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Ursolic acid benzaldehyde chalcone, leads to inhibition of cell proliferation and arrests cycle in G1/G0 phase in colon cancer

Xinying Zhuang [a,1], Aihua Dong [a,1], Ruicai Wang [b], Hongyun Cao [c,*]

[a] Department of Gastroenterology, People's Hospital of Linzi District, Zibo, Shandong Province 255400, China
[b] Department of Pathology, People's Hospital of Linzi District, Zibo, Shandong Province 255400, China
[c] Department of Clinical Microbiology, People's Hospital of Linzi District, Zibo, Shandong Province 255400, China

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ABSTRACT

The present study investigates the effect of matrine on colon cancer cell viability and apoptosis and tumor growth in mice xenograft model. The results from MTT assay revealed a concentration and time dependent reduction in viability of HCT8 and HT29 colon cancer cells by matrine. The viability of HCT8 and HT29 cells was reduced to 24.67 and 29.32% on treatment with 4 μM/ml concentration of matrine after 48 h ($P < 0.05$). The results from flow cytometry revealed increase in population of HCT8 and HT29 cells to 77.6 ± 0.3 and 54.0 ± 5.4%, respectively compared to 1.4 ± 0.3 and 2.4 ± 0.7% in control on exposure to 1 μM/ml concentration of matrine. Histone H2AX phosphorylation and expression of Myt1, cyclin A2, cyclin B1 and p53 were increased in HCT8 and HT29 cells on treatment with matrine for 48 h. Matrine treatment also increased the phosphorylation of cdc2 significantly compared to control cells at 48 h ($P < 0.05$). Results from Annexin-V/FITC-staining showed increase in proportion of apoptotic cells in HCT8 and HT29 cells 67.52 and 68.56 on treatment with 1 μM/ml of matrine. Matrine treatment caused a marked reduction in the growth of HCT8 cell xenograft after 21 days. Thus matrine inhibits cell viability, induces apoptosis and inhibits tumor growth in colon cancer.

1. Introduction

Colon cancer constitutes the third most commonly detected cancer throughout the world and efforts have been put constantly to control its growth and progression. Despite the development of new treatment strategies the rate of mortality in colon cancer patients is very high (Siegel et al., 2013). Initially, colon cancer has been treated mainly using 5-fluorouracil containing therapeutic strategies. However, the rate of response in the patients was found to be less than 20% which demands for development of new and effective strategies (Jones et al., 1995). The failure of 5-fluorouracil to prevent colon cancer has been attributed to the development of resistance (Szoke et al., 2007). Studies have revealed that increased uptake of fruits and phytochemicals facilitates the prognosis and reduce the harmful effects of colon cancer (Ali et al., 2012). Colon cancer growth is inhibited by various biological active molecules through alterations of various pathways (Parkinson et al., 1994). Taking into consideration the role of various molecules in colon cancer suppression, the phytochemical are being screened for the development of novel and more effective carcinoma treatment strategy.

Matrine is a very interesting molecule having formula $C_{15}H_{24}N_2O$ and is obtained by phytochemical investigation of the whole plant genus *Sophora*. In traditional Chinese medicine matrine great importance in the treatment of inflammatory diseases (Li et al., 2007). In addition, matrine has also been used for the treatment of virus induced hepatitis, neuropathic pain and cardiotoxicity induced by isoproterenol (Long et al., 2004; Li et al., 2010; Haiyan et al., 2013). Furthermore, matrine exhibits inhibitory effect on the cancer growth in gastric carcinoma, rhabdomyosarcoma, acute myeloid leukemia and breast carcinoma (Li et al., 2013; Guo et al., 2012; Zhang et al., 2012). Current study demonstrates the effect of matrine on cell viability and induction of apoptosis in colon cancer cells and tumor growth in mice xenograft model. Matrine treatment reduced the viability and induced apoptosis in colon cancer cells. It also inhibited tumor growth in mice HCT8 xenograft model.

