In negative group, the expression rates of Mtp53 and PCNA were about 79.48 and 84.26%. But their expression rates were about 32.47 and 23.13% of low-dosage BBE and 41.62 and 31.66% of high-dosage BBE. Data are means ± SD of three independent experiments. (B) After the proteins of tumor tissues were prepared, Mtp53 and PCNA were analyzed by western blot. Data are representative of three independent experiments.

known. To evaluate anti-tumor (especially solid tumor) effect, the tumor inhibition rate was often counted. The growth of the solid tumors of BBE-treated mouse was inhibited, which indicates that BBE resists UCC.

Drug absorption rate was related with the ways of the drugs used. Usually, the absorption rate of peritoneal injection exceeds the one of intragastric administration. In this research, CTX was given to mice by peritoneal injection and BBE was given to mice by intragastric administration. The results showed that BBE action was inferior to the action of CTX, although BBE was high anti-tumor activity. Therefore, it was considered that the action of BBE was affected by the way of intragastric administration. In addition, many research results also showed that the activity function in natural plants only was generated by some effective substances. Since BBE was not purified in the study, possibly, the effect of BBE anti-tumor is not high.

Flavonoids have strong anti-tumor activity. By analyzing the chemical constituents of BBE, it was confirmed that there were flavonoids, saccharide, saponins, etc. Flavonoids content was 6.304% in *B. bipircadta* L. Since BBE had obvious activity of anti-uterine cervix cancer, whether flavonoids have great action in resisting UCC which should be further studied.

Cell apoptosis depends on the proportion between Bcl-2 protein and Bax protein. When the Bcl-2 protein expression surpasses the Bax protein expression, cell apoptosis is inhibited. Otherwise, cell apoptosis is developed. Since BBE clearly down-regulated Bcl-2 protein expression and up-regulated Bax protein expression, we concluded that the apoptosis of U14 cells is promoted.

Wtp53 negatively regulates the cell that enters into G_1 time from G_0 time. When p53 is mutated, its function is lost so that the cells endlessly are proliferated. The Mtp53 in BBE-treated mice was less than the one in negative control group. Therefore, Mtp53 expression is blocked by BBE and the apoptosis of U14 cells is developed.

PCNA promotes cell proliferation (Finlaly et al., 1989). The detection of PCNA in the tumors acted as a good target to evaluate the proliferation of cells (DAI, 1996; Yang et al., 1997). In the communication, PCNA were excessively expressed in negative control group and it is speculated that the proliferation activity of U14 cells was very strong. Nevertheless, PCNA were lowly expressed in BBE-treated mice and it is believed that the proliferation of U14 cells was inhibited. In brief, it is inferred that the anti-U14 cell function of BBE partly carries on through the intervention to cell multiplication.

In summary, it is known from the discussion that *in vivo* BBE inhibits UCC and results in cell apoptosis via down-regulating Bcl-2, Mtp53 and PCNA expression and up-regulating Bax expression. And BBE has the powerful activities in controlling UCC. These data provide a new insight in treating UCC and are useful to develop *B. bipircadta* L. as a new anti-cancer agent.

ACKNOWLEDGEMENT

This paper was supported by the grant “Study of EGF-mediated signal pathways in cell mitosis” (QN2011010) from Northwest A&F University, Shaanxi Yangling, China.

REFERENCES

Bi L, Li H, Zhang Y (1998). Effect of trichosanthin of cell cycle and apoptosis of murine melanoma cells. Zhongguo Zhong Xi Yi Jie He Za Zhi, 18(1): 35-37.

Boise LH, Gonzalez-Garcia M, Postema CE, Ding L, Lindsten T, Turka LA, Mao X (1993). Bcl-x, a bcl-2 -related gene that functions as adominant regulator of apoptotic cell death. Cell, 74(4): 597-608.

Chen G, Zhu J, Shi X, Zhong H, Liu W, Jin X (1997). Preliminary study on the arsenic trioxide-induced NB4cell apoptosis and its molecular mechanisms. Chin. J. Hematol., 18(1): 25-28.

Chen GQ, Shi XG, Tang W, Xiong SM, Zhu J, Cai X (1997). Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL): I. As_2O_3 exerts dose-dependent dual effects on APL cells. Blood, 89 (9): 3345-3353.

