Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Juglone Induces Michigan Cancer Foundation-7 Human Breast Cancer Cells Apoptosis through Bcl-2-Associated X protein/B-cell lymphoma/leukemia-2 Signal Way

Qing Luo, Kang Hu[1], Fang Chen, Feng-Jiao Gan[1], Yuan-Xiu Leng, Xu-Mei Chen, Su-Hong Sun[1]

Department of Oncology Laboratory, Affiliated Hospital of Zunyi Medical University, [1]Breast and Thyroid Disease Medical Center, Affiliated Hospital of Zunyi Medical University, Zunyi, China

Submitted: 01-12-2018 Revised: 02-01-2019 Published: 19-09-2019

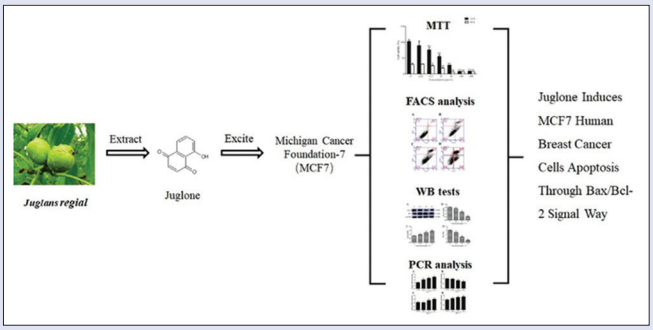
ABSTRACT

Background: Juglone is a natural pigment, which has a cytotoxic effect against tumor cells. However, its cytotoxicity to human breast cancer cells (Michigan Cancer Foundation-7 [MCF7]) has not been demonstrated. **Objective:** The objective was to observe the effect of Juglone on the protein expression of caspase-3, 9 in the apoptosis of Human Breast Cancer Cells (MCF7) and apoptosis-related protein, Bcl-2-associated X protein (Bax), and B-cell lymphoma-2 (Bcl-2) and investigate in the inhibitory effect of MCF7. **Materials and Methods:** Methyl thiazolyl tetrazolium experiment was performed for the detection of the cell growth inhibition effect of MCF7. The apoptosis rate was detected by flow cytometry. Western Blot was used to detect the protein expression of Bax, Bcl-2, and caspase. The messenger RNA (mRNA) was tested using quantitative real-time polymerase chain reaction. **Results:** Juglone inhibited the growth of MCF7 cells in a concentration- and time-dependent manner and promotes the apoptosis of MCF7 cells in a concentration-dependent manner. Compared with control group, the Juglone group raised the expression of Bax protein ($^*P < 0.05$), and the protein expression of Bcl-2 was decreased ($^*P < 0.05$). The expression of caspase-3 protein was not changed, and the caspase-9 was significantly elevated in the high concentration of Juglone. Cleaved caspase-3, -9 protein was significantly raised ($^*P < 0.05$). The mRNA levels of Bax, caspase-3, and caspase-9 in MCF7 cells of Juglone group were significantly increased compared with control, while the expression of Bcl-2 was suppressed obviously. **Conclusion:** Juglone inhibited the growth of MCF7 cells and promotes the apoptosis of MCF7 cells. Its mechanism in promoting MCF7 cell apoptosis may be related to the decrease of the expression of the mitochondrial pathway-associated apoptosis factor Bcl-2, increase of the protein expression of Bax, and mitochondrial pathway downstream caspase-3, 9.

Key words: Apoptosis, B-cell lymphoma/leukemia-2, Bcl-2-associated X protein, Juglone, Michigan Cancer Foundation-7

SUMMARY

- Juglone is an agonist of Bcl-2-associated X protein/B-cell lymphoma-2 pathway in Michigan Cancer Foundation-7 proliferative and that application of Juglone may be useful for the treatment of breast cancer and other cancers.



Abbreviations used: TCM: Traditional Chinese medicine; Pin1: Peptidyl-proly1 cis/trans isomerase; MCF7: Michigan Cancer Foundation-7; MTT: Methyl thiazolyl tetrazolium; FACS: Fluorescence-activated cell sorting; RT-PCR: Real-time polymerase chain reaction; WB: Western blot; Bcl-2: B-cell lymphoma/leukemia-2; Bax: Bcl-2-associated X protein.