* Corresponding author.
E-mail address: hongyuncao145@hotmail.com (H. Cao).
[1] These two authors contribute to this work equally.
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2. Materials and methods

2.1. Chemicals and reagents

Matrine and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The stock solution of matrine was prepared in DMSO and stored at −10 °C prior to use in the experiment.

2.2. Animals and ethics statement

Thirty 6 week-old male BALB/c nude mice were obtained from the Experimental Animal Center of Xi'an Jiaotong University (Xi'an, China). The animals were adjusted to the laboratory conditions one week before the start of the actual experiment. Mice were maintained under 12 h of light and dark cycles and had free access to the food and water. All the experimental procedures were performed according to the guidelines of the Xi'an Jiaotong University. The study was approved by the Animal Care and Use Committee of the Medical School of Xi'an Jiaotong University.

2.3. Cell lines and culture

The HCT8 and HT29, colon cancer cell lines were purchased from the American Type Culture Collection (Rockville, MD, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich) supplemented with 10% FBS, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

2.4. MTT assay

Reduction of viability in HCT8 and HT29 colon cancer cells by matrine treatment was analysed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich, St. Louis, MO, USA) assay. HCT8 and HT29 colon cancer cells, seeded at a density of 2×10^6 per well in 2 ml DMEM in 6-well plates (Nunc A/S, Roskilde, Denmark) were incubated for 24 h. Following incubation, the cells were treated with 1, 2, 3, 4 and 5 μM/ml concentrations of matrine for 12, 24, 48 and 72 h. Then to each of the well 50 μl MTT (5 μg/ml) solution was added and incubated was continued for 2 h. DMSO (100 μl) was put in each of the well and cells were incubated for 5 min more. The optical density (OD) was recorded for each well at 550 nm by using an EL800 Universal Microplate Reader (BioTek Instruments Inc., Winooski, VT, USA).

2.5. Analysis of apoptosis by flow cytometry

Colon cancer cells after incubation with matrine were analysed for induction of apoptosis using Annexin V-FITC staining apoptosis detection kit (MBL). HCT8 and HT29 cells were distributed on tissue culture slides and left un-disturbed for 24 h for the attachment. The cells were incubated for 48 h with matrine followed by suspension in 500 μl of 1X binding buffer. Annexin V-FITC (5 μl) and propidium iodide (5 μl) were added to each of the dish and incubation was continued for 10 min under dark conditions. The stained cells were analysed using flow cytometry (BD FacsCalibur).

2.6. Cell cycle analysis

The colon cancer cell lines, HCT8 and HT29 were seeded in 100 mm culture dishes at a density of 2×10^5 cells per well. The cells were incubated for 48 h with matrine and then centrifuged for 15 min at 500*g*. Then the cells were rinsed in ice-cold PBS followed by fixing in 70% ethyl alcohol. The cells were twice washed with PBS and put in 300 μl PI/RNase Staining Buffer (BD Biosciences). FACScan was used for the analysis of cells in the various phases of cell cycle using laser excitation set at 480 nm.

2.7. Western blot analysis

HCT8 and HT29 colon cancer cells were incubated in DMEM medium supplemented with 2% FBS and matrine for 48 h. The cells were then lysed with RIPA buffer (Rockland, Gilbertsville, PA, USA) containing 5 μM AEBSF, 10 μM E-64, 1.5 μM aprotinin, 0.01 μM leupeptin and phosphatase inhibitors. Protein concentration was determined by RC/DC Bio-Rad assay kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the instructions for user's. The protein samples were separated by electrophoresis on a 10% polyacrylamide-sodium dodecyl sulfate gel. The samples were then transferred onto a polyvinylidene fluoride membranes (Pall Corporation, Port Washington, NY, USA). The non-specific sites on the membranes were blocked by incubation with skimmed milk in Tris-buffered saline containing 0.1% Tween-20. The membranes after PBS washing were incubated with primary antibodies for overnight. The antibodies used were against β-actin, p53 (monoclonal), phospho-H3 (monoclonal), cyclin B1 (polyclonal), cyclin A2 (monoclonal), cdc2 (polyclonal), Myt1 (polyclonal) or phospho H2AX (polyclonal) (1:1000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). The membranes were again washed with PBS and then incubated with peroxidase-conjugated anti-rabbit or anti-mouse immunoglobulin G antibody (1:5000 dilution in blocking solution; Santa Cruz Biotechnology, Inc.). The bands were detected by using an enhanced chemiluminescence western blotting detection reagent (GE Healthcare Life Sciences, Stockholm, Sweden).

