International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2019.844.850

Research Article
Inhibition of U87 Glioma Cell Growth by Baicalein Through Apoptosis Induction and Cell Cycle Arrest

[1]Yonghai Zhang, [2]Junzhao Li, [3]Xianfeng Zhang, [1]Guangzhong Gao, [1]Jun Lu, [1]Qin Zhang and [1]Yasuo Ding

[1]Department of Neurosurgery, Jiangsu Taizhou People's Hospital, 225300 Taizhou, Jiangsu, China
[2]Department of Paediatrics, Jiangsu Taizhou People's Hospital, 225300 Taizhou, Jiangsu, China
[3]Department of Neurosurgery, The First Hospital of Jilin University, 130000 Changchun, Jilin, China

Abstract

Background and Objectives: Malignant glioma is the frequently detected brain tumor with high morbidity and mortality rates. The present study investigated the effect of baicalein, a chemical constituent of *Scutellaria baicalensis* on glioma cell proliferation. **Materials and Methods:** The MTT assay was used to determine changes in C6 and U87 glioma cell proliferation by baicalein. Apoptosis induction in U87 cells by baicalein treatment was assessed by commercially available Annexin V-FITC Detection kit (BD Biosciences, San Jose, CA, USA). Effect of baicalein on Mcl-1, caspase-3, PARP and cytochrome c proteins was analyzed by western blotting. **Results:** The results revealed that baicalein exposure of C6 and U87cells significantly ($p<0.05$) decreased cell viability. Baicalein treatment of U87 cells caused significant enhancement in apoptotic cell percentage and lead to release of cytochrome c from mitochondria. Its exposure caused cleavage of caspase-3, enhanced PARP activation and increased percentage of cells in G2/M phase. Baicalein treatment caused concentration dependent decrease in myeloid cell leukemia-1 (Mcl-1) protein expression in U87 cells. **Conclusion:** The study concluded that baicalein treatment inhibits glioma cell viability through induction of apoptosis and arrest of cell cycle. It inhibited Mcl-1 and anti-apoptotic protein expression. Therefore, baicalein can be of immense significance for glioma treatment.

Key words: Brain tumor, glioma treatment, infiltration, cell proliferation, anti-apoptotic, Malignant glioma, *Scutellaria baicalensis*

Citation: Yonghai Zhang, Junzhao Li, Xianfeng Zhang, Guangzhong Gao, Jun Lu, Qin Zhang and Yasuo Ding, 2019. Inhibition of U87 glioma cell growth by baicalein through apoptosis induction and cell cycle arrest. Int. J. Pharmacol., 15: 844-850.

Corresponding Author: Junzhao Li, Department of Paediatrics, Jiangsu Taizhou People's Hospital, 225300 Taizhou, Jiangsu, China
Tel/Fax: 0086-0523-86606022

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

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

INTRODUCTION

Malignant glioma is the frequently detected tumor of brain and has high morbidity and mortality rate[1]. Although gliomas do not undergo metastasis to distant organs, but usually infiltrate into local organs and invade tissues of the adjacent organs. Invasion of the glioma to adjacent tissues is responsible for its poor prognosis rate[2]. The glioma treatment at present involves use of radio and chemotherapy in combination as well as surgical resection[3]. Although, huge advancement has been made in the fields of radiotherapy and surgical techniques but satisfactory treatment strategy for glioma is yet to be developed. Natural product chemistry has provided many molecules that efficiently inhibit growth of glioma cells *in vitro*. Most potent among these compounds have been investigated in detail against glioma growth and are presently in clinical trials stage[3-5].

The major limitation to currently available treatment strategies is the extreme resistance of the glioma cells to the apoptotic stimuli[6,7]. The higher resistance of glioma cells to the treatment strategy is believed to be associated with the over-expression of anti-apoptotic proteins[7,8]. Some of the anti-apoptotic proteins over expressed in glioma cells include Mcl-1, Bcl-2 and Bcl-xL[7,8]. Mcl-1 is an anti-apoptotic protein which is differentiated from the proteins of Bcl-2 family by its ability to resist various types of apoptotic stimuli[9]. It has been found that Mcl-1 plays an important role for the multiple myeloma carcinoma cell survival and proliferation[10]. Studies have shown compounds that increase pro-apoptotic protein expression and inhibit of anti-apoptotic proteins like Bcl-xL, Bcl-2 and Mcl-1 can be therapeutic importance for glioma treatment[11-14].