Correspondence:

Dr. Su-Hong Sun,
Breast and Thyroid Disease Medical Center,
Affiliated Hospital of Zunyi Medical University, No. 149, Dalian Road, Zunyi, Guizhou Province, China.
E-mail: zyyxy_ssh@sina.com

DOI: 10.4103/pm.pm_604_18

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Cancer change over time through a process of cell-level evolution, dictated by genetic and epigenetic alterations.[1] Cancer is a part of the major problems affecting public health worldwide. As other cultures, the populations on medicinal herbs and their preparations to combat cancer.[2,3] Traditional Chinese Medicine (TCM) has been tailoring herbal mixtures for individualized health care for 2000 years. A systematic study of the patterns of TCM formulas and herbs prescribed for cancers is useful. Targeted cancer therapies, with specific molecular targets, ameliorate the side effect issue of radiation and chemotherapy and point to the development of personalized medicine. Combination of drugs targeting multiple pathways of carcinogenesis is potentially more fruitful.[4]

Breast cancer is the most common cancer in women, and it is the second leading cause of death in women after lung cancer.[5] *Juglans regia* has been found to exhibit significant anticancer activity against various human cancer cell lines. Juglone was an active chemical constituent which was isolated from *Juglans regia* had cytotoxic activity along with its various analogs against different human cancer cell lines.[6] Juglone

Cite this article as: Luo Q, Hu K, Chen F, Gan FJ, Leng YX, Chen XM, *et al.* Juglone induces Michigan Cancer Foundation-7 Human breast cancer cells apoptosis through Bcl-2-associated X protein/B-cell lymphoma/leukemia-2 signal way. Phcog Mag 2019;15:573-8.

has been extensively used as a chemotherapeutic agent in Chinese herbal medicine against various tumors, including prostate cancer[7] leukemia,[8] melanoma,[9] gastric cancer,[10] and pancreatic cancer.[11] Juglone, also named 5-hydroxy-1,4-naphthoquinone (molecular weight of 174.15Da), is an important component of herb *Juglans regia*. The present study suggested that Juglone can inhibit the growth of Michigan Cancer Foundation-7 (MCF7) cells and promotes the apoptosis of MCF7 cells. Its mechanism in promoting MCF7 cell apoptosis may be linked to the decrease of the expression of the mitochondrial pathway-associated apoptosis factor Bcl-2, increase of the protein expression of Bcl-2-associated X protein (Bax), and mitochondrial pathway downstream caspase-3,9. These findings have significant implications for the potential use of the promising Juglone as a therapeutic or prophylactic treatment of cancer in humans.

MATERIALS AND METHODS

Materials and reagents

All the chemicals and reagents used in this experiment were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cell culture

Human Caucasian breast adenocarcinoma (MCF7) cells provided from the American Type Culture Collection, Rockville, MD, USA. were grown in Dulbecco's Modified Eagle's medium (DMEM) with 10% fetal bovine serum (Atlanta Biological, Atlanta, GA, USA), L-glutamine, MEM nonessential amino acid solution, sodium bicarbonate, and penicillin G (100 U/mL), streptomycin (100 μg/mL), under the condition of 37°C in a humidified atmosphere containing 95% air and 5% CO_2. culture media were changed every 3–4 days, and doubling time was 36 h.

Methyl thiazolyl tetrazolium assay

A 15 × 10^3 MCF7 cells were loaded into a 96-well plate, and 200 μL of DMEM medium containing 7% serum was added. After 24-h incubation, 200 μL of various concentrations of the extract (0, 6.25, 12.5, 25, 50, 100, and 200 μmol/mL) dissolved in serum-free DMEM medium was added to the wells. The cells were separately incubated with different doses for 24 and 48 h. The supernatant from each well was removed after incubation. For each well, 500 μL of methyl thiazolyl tetrazolium (MTT) solution (5 mg/mL) was added and incubated for 3 h. The supernatant from each well was then added with 100 μL of dimethyl sulfoxide and kept at room temperature for 30 min. The optical density of each well was measured at 570 (for sample test and calculation) and 630 nm (as a reference wavelength). The viability of the cells for each concentration was calculated using the following formula:

Cell viability (%) = (A570 [sample]/A [control]) × 100

Fluorescence-activated cell sorting analysis

MCF7 cells were washed twice with cold phosphate-buffered saline and then resuspended in 1× binding buffer (0.01 M Hepes/NaOH (pH 7.4), 0.14 M NaCl, and 2.5 mM $CaCl_2$) at a concentration of 1 × 10^6 cells/mL. Transferred 100 μL of the solution (1 × 10^5 cells) to 5 mL culture tube. Added 5 μL of FITC Annexin V and 5 μL PI (BD Pharmingen, USA) into the tube. the cells were gently vortexed and incubated for 15 min at room temperature in the dark. Added 400 μL of 1× binding buffer to each tube and carried out fluorescence-activated cell sorting (FACS) by Guava® easyCyte™ flow cytometer (Millipore) within 1 h.

Western blot analysis

Cells were lysed with lysis buffer. Then, equal amount of proteins extracted from each sample were heat denatured and submitted to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 12% (v/v) polyacrylamide gel. After electrophoresis, proteins were electrotransferred onto polyvinylidene fluoride membranes. The membranes were blocked with 5% (w/v) skimmed milk at room temperature for 2 h and probed with primary antibody and at 4°C overnight. The primary antibodies used were Bax, Bcl-2, caspase-3,9, and cleaved caspase-3,9 (Proteintech Group, USA). After washing, membranes were incubated with the appropriate secondary antibody in 5% (w/v) skimmed milk/tris-buffered saline containing 0.05% Tween® 20 for 1 h at 4°C. Proteins were detected using SignalFire™ ECL Reagent (Cell Signaling Technology, USA).