Chen H, Zhang ZS, Zhang YL (1999). Anticancer function and inducing tumor cells Apoptosis of curcumin. Chin. J. Oncol., 21(2): 118.
Chen HZ, Shi YF, Zheng W (1999). Mechanism of taxol-induced apoptosis in chorioepithelioma cancer JAR cells. Chin. J. Obstet. Gynecol., 34(2): 121.
Chen L, Zheng S, Willingham MC (1997). Mechanism of taxol-induced apoptosis in human breast cancer cells. Chin. J. Oncol., 19(2): 103-106.
Cheng J (1995). The research Progress of cell apoptosis. Foreign Medical Sciences Epidemiology Lemology, 22 (3): 101-104.
DAI L. (1996) PCNA and gastric cancer. Foreign Medical Sciences: Digestive System Diseases, 16(1): 3-5.
Deng ZH, Cai HP, Li S (2000). Studying of Molecular Mechanism of Apoptosis of Colorectal Cancer Cell Line Induced by Arsenic Trioxide. Cancer research on prevention and treatment, 27(2): 105-108.
Finlaly CA, Hinds P W, Levine AJ (1989). The p53 protooncogene can act as a suppressor of transformation. Cell, 57: 1083-1093.
Gautam SC, Xu YX, Pindolia KR, Janakiraman N, Chapman RA (1998). Nonselective Inhibition of Proliferation of Transformed and Nontransformed Cells by the Anticancer Agent, Cureumin (Diferuloylinethane). Biochem. Pharmacol., 55 (8): 1333-1337.
Huang RM, Yuan SL, Song Y (1998). Apoptosis of human leukemic HL-60 cells induced tanshinone II. Cancer., 17(3): 164-166.
Jiang MC, Yang-Yen HF, Yen JJ, Lin JK (1996). Curcumin induces apoptosis in immortalized NIH 3T3 and malignant cancer cell lines. Nutr. Cancer., 26(1): 111-120.
Kong M, Ke YB, Zhou MY, Ke XY, Lu B, Nie HL (1998). Study on trichosanthin induced apoptosis of leukemia K562cells. Shi Yan Sheng Wu Xue Bao, 31(3): 233-243.
Kuo ML, Huang TS, Lin JK (1996). Curcmnin, an antioxidant and anti-tumor promotor, induces apoptosis in human leukemia cells. Biochim. Biophys. Acta., 1317(2): 95-100.
Li GX, Zhang L, Wang Y (1997). Studies of the icarrin-induced tumor cells apoptosis and its mechanisms. Chin. J. Cancer Biother., 4(3): 235-236.
Ma M, Wang JP, Xu LC (2005). Chemical constituents of *Bidens bipinnata*. Chin. Tradit. Herbal Drugs. 36(1): 7-9.
Peng WD, Zhang J, Hui HX (1998). Taxol-induced apoptosis in human esophageal carcinoma cell line. J. Fourth Milit. Med. Univ., 19(2): 129-133.
Sachs L, Lotem J (1993). Control of programmed cell death in normal and leukemic cells: new implications for therapy. Blood, 82(1): 15-21.
Scatena CD, Stewart ZA, Mays D, Tang LJ, Keefer CJ, Leach SD (1998). Mitotic Phosphorylation of bcl-2 during Nomral Cell Cycle Progression and Taxol-induced Growth Arrest. J. Biol. Chem., 273(46): 30777-30784.
Shen ZY, Tan LJ, Cai WJ, Shen J, Chen C, Tang XM (1999). Arsenic trioxide induces apoptosis of oesophageal carcinoma *in vitro*. Int. J. Mol. Med., 4(1): 33-37.
Su H, Sun GL, Lai ZB (2000). Preliminary studies on the effect of arsenic trioxide on proliferation and apoptosis of K562 cells and it's mechanism. J. Leukemia, 9(5): 267-270.
Wang J, Qin H, Zhang H, Wang M, Zhang L, Wang Y (1997). Inhibition of 5 Compounds from *Bidens bipinnata* on Leukemia Cells *in vitro*. Zhong Yao Cai, 20(5): 247-249.
Wei YQ, Zhao X, Kariya Y, Fukata H, Teshigawara K, Uchida A (1994). Induction of Apoptosis by Quercetin: Involemeat of Heat Shock Protein. Cancer Res., 54(18): 4952-4957.
Xiao D, Zhu SP, Gu ZL (1997). Quercetin induced apoptosis in human leukemia HL-60 cells. Zhongguo Yao Li Xue Bao, 18(3): 280-283.
Xie P, Luo YM (1993). Natural Pharmaceutical chemistry Experiment Technology. Jiangxi: Jiangxi Science and Technology Press. pp. 165-269.
Xu JH, Chen YZ, Zhao R (1998). Effects of Curcumin on inducing Apoptosis of K562 Cells. Pharm. Clinics Chin. Mater. Med., 14(6): 19-22.
Yang H, Wang X, Yu L (1996). The antitumor activity of elemene is associated with apoptosis. Zhonghua Zhong Liu Za Zhi. 18(3): 169-72.
Yang Y, Liu Z, Tolosa E, Yang J, Li L (1998). Triptolide induces apoptosis death of T lymphocyte. Immunopharmacology, 40(2): 139-149.
Yang ZL, Li YG, Huang S (1997). The relationship between scoring of PCNA and expressions of bcl-2, p53 oncoprotein in gastric cancer. Chin. J. Clinical Oncol., 24(11): 811-814.
Yeung TK, Germond C, Chen X, Wang Z (1999). The Mode of Action of Taxol: Apoptosis at Low Concentration and Necrosis at High Concentration. Biochem. Biophys. Res. Commun., 263(2): 398-404.
Yin C, Knudson CM, Korsmeyer SJ, Van Dyke T (1997). Bax suppresses tumorgenesis and stimulates apoptosis *in vivo*. Nature, 385 (6617): 637-640.
Yuan J, Gu ZL, Chou WH, Kwok CY (1999). Elemene induces apoptosis and regulates expression of bcl-2 protein in human leukemia K562 cells. Zhongguo Yao Li Xue Bao, 20(2): 103-106.
Zhang TC, Cao EH, Li JF, Ma W, Qin JF (1999). Induction of apoptosis and inhibition of human gastric cancer MGC-803 cell growth by arsenic trioxide. Eur. J. Cancer., 35(8): 1258-1263.
Zheng J, Deng YP, Lin C, Fu M, Xiao PG, Wu M (1999). Arsenic trioxide induces apoptosis of HPV16 DNA-immortalized human cervical epithleial cells and selectively inhibits viral gene expmssion. Int. J. Cancer., 82(2): 286-292.