In traditional Chinese medicine the plant *Scutellaria baicalensis* belonging to Labiatae family has been used for the treatment of various disease such as heart and bleeding disorders[15,16]. As a component of combination therapies *Scutellaria baicalensis* has been found to be great significance for the treatment of a number of diseases[17,18]. Taking into consideration the therapeutic significance the plant was subjected to phytochemical investigation which lead to isolation of baicalein and many other compounds[19,20]. Previous study showed that baicalein treatment inhibits the proliferation of lung squamous carcinoma cells through cell cycle arrest in S-phase and by the induction of apoptosis[21]. The current study was the first to describe the effect of baicalein on growth of glioma cells. The present study was designed to investigate the effect of baicalein on the glioma cell viability and expression of apoptosis related protein. The study demonstrated that baicalein exposure of glioma cells lead to apoptosis induction and arrest of cell cycle in G0/G1 phase.

MATERIALS AND METHODS

Cell lines and culture: The C6 and U87 glioma cell lines were supplied by the Cell Bank of the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The cell culture was performed in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal calf serum (Gibco; Thermo Fisher Scientific, Inc.). The medium was mixed with antibiotics, penicillin (100 U mL^{-1}) and streptomycin (100 μg mL^{-1}). The cell culture was carried out under humidified atmosphere of 5% CO_2 in an incubator at 37°C. The study was performed over 1 year and 7 months, i.e., from May, 2017-November, 2018.

3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay: The C6 and U87 cells at 1×10^5 cells per well density were distributed into 96-well microtiter plates in RPMI-1640 medium. The cells were cultured overnight and then incubated for 24 h with 1, 2, 3, 4, 5 and 6 μM concentrations of baicalein or dimethyl sulfoxide (control). The MTT (Sigma-Aldrich, St. Louis, MO, USA) solution (5 mg mL^{-1}) was added to each well of the plate and incubation at 37°C was continued for 4 h more. The medium was discarded and dimethyl sulfoxide was added to the plates for dissolving any formazan crystals formed. The absorbance measurements were performed for each plate three times independently at 485 nm using a microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Analysis of apoptosis: Apoptosis induction in U87 cells by baicalein treatment was determined by using the commercially available Annexin V-FITC Detection kit (BD Biosciences, San Jose, CA, USA)[21]. The cells were cultured for 24 h at a density of 2×10^6 cells/well into the 6 well plates and grown for 24 h. The medium in the plates was then replaced with fresh medium containing various concentrations of baicalein or dimethyl sulfoxide alone (as control) and cultured for 24 h. Then ice-cold PBS was used for washing the cells twice and the cells were re-suspended in 100 μL of the binding buffer. The cells were subjected to incubation at room temperature for 20 min with 5 μL of Annexin V-fluorescein isothiocyanate (FITC; BD Biosciences) and 10 μL propidium iodide (PI; BD Biosciences).

Analysis of cell cycle: The U87 cells at 2×10^6 density were distributed into the 10 cm culture dishes containing RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.)[21]. The cells were grown for 24 h and then treated with various concentrations of baicalein or dimethyl sulfoxide alone (as control) for 24 h. After baicalein treatment cells were harvested and then fixed for 24 h in 70% ethyl alcohol at -2°C. The cells washed with PBS were treated with 5% PI solution for staining. Distribution of the cells in various phases were determined by FACS Calibur flow cytometer using Cell Quest software Pro (5.1 version; BD Biosciences, Franklin Lakes, NJ, USA). The data was analyzed by Mod Fit LT software package (version 2.0; Verity Software House, Inc., Topsham, ME, USA).