Quantitative real-time polymerase chain reaction

Total RNA in cell was extracted using spin column (Shanghai Generay Biotech Co., Ltd., China) and reverse transcription into cDNA (TaKaRa, Japan) according to the manufacturer's protocol. The gene expression levels were determined by quantitative real-time polymerase chain reaction (RT-PCR) conducted using the Applied Biosystems ViiA™ 7 Real-Time PCR system (Life Technologies, USA). The primers used in the experiments were listed in Table 1. The cDNA in a 10 μL reaction volume was denatured at 95°C for 30s followed by 40 cycles of PCR stage (95°C, 5s, 60°C, 30s). β-actin was used as an internal control to normalize the expression levels of genes.

Statistical analysis

All values are presented as the mean ± standard error. One-way analysis of variance was performed by SPSS 16.0 software (SPSS Corp., Chicago, IL, USA), and differences between groups were defined as significant (*) when $P < 0.05$.

RESULTS

Michigan Cancer Foundation-7 viability

After incubation for 24 and 48 h, the mean viabilities of MCF7 cells revealed dose dependently differences between the Juglone and the control [Figure 1]. The cell viability was significantly reduced after incubated with 12.5, 25, and 50 μmol/L of Juglone compared with the control group. We found the cell viability of MCF7 with incubation time in 48 h was too low to be detected. In that case, 12.5, 25, and 50 μmol/L of Juglone concentrations and 24 h of incubation time were used in subsequent experiments.

Fluorescence-activated cell sorting analysis for Michigan Cancer Foundation-7

In the blank control group, the cell survival rate was up to 99% [Figure 2a]. After cocultured with Juglone, the survival rate of the cells was decreased and the apoptosis rate was increased. Meanwhile, with the increasing concentrations of drug serum, the

Table 1: The primers used in the experiments

mRNA	qRT-PCR primers	Sequence (5'-3')
Bcl-2	Forward primer	GGTGGGGTCATGTGTGTGG
	Reverse primer	CGGTTCAGGTACTCAGTCATCC
Bax	Forward primer	CCCGAGAGGTCTTTTTCCGAG
	Reverse primer	CCAGCCCATGATGGTTCTGAT
Caspase-3	Forward primer	GAAATTGTGGAATTGATGCGTGA
	Reverse primer	CTACAACGATCCCCTCTGAAAAA
Caspase-9	Forward primer	CTCAGACCAGAGATTCGCAAAC
	Reverse primer	GCATTTCCCCTCAAACTCTCAA

Bcl-2: B cell lymphoma-2; Bax: Bcl-2 associated X protein

cell apoptosis rates were increased successively. The apoptosis rate was 10.5%, 20.6%, and 62.2%, the early apoptosis rate was 6.8%, 9.8%, and 22.6%, and the late apoptosis rate was 3.7%, 10.8%, and 39.6%, respectively [Figure 2b-d], with the serum concentration of Juglone at 12.5, 25, and 50 μmol/L.

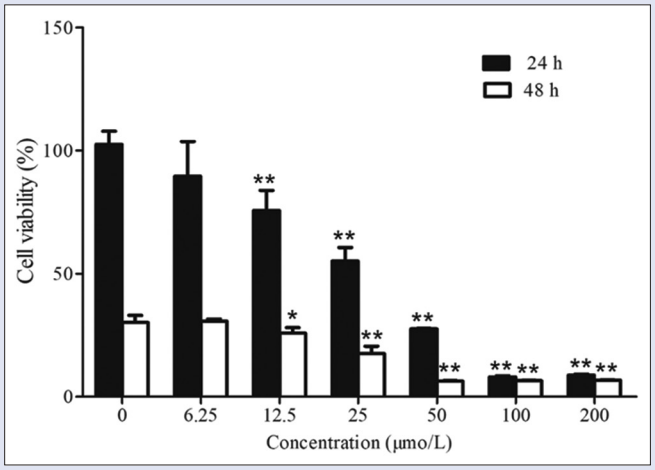


Figure 1: Michigan Cancer Foundation-7 viability (%) exposed to different concentrations and different incubation periods. Significant difference between groups, *$P < 0.05$; **$P < 0.01$

Juglone administration Michigan Cancer Foundation-7 protein expression

The Bax/Bcl-2 signaling way has been demonstrated to play an important role in MCF7 apoptosis. To determine the mechanism of Juglone on MCF7 apoptosis, we examined the effect of juglone on the expressive activity of Bax/Bcl-2 signaling way. Compared with the control group, the Bax protein expression showed a significant increase in the administrative groups (*$P < 0.05$) [Figure 3a and c]. The expression of Bax protein in the 50 μmol/L group, 25 μmol/L group, and 12.5 μmol/L group increased on the quantity–effect relationship. The expression level of Bcl-2 anti-apoptotic protein significantly decreased between the control group and the treated group (*$P < 0.05$) [Figure 3b]. Caspase-9, cleaved caspase-9, caspase-3, and cleaved caspase-3 were the critical proteins of apoptosis. The results were presented in Figure 4. With the increase of drug concentration, caspase 3/9 protein expressions were not obvious changed compared with the blank group, but the protein expressions of cleaved caspase-9 and cleaved caspase-3 increased successively, showing significant differences among all groups.