2.8. Animal and tumor xenograft assays

A suspension containing 2×10^6 HCT8 carcinoma cells were implanted into the BALB/c nude mice in the right flank region. The mice in the treatment group were injected 4 mg/kg doses of matrine daily for 21 days while as control and the untreated groups received same volume of normal saline. On alternate days, general characteristics and the body weight for each of the animal was recorded. Tumor volume was also measured using calipers. On completion of the treatment, the animals were sacrificed to extract the tumor mass. Wet and dry weight of the tumor in each of the mice was recorded.

2.9. Statistical analysis

The data expressed are the mean ± standard deviation (SD). Data for three groups were compared using Student's *t*-test and analyzed using Microsoft Excel 2010 software (Microsoft Corporation, Redmond, WA, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. Matrine inhibits cell viability in HCT8 and HT29 colon cancer cell lines

HCT8 and HT29 colon cancer cells were incubated with 1, 2, 3, 4 and 5 μM/ml concentrations of matrine for 12, 24, 48 and 72 h. The results from MTT assay revealed a concentration and time-dependent reduction in HCT8 and HT29 cell viability on treatment with matrine (Fig. 1). Although no significant reduction was observed at 1 μM/ml concentration but the effect was significant after 2 μg/ml ($P < 0.05$). The viability of HCT8 and HT29 cells was

reduced to 24.67 and 29.32% on treatment with 4 μM/ml concentration of matrine after 48 h.

3.2. Effects of matrine on cell cycle distribution in HCT8 and HT29 colon cancer cells

The effect of matrine on cell cycle distribution was analysed using 1 μM/ml concentration after 48 h. The results from flow cytometry showed a significant increase in the population of HCT8 and HT29 cells in G2/M phase at 48 h compared to the untreated control cells (Fig. 2). The population of HCT8 and HT29 cells in the G2/M phase at 48 h was 77.6 ± 0.3 and 54.0 ± 5.4%, respectively. In control cell cultures the population of HCT8 and HT29 cells was 1.4 ± 0.3 and 2.4 ± 0.7%, respectively at 48 h on treatment with 1 μM/ml concentration of matrine (Fig. 2).

3.3. Effects of matrine on expression of cell cycle regulatory proteins

We used western blot assay to analyse the effect of matrine (1 μM/ml) on expression of cell cycle regulatory proteins in HCT8 cells. The results revealed a significant increase in the histone H2AX phosphorylation in HCT8 cells on treatment with matrine compared to the control cells. The expression of Myt1, cyclin A2, cyclin B1 and p53 was increased in HCT8 cells on exposure to 1 μM/ml concentration of matrine for 48 h (Fig. 3). Matrine treatment also increased the phosphorylation of cdc2 significantly compared to control cells at 48 h ($P < 0.05$) (Fig. 3). Treatment of HCT8