Western blot analysis: The U87 cells after 24 h incubation with baicalein were lysed over 10 min using ReadyPrep Protein Extraction kit (Bio-Rad Laboratories, Inc.)[21]. The lysate was centrifuged for 20 min at 12,000 xg to separate the cell debris and collect the supernatant. The protein separation was performed by gel electrophoresis using 10% sodium dodecyl sulfate polyacrylamide and the specific proteins were subsequently transferred onto polyvinylidene fluoride membranes (Roche Diagnostics, Basel, Switzerland). The membrane non-specific sites were blocked by 1 h treatment with 0.05% Tween-20 and 5% skim milk in PBS at room temperature. The membranes were washed three times with Tris-buffered saline followed by overnight incubation at 4°C with primary antibodies against Mcl-1, caspase-3, PARP and cytochrome c (all from Sigma). The membranes after washing were exposed to goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (KPL, Inc., Gaithersburg, MD, USA) at room temperature for 1 h. The detection of immune reactive bands was performed using the enhanced chemiluminescence system (GE Healthcare Life Sciences, Little Chalfont, UK).

Statistical analysis: The values are presented as the Mean±SD of three independently performed experiments. Statistical analysis of the data was carried out using SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA). Data was analyzed by One-way analysis and the Student-Newman-Keuls method. The $p<0.05$ was considered to indicate a statistically significant difference.

RESULTS

Glioma cell viability reduction on exposure to baicalein: The C6 and U87 cell lines were exposed for 24 h to dimethyl sulfoxide or 1, 2, 3, 4, 5 and 6 μM concentrations of baicalein (Fig. 1). The viability of C6 and U87 cells kept on decreasing with the increase in baicalein concentration up to 5 μM and then remained constant. On exposure to 4, 5 and 6 μM concentrations of baicalein the viability of C6 cells was recorded as 44.46, 27.75 and 27.73, respectively. Similarly, for U87 cells viability was measured as 47.23, 29.71 and 29.65 on exposure to 4, 5 and 6 μM concentrations of baicalein, respectively.

Baicalein exposure lead to apoptosis of glioma cells: To investigate baicalein mediated reduction of glioma cell viability, U87 cells exposed for 24 h to baicalein were examined for apoptosis. Increasing the concentration of baicalein from 2-5 μM significantly enhanced apoptotic cell proportion in U87 cell cultures (Fig. 2).

Baicalein exposure of U87 cells causes activation of apoptotic proteins: Baicalein exposure of U87 cells lead to cleavage of caspase-3 in concentration based manner (Fig. 3). The caspase-3 activation in U87 cells by baicalein was significant from 3 μM concentration at 24 h. Compared to untreated cells the activation of caspase-3

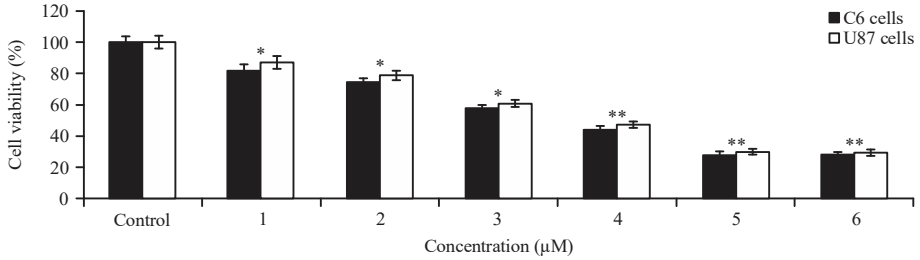

Fig. 1: Baicalein suppresses glioma cell viability

Exposure of C6 and U87 cells to baicalein or DMSO for 24 h was followed by measurement of viability by MTT method. For both cell lines viability measurements were performed in triplicates for each concentration of baicalein, Values presented are Mean±SD of the triplicates measurements, *$p<0.05$, **$p<0.02$ vs. control cells