Quantitative real-time polymerase chain reaction

We evaluated the messenger RNA (mRNA) expressions of Bax, Bcl-2, caspase-3, and caspase-9 in MCF7 cells which deal with Juglone. The mRNA levels of Bax, caspase-3, and caspase-9 in MCF7 cells of juglone group were significantly increased compared with control, while the expression of Bcl-2 was suppressed obviously [Figure 5]. The changes of mRNA expression for the Bcl-2, Bax, caspase-3, and caspase-9 in the MCF7 showed a certain dose–effect relationship.

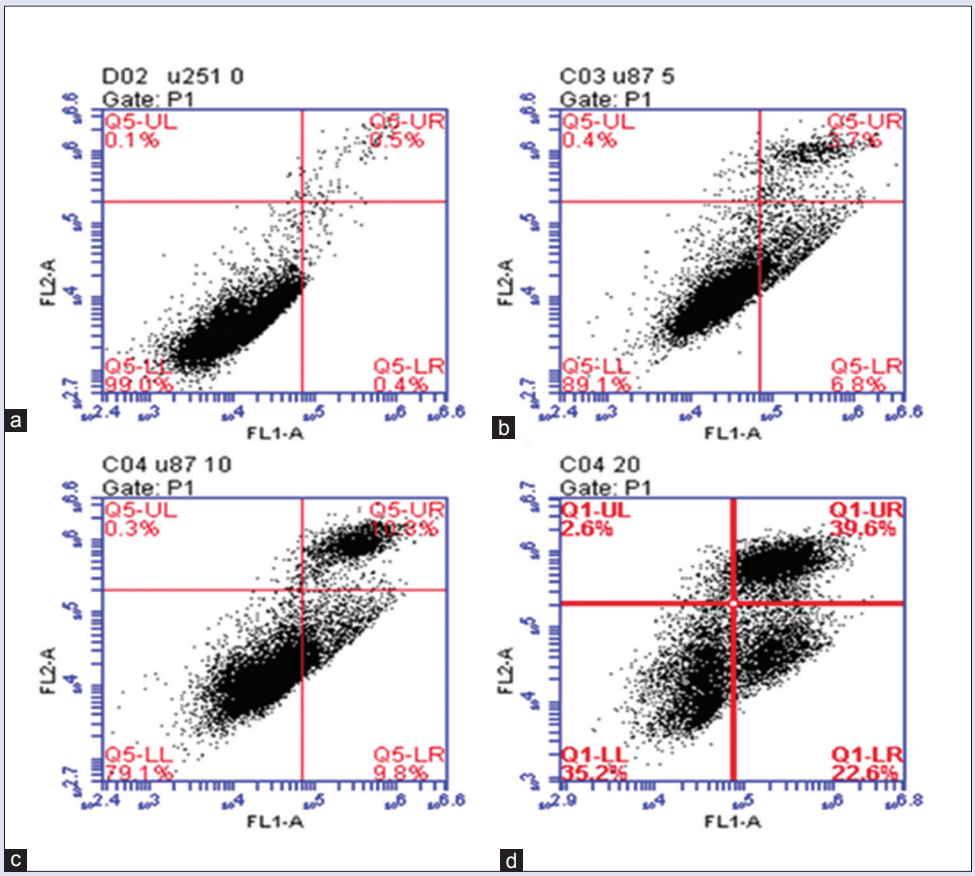


Figure 2: Evaluation of cell mortality in Dulbecco's Modified Eagle's medium cultures exposed to Juglone. (a) Fluorescence-activated cell sorting analysis of blank control group. (b-d) Fluorescence-activated cell sorting analysis of Michigan Cancer Foundation-7 in cultures exposed to Juglone of 12.5, 25, and 50 μmol/L