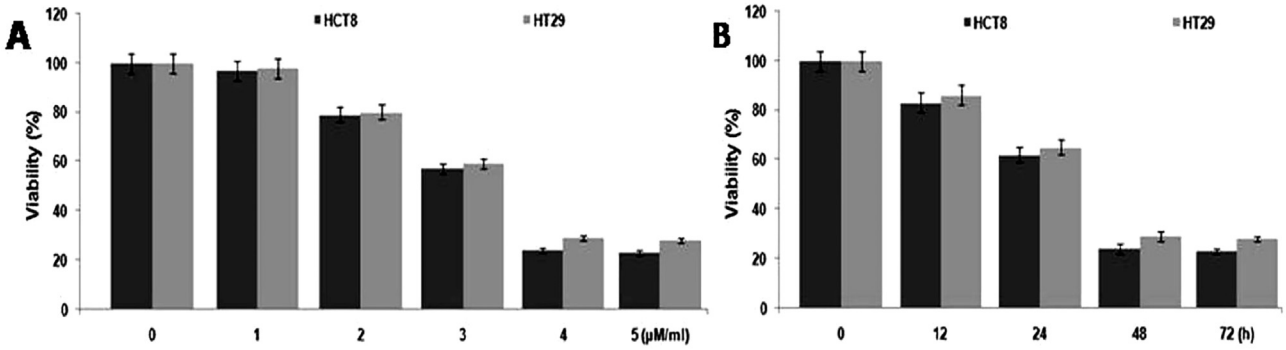

Fig. 1. Cell viability following matrine treatment. The cells were incubated with various concentrations of matrine (0, 1, 2, 3, 4 and 5 μM/ml) for 12, 24, 48 and 72 h. Cell viability was determined using an MTT assay. HCT8 and HT29 cell lines exhibited a significant reduction in viability, dependent on matrine treatment time and dose. The data are presented as the mean percentage of viable cells ± standard.

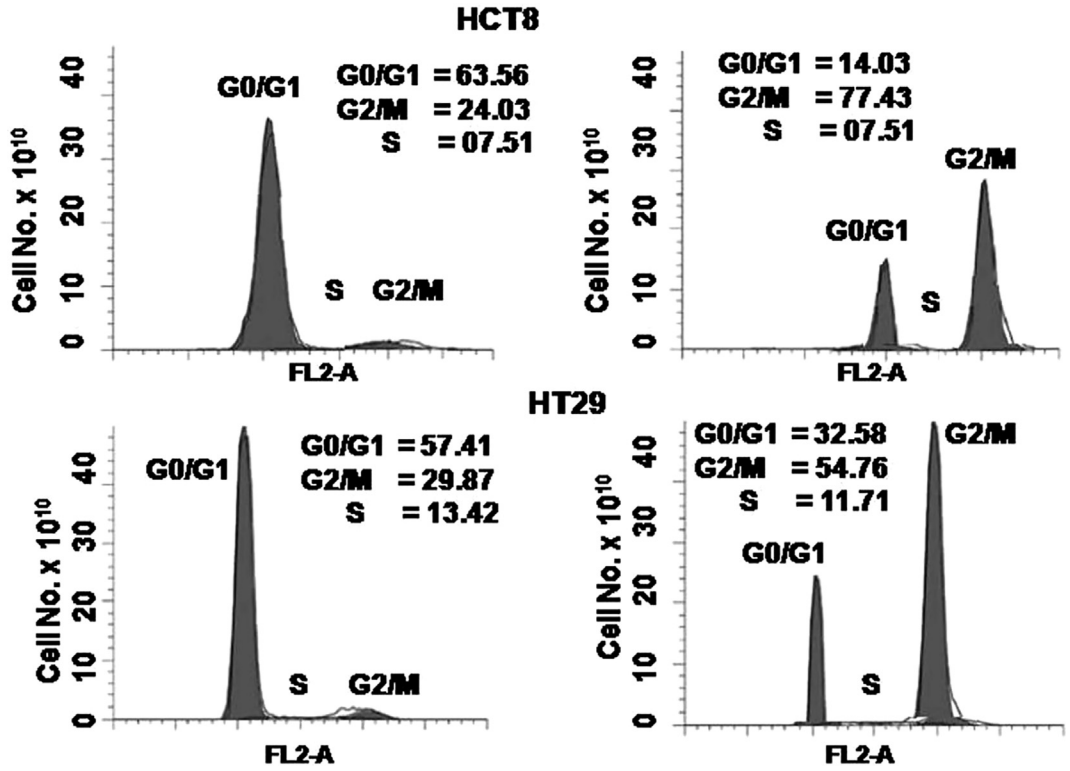

Fig. 2. Matrine induces G2/M phase arrest in oral squamous cell cancer cells. HCT8 and HT29 cell lines were incubated with 1 μM/ml concentration of matrine for 48, fixed and stained with propidium iodide, and analyzed for DNA content.