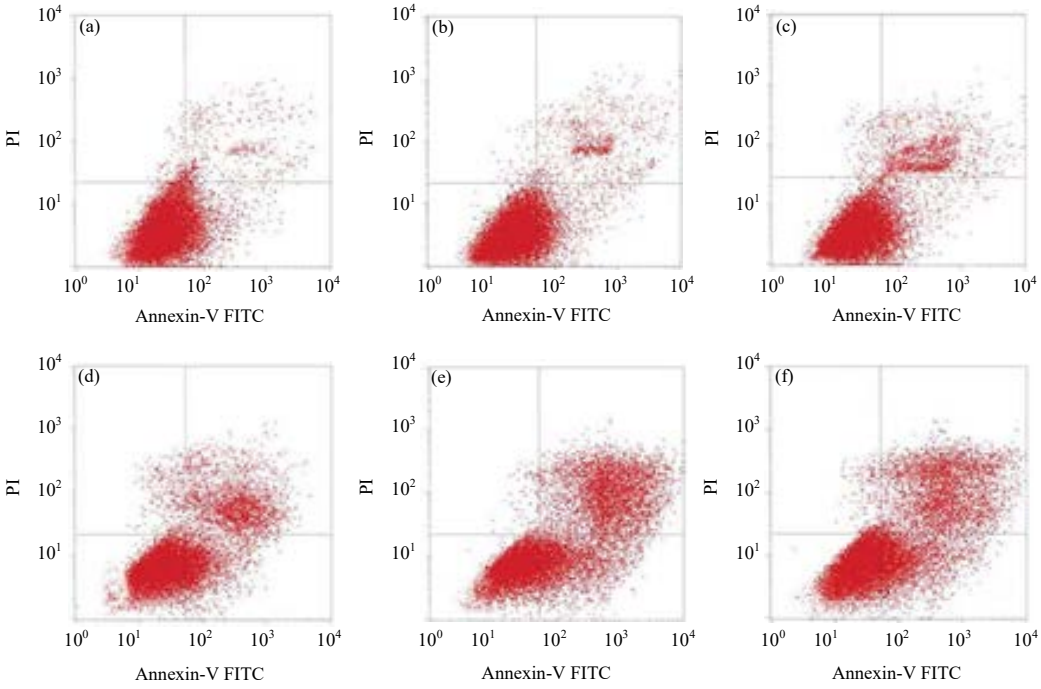

Fig. 2(a-f): Apoptosis induction in U87 cells by baicalein, the cells were exposed to various concentrations of baicalein for 24 h and then analyzed by flow cytometry for apoptosis induction, (a) Control, (b) 1 μM, (c) 2 μM, (d) 3 μM, (e) 4 μM and (f) 5 μM

PI: Propidium iodide

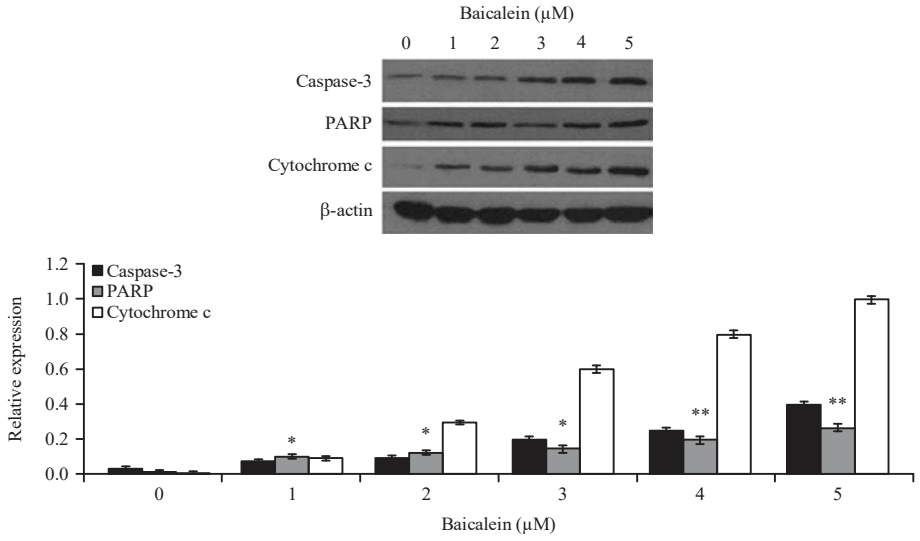

Fig. 3: Baicalein activates proteins associated with apoptosis in glioma cells. U87 cells treated with baicalein for 24 h were subjected to analysis of caspase-3 cleavage, cytochrome c release and activation of PARP by western blotting

*p<0.05, **p<0.01 vs. control cells

in U87 cells was significantly higher on exposure to 5 μM concentration of baicalein. The release of cytochrome c was also increased from mitochondria into the cytoplasm in U87 cells on exposure to baicalein. Treatment of U87 cells with baicalein for 24 h enhanced the activation of PARP.