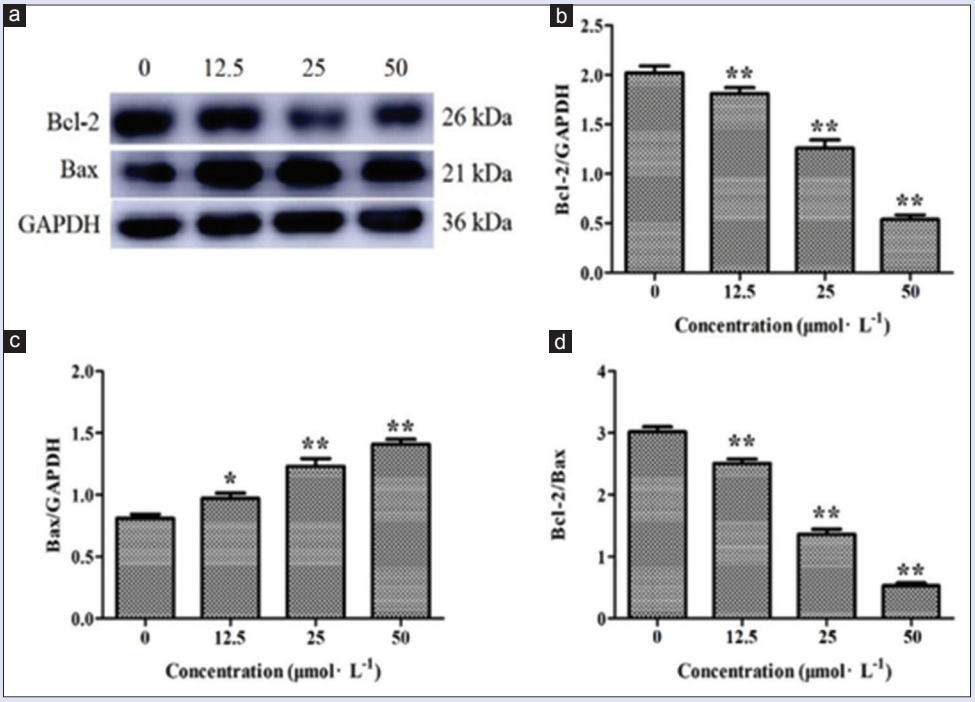


Figure 3: Effect of Juglone at different concentrations on the expression of B-cell lymphoma-2-associated X protein (a, c), and B-cell lymphoma-2, anti--apoptotic protein (b) in Michigan Cancer Foundation-7 cells. The data were expressed as mean ± standard error per group. *$P < 0.05$ versus the blank group, **$P < 0.01$ versus the blank group

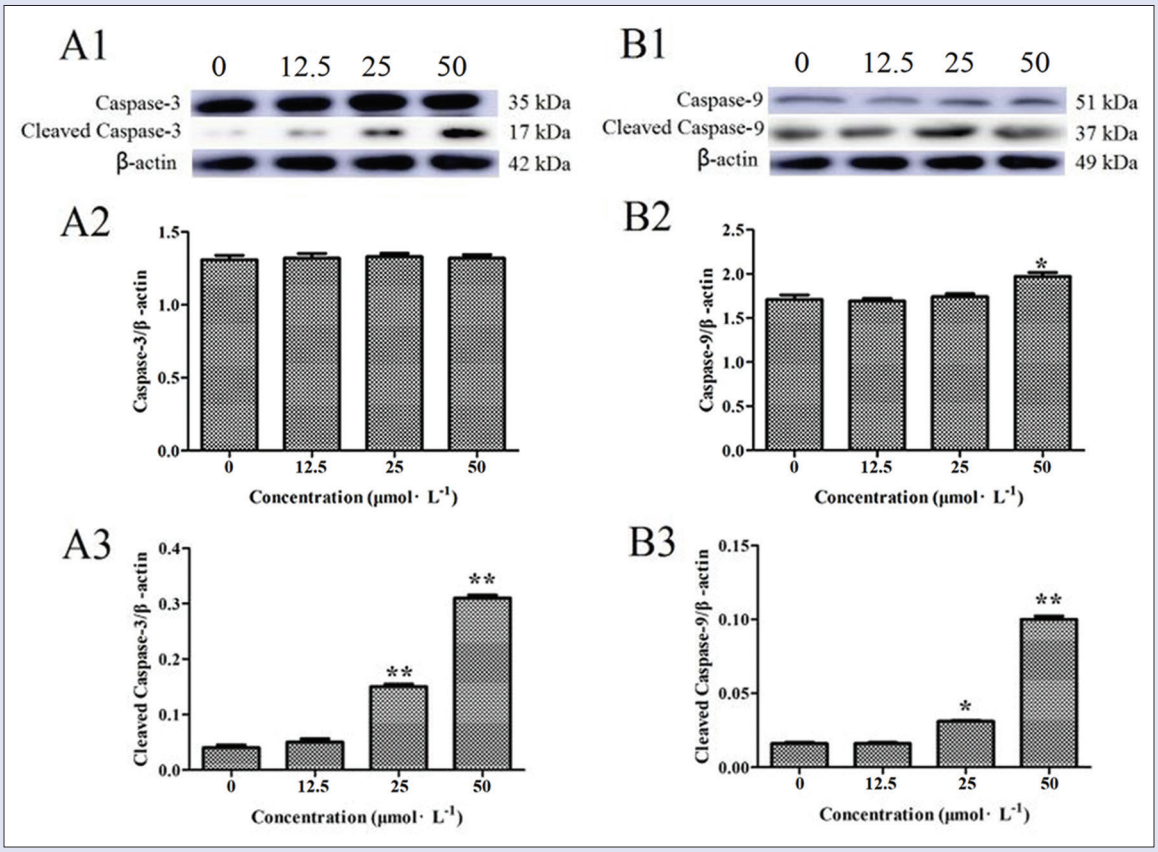


Figure 4: Effect of Juglone at different concentrations on the expression of caspase 3 (A1, A2)/9 (B1, B2) and cleaved caspase 3 (A3)/9 (B3) protein in Michigan Cancer Foundation-7 cells. The data were expressed as mean ± standard error per group. *$P < 0.05$ versus the blank group, **$P < 0.01$ versus the blank group