cells with matrine for 48 h reduced the expression level of phosphorylated histone H3 Ser 10 at 48 h ($P < 0.05$).

3.4. Matrine induces apoptosis in HCT8 and HT29 colon cancer cell lines

Apoptosis in HCT8 and HT29 cells by matrine was analysed using Annexin-V/FITC-staining after 48 h. The results revealed a concentration and time dependent increase in the proportion of apoptotic cells on treatment with matrine for 48 h (Fig. 4). The proportion of apoptotic cells in HCT8 cells was found to be 1.23, 11.64, 33.79, 67.52 and 68.23% on treatment with 1, 2, 3, 4 and 5 μM/ml concentration of matrine for 48 h. In HT29 cells, treatment with 1, 2, 3, 4 and 5 μM/ml concentration of matrine induced apoptosis in 2.06, 9.52, 29.32, 68.56 and 69.67% cells (Fig. 4).

3.5. Matrine inhibits in vivo colon tumor growth

The effect of matrine on *in vivo* tumor growth was evaluated in HCT8 cell xenografted nude mice model using the reported protocol (18). Matrine treatment at a concentration of 4 mg/kg daily caused a marked reduction in the growth of HCT8 cell xenograft

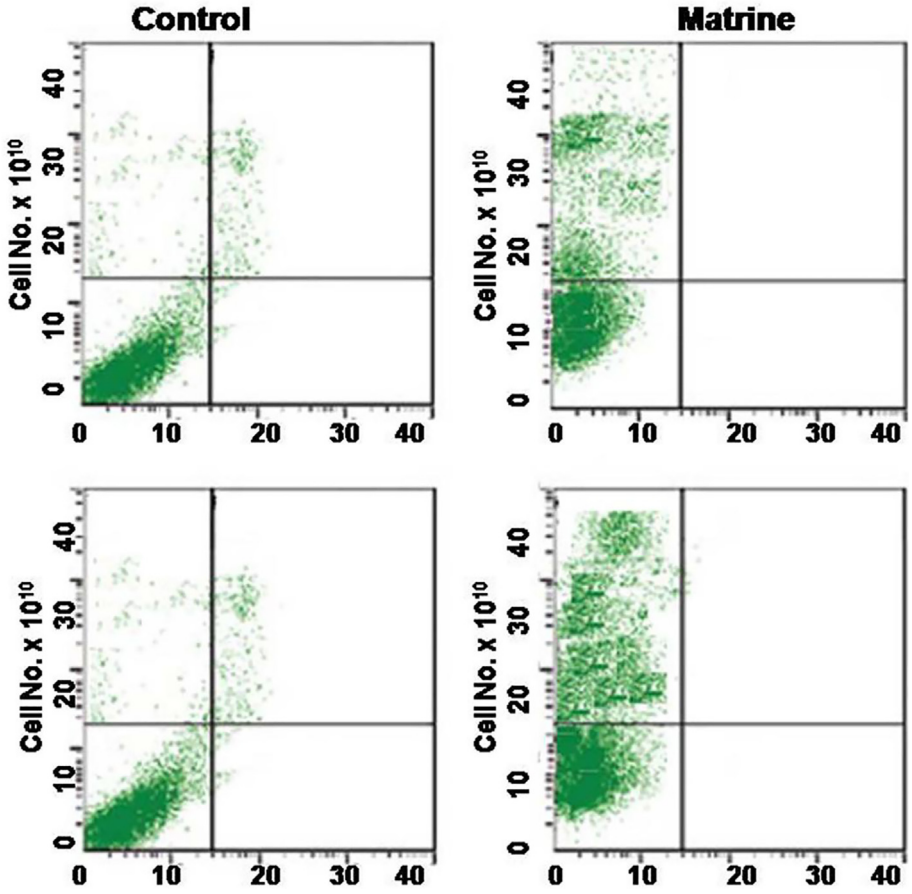

Fig. 3. Effects of matrine on cell cycle regulating molecules in HCT8 and HT29 cell lines. The cells were treated with 1 μM/ml concentration of matrine and the protein expression levels at 48 h following treatment were examined by western blot analysis.

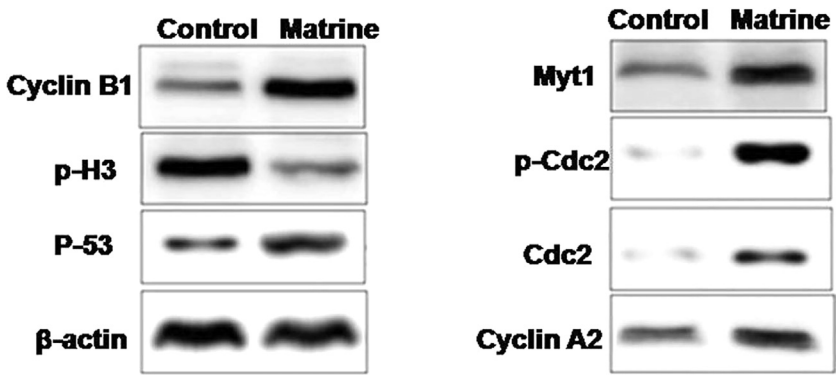

Fig. 4. Induction of apoptosis in matrine-treated HCT8 and HT29 cell lines. The cells were harvested following 48 h incubation with 1, 2, 3, 4 and 5 μM/ml concentrations of matrine. Apoptosis was determined by staining the cells with Annexin V-FITC and PI labeling. The percentage figure in each window indicates the proportion of Annexin V-FITC positive cells. FITC, fluorescein isothiocyanate; PI, propidium iodide.

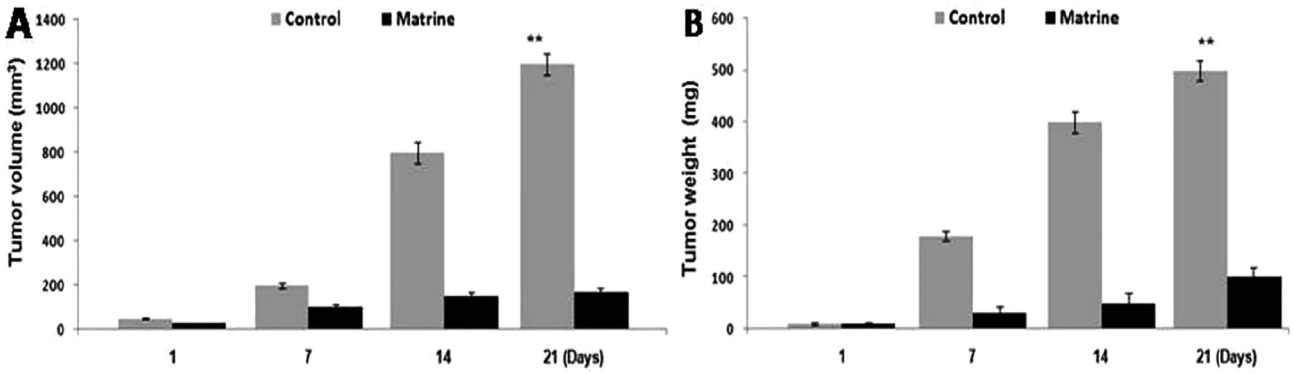

Fig. 5. Inhibition of tumor growth in HCT8 xenograft mice model by matrine treatment after 21 days. The mice were treated for 21 days with 4 mg/kg doses of matrine daily and tumor growth was examined.

after 21 days (Fig. 5A). The weight of tumor extracted from the mice with HCT8 xenograft after completion of treatment matrine was significantly lower compared to the control group (Fig. 5B).