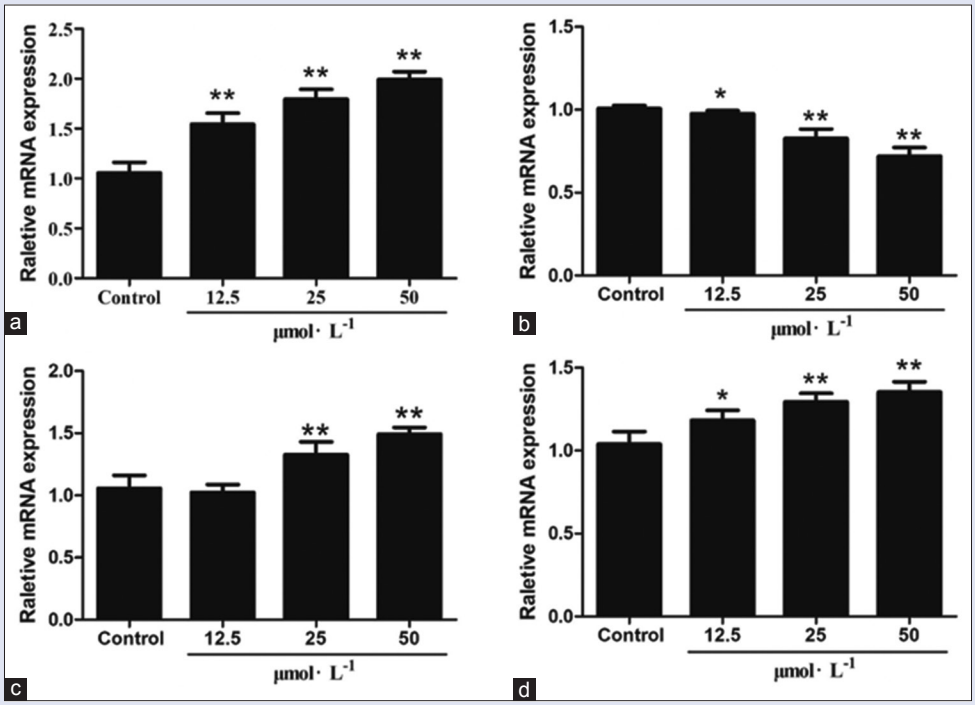


Figure 5: Juglone promoted apoptosis of Michigan Cancer Foundation-7 on the B-cell lymphoma-2-associated X protein (a), B-cell lymphoma-2 (b), caspase-3 (c), caspase-9 (d), and in Michigan Cancer Foundation-7 mRNA expression levels of genes in Michigan Cancer Foundation-7. β-actin was used as the internal control. The data were expressed as mean ± standard error per group. **P* < 0.05 versus the blank group, ***P* < 0.01 versus the blank group

DISCUSSION

Juglone (5-hydroxy-1,4-naphthoquinone) is a natural compound isolated from *Juglans mandshurica* and *Juglans regia*.[6,12] Previous studies have shown that it have diverse biological effects such as antiviral, antibacterial, and anticancer properties.[13,14] Furthermore, numerous reports have demonstrated that it irreversibly inhibits the enzymatic activity of peptidyl-prolyl cis/trans isomerase (Pin1).[12,15,16] Juglone could inhibit tumor stem-like cells growth in gliomas through the activation of Reactive oxygen species-p38-mitogen activated protein kinase (ROS-p38-MAPK) pathway *in vitro*.[17] Juglone inhibited U251 human glioma cell line migration and the formation of new blood vessels. At the molecular level, Juglone markedly suppressed Pin1 levels in a time-dependent manner by transforming growth factor beta 1/Smad signaling, a critical upstream regulator of miR-21.[16] Juglone as a Pin1 inhibitor to inhibition suppresses proliferation of LNCaP (androgen-dependent) and DU145 (androgen-independent).[15] Pin1 is an enzyme that specifically binds to the motifs containing phosphorylated serine or threonine, immediately preceding proline, in numerous proteins. The association with Pin1 promotes cis/transisomerization of the peptide bond.[15] Pin1 was associated with many cellular processes, the aberrance of which lead to both degenerative and neoplastic diseases. Pin1 in diverse cancer capabilities and certain Pin1-targeted small-molecule compounds that exhibit anticancer activities, expecting to facilitate anticancer therapies by targeting Pin1.[18] The previous study has shown that ERα protein is posttranslationally regulated by Pin1 in a proportion of breast carcinomas. Since Pin1 impacts both ERα protein levels and transactivation function, these data implicate Pin1 as a potential surrogate marker for predicting outcome of ERα-positive breast cancer.[19] These previous findings indicate that Juglone potential effectiveness of Pin1 inhibitors as therapeutic agents for different cancers.