4. Discussion

Present study investigates the effect of matrine on viability and induction of apoptosis in colon cancer cell lines and growth of tumor in HCT8 xenograft model. Matrine exhibits inhibitory effect on the cancer growth in gastric carcinoma, rhabdomyosarcoma and breast carcinoma (Li et al., 2012). The results showed a significant reduction in the cell viability in HCT8 and HT29 colon cancer cells on treatment with matrine in dose and time dependent manner. The effect of matrine on distribution of HCT8 and HT29 cells in various phases of cell cycle was also analysed. Our results showed a significant increase in the population of HCT8 and HT29 cells in G2/M phase at 48 h of matrine treatment.

Progress of cells to various phases of the cell cycle is regulated by the activation of different complexes of the cyclin and cyclin-dependent kinases. Studies have demonstrated that regulation of G2/M phase is associated with cyclin A- and cyclin B in combination with cdc2 as the activation of cdc2 Tyr 15 leads to inactivation of cdc2/cyclin B complex in G2 phase (Parker and Piwnica-Worms, 1992; Booher et al., 1997; Liu et al., 1997). Current study demonstrated a significant increase in the expression of Myt1, cyclin A2, cyclin B1 and p53 in both HCT8 and HT29 cells on exposure to matrine for 48 h. Matrine exposure also increased the phosphorylation of cdc2 significantly compared to control cells at 48 h ($P < 0.05$). It is reported that histone H2AX phosphorylation is an important checkpoint in the cells for entry to the mitosis (Van Hooser et al., 1998; Xi et al., 2015). Our results showed that matrine treatment promoted the activation of histone H2AX in HCT8 and HT29 cells after treatment for 48 h. The results from the present study induction of apoptosis in colon cancer cells in a concentration and time dependent manner on treatment with matrine for 48 h.

In conclusion, the present study demonstrated that matrine inhibits viability and induces apoptosis in colon cancer cells and inhibits tumor growth in tumor xenograft mice model. Thus matrine can be used for the treatment of colon cancer.

Conflict of interest

The authors declare no conflict of interest.

References

Ali, R., Mirza, Z., Ashraf, G.M., Kamal, M.A., Ansari, S.A., Damanhouri, G.A., Abuzenadah, A.M., Chaudhary, A.G., Sheikh, I.A., 2012. New anticancer agents: recent developments in tumor therapy. Anticancer Res. 32, 2999–3005.

Booher, R.N., Holman, P.S., Fattaey, A., 1997. Human Myt1 is a cell cycle-regulated kinase that inhibits Cdc2 but not Cdk2 activity. J. Biol. Chem. 272, 22300–22306.

Guo, L., Xue, T.Y., Xu, W., Gao, J.Z., 2012. Effects of matrine on the proliferation and apoptosis of human rhabdomyosarcoma RD cells. Zhongguo Dang Dai Er Ke Za Zhi 14, 780–784 (In Chinese).

Haiyan, W., Yuxiang, L., Linglu, D., Tingting, X., Yinju, H., Hongyan, L., Lin, M., Yuanxu, J., Yanrong, W., Jianqiang, Y., 2013. Antinociceptive effects of matrine on neuropathic pain induced by chronic constriction injury. Pharm. Biol. 51, 844–850.

Jones Jr., D.V., Winn, R.J., Brown, B.W., Levy, L.B., Pugh, R.P., Wade, J.L., Gross, H.M., Pendergrass, K.B., Levin, B., Abbruzzese, J.L., 1995. Randomized phase III study of 5-fluorouracil plus high dose folinic acid versus 5-fluorouracil plus folinic acid plus methyl-lomustine for patients with advanced colorectal cancer. Cancer 76, 1709–1714.

Li, L.Q., Li, X.L., Wang, L., Du, W.J., Guo, R., Liang, H.H., Liu, X., Liang, D.S., Lu, Y.J., Shan, H.L., Jiang, H.C., 2012. Matrine inhibits breast cancer growth via miR-21/PTEN/Akt pathway in MCF-7 cells. Cell. Physiol. Biochem. 30, 631–641.

Li, X., Zhou, R., Zheng, P., Yan, L., Wu, Y., Xiao, X., Dai, G., 2010. Cardioprotective effect of matrine on isoproterenol-induced cardiotoxicity in rats. J. Pharm. Pharmacol. 62, 514–520.

Li, Y., Wang, B., Zhou, C., Bi, Y., 2007. Matrine induces apoptosis in angiotensin II-stimulated hyperplasia of cardiac fibroblasts: effects on Bcl-2/Bax expression and caspase-3 activation. Basic Clin. Pharmacol. Toxicol. 101, 1–8.

Li, Y., Zhang, J., Ma, H., Chen, X., Liu, T., Jiao, Z., He, W., Wang, F., Liu, X., Zeng, X., 2013. Protective role of autophagy in matrine-induced gastric cancer cell death. Int. J. Oncol. 42, 1417–1426.

Liu, F., Stanton, J.J., Wu, Z., Piwnica-Worms, H., 1997. The human Myt1 kinase preferentially phosphorylates Cdc2 on threonine 14 and localizes to the endoplasmic reticulum and Golgi complex. Mol. Cell. Biol. 17, 571–583.

Long, Y., Lin, X.T., Zeng, K.L., Zhang, L., 2004. Efficacy of intramuscular matrine in the treatment of chronic hepatitis B. Hepatobiliary Pancreat. Dis. Int. 3, 69–72.

Parker, L.L., Piwnica-Worms, H., 1992. Inactivation of the p34cdc2-cyclin B complex by the human WEE1 tyrosine kinase. Science 257, 1955–1957.

Parkinson, D.R., Arbuck, S.G., Moore, T., Pluda, J.M., Christian, M.C., 1994. Clinical development of anticancer agents from natural products. Stem Cells 12, 30–43.

Siegel, R., Naishadham, D., Jemal, A., 2013. Cancer statistics, 2013. CA Cancer J. Clin. 63, 11–30.

Szoke, D., Gyorffy, A., Surowiak, P., Tulassay, Z., Dietel, M., Gyorffy, B., 2007. Identification of consensus genes and key regulatory elements in 5-fluorouracil resistance in gastric and colon cancer. Onkologie 30, 421–426.

Van Hooser, A., Goodrich, D.W., Allis, C.D., Brinkley, B.R., Mancini, M.A., 1998. Histone H3 phosphorylation is required for the initiation, but not maintenance, of mammalian chromosome condensation. J. Cell Sci. 111, 3497–3506.

Xi, S.-Y., Teng, Y.-H., Li, Jie.-p., Zhang, Y.-y., Liu, S.-l., Zou, X., Zhou, J.-y., Wu, J., Wang, R.-p., 2015. Jianpi Huayu Decoction Inhibits Proliferation in Human Colorectal Cancer Cells (SW480) by Inducing G0/G1-Phase Cell Cycle Arrest and Apoptosis. Evidence-Based Complementary and Alternative Medicine 2015. http://dx.doi.org/10.1155/2015/236506.

Zhang, S., Zhang, Y., Zhuang, Y., Wang, J., Ye, J., Zhang, S., Wu, J., Yu, K., Han, Y., 2012. Matrine induces apoptosis in human acute myeloid leukemia cells via the mitochondrial pathway and Akt inactivation. PLoS ONE 7, e46853.