Bax is a fellow of a larger gene family based on amino acid domain homologies found in the prototypical member.[20] Proapoptotic Bcl-2 protein Bax is normally in a dynamic equilibrium between cytosol and mitochondria, enabling fluctuations in survival signals to finely adjust apoptotic sensitivity and translocate to mitochondria following an apoptotic stimulus.[21] In this study, MTT assay and FACS analysis revealed that Juglone promoted apoptosis of MCF7, and WB detection showed that Juglone increased the content of Bax protein in MCF7 cells. Meanwhile, the mRNA expression of Bax in MCF7 cells was raised. Bcl-2, the founding member of a family of apoptotic regulators, confers resistance to apoptotic stimuli. Analogous mechanisms, i.e., genomic changes that enhance expression, altered regulation by miRNAs, and diminished ubiquitin-mediated turnover, contribute to upregulation of the other anti-apoptotic Bcl-2 family members in various cancers as well.[22] In this research, the protein and mRNA expression of Bcl-2 in MCF7 was decreased. Overexpressed Bax also counters the death repressor activity of Bcl-2. The results suggest a model in which the ratio of Bcl-2 to Bax determines survival or death following an apoptotic stimulus.[23] Therefore, we also calculated the ratio of Bcl-2 to Bax and found that the ratio decreased with the increase of Juglone concentration and presented a dose–effect relationship. Therefore, Juglone-induced apoptosis of MCF7 cells by affection Bcl-2/Bax [Figure 3d].

Caspases are proteases with a well-defined role in apoptosis. However, increasing evidence indicates multiple functions of caspases outside apoptosis.[24] Caspase-3 has long been recognized as the key proteases involved in cell demolition during apoptosis. Caspase-9 is the apoptotic initiator protease of the intrinsic or mitochondrial apoptotic pathway, which is activated at multiprotein activation platforms. Caspase activation also modulates signal transduction inside cells, through activation or inactivation of kinases, phosphatases, and other signaling molecules.[25,26] Essential to make sure that caspase-3 is fully activated to indicate that apoptosis has occurred.[27] In this research, we found

that protein concentration of caspase-3 was not changed by different concentrations of Juglone, while the protein concentration of caspase-9 was increased at high concentrations of Juglone. The concentration of cleaved caspase-3/9 proteins increased with the increase of Juglone concentration and presented a dose-effect relationship. Through Q-PCR detection, it was found that there was a positive ratio between the mRNA content of caspase-3,9 and the concentration of Juglone and showed a certain amount of dose–effect relationship.

CONCLUSION

MTT assay and FACS assay showed that Juglone significantly inhibited the proliferation of MCF7. Its function is to increase the Bax gene and protein expression and inhibiting the Bcl-2 gene and protein expression at the same time. Meanwhile, the gene and protein expression of caspase-3,9 are increased. The results showed that Juglone had the ability to treat breast cancer, and the effect of Juglone on Bax/Bcl-2 pathway indicated that Juglone might also have the ability to treat other cancers.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (No. 81860469); University Excellent Science and Technology Innovation Talent Support Plan Fund of Guizhou Province (Qian Jiao He KY zi [2015]495); The Key Technologies R and D Program of Huichuan Zunyi (Zun Hui Ke He [2014]30; and The Key Technologies R and D Program of Guizhou (Qian Ke He LH zi[2016]7479); Project of Science and Technology, Department of Guizhou Province (Qian Ke He LH[2015]7485).

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Maley CC, Aktipis A, Graham TA, Sottoriva A, Boddy AM, Janiszewska M, *et al.* Classifying the evolutionary and ecological features of neoplasms. Nat Rev Cancer 2017;17:605-19.
2. Abu-Darwish MS, Efferth T. Medicinal plants from near east for cancer therapy. Front Pharmacol 2018;9:56.
3. Pan SY, Zhou SF, Gao SH, Yu ZL, Zhang SF, Tang MK, *et al.* New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. Evid Based Complement Alternat Med 2013;2013:627375.
4. Chiu PH, Hsieh HY, Wang SC. Prescriptions of traditional Chinese medicine are specific to cancer types and adjustable to temperature changes. PLoS One 2012;7:e31648.
5. Han X, Deng S, Wang N, Liu Y, Yang X. Inhibitory effects and molecular mechanisms of tetrahydrocurcumin against human breast cancer MCF-7 cells. Food Nutr Res 2016;60:30616.
6. Zhang XB, Zou CL, Duan YX, Wu F, Li G. Activity guided isolation and modification of juglone from *Juglans regia* as potent cytotoxic agent against lung cancer cell lines. BMC Complement Altern Med 2015;15:396.
7. Fang F, Chen S, Ma J, Cui J, Li Q, Meng G, *et al.* Juglone suppresses epithelial-mesenchymal transition in prostate cancer cells via the protein kinase B/glycogen synthase kinase-3β/Snail signaling pathway. Oncol Lett 2018;16:2579-84.
8. Xu HL, Yu XF, Qu SC, Qu XR, Jiang YF, Sui da Y. Juglone, from *Juglans mandshurica* maxim, inhibits growth and induces apoptosis in human leukemia cell HL-60 through a reactive oxygen species-dependent mechanism. Food Chem Toxicol 2012;50:590-6.
9. Liu X, Chen Y, Zhang Y, Du J, Lv Y, Mo S, *et al.* Juglone potentiates TRAIL-induced apoptosis in human melanoma cells via activating the ROS-p38-p53 pathway. Mol Med Rep 2017;16:9645-51.
10. Benites J, Toledo H, Salas F, Guerrero A, Rios D, Valderrama JA, *et al. In vitro* inhibition of *Helicobacter pylori* growth by redox cycling phenylaminojuglones. Oxid Med Cell Longev 2018;2018:1618051.
11. Avcı E, Arıkoğlu H, Erkoç Kaya D. Investigation of juglone effects on metastasis and angiogenesis in pancreatic cancer cells. Gene 2016;588:74-8.
12. Costantino S, Paneni F, Lüscher TF, Cosentino F. Pin1 inhibitor juglone prevents diabetic vascular dysfunction. Int J Cardiol 2016;203:702-7.
13. Jha BK, Jung HJ, Seo I, Suh SI, Suh MH, Baek WK. Juglone induces cell death of *Acanthamoeba* through increased production of reactive oxygen species. Exp Parasitol 2015;159:100-6.
14. Fang F, Qin Y, Qi L, Fang Q, Zhao L, Chen S, *et al.* Juglone exerts antitumor effect in ovarian cancer cells. Iran J Basic Med Sci 2015;18:544-8.
15. Kanaoka R, Kushiyama A, Seno Y, Nakatsu Y, Matsunaga Y, Fukushima T, *et al.* Pin1 inhibitor juglone exerts anti-oncogenic effects on LNCaP and DU145 cells despite the patterns of gene regulation by pin1 differing between these cell lines. PLoS One 2015;10:e0127467.
16. Wang J, Liu K, Wang XF, Sun DJ. Juglone reduces growth and migration of U251 glioblastoma cells and disrupts angiogenesis. Oncol Rep 2017;38:1959-66.
17. Wu J, Zhang H, Xu Y, Zhang J, Zhu W, Zhang Y, *et al.* Juglone induces apoptosis of tumor stem-like cells through ROS-p38 pathway in glioblastoma. BMC Neurol 2017;17:70.
18. Chen Y, Wu YR, Yang HY, Li XZ, Jie MM, Hu CJ, *et al.* Prolyl isomerase pin1: A promoter of cancer and a target for therapy. Cell Death Dis 2018;9:883.
19. Rajbhandari P, Schalper KA, Solodin NM, Ellison-Zelski SJ, Ping Lu K, Rimm DL, *et al.* Pin1 modulates ERα levels in breast cancer through inhibition of phosphorylation-dependent ubiquitination and degradation. Oncogene 2014;33:1438-47.
20. Maes ME, Schlamp CL, Nickells RW. BAX to basics: How the BCL2 gene family controls the death of retinal ganglion cells. Prog Retin Eye Res 2017;57:1-25.
21. Schellenberg B, Wang P, Keeble JA, Rodriguez-Enriquez R, Walker S, Owens TW, *et al.* Bax exists in a dynamic equilibrium between the cytosol and mitochondria to control apoptotic priming. Mol Cell 2013;49:959-71.
22. Correia C, Lee SH, Meng XW, Vincelette ND, Knorr KL, Ding H, *et al.* Emerging understanding of Bcl-2 biology: Implications for neoplastic progression and treatment. Biochim Biophys Acta 2015;1853:1658-71.
23. Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes *in vivo* with a conserved homolog, bax, that accelerates programmed cell death. Cell 1993;74:609-19.
24. Shalini S, Dorstyn L, Dawar S, Kumar S. Old, new and emerging functions of caspases. Cell Death Differ 2015;22:526-39.
25. Boland K, Flanagan L, Prehn JH. Paracrine control of tissue regeneration and cell proliferation by caspase-3. Cell Death Dis 2013;4:e725.
26. Kim B, Srivastava SK, Kim SH. Caspase-9 as a therapeutic target for treating cancer. Expert Opin Ther Targets 2015;19:113-27.
27. Crowley LC, Waterhouse NJ. Detecting cleaved caspase-3 in apoptotic cells by flow cytometry. Cold Spring Harb Protoc 2016;11:1064-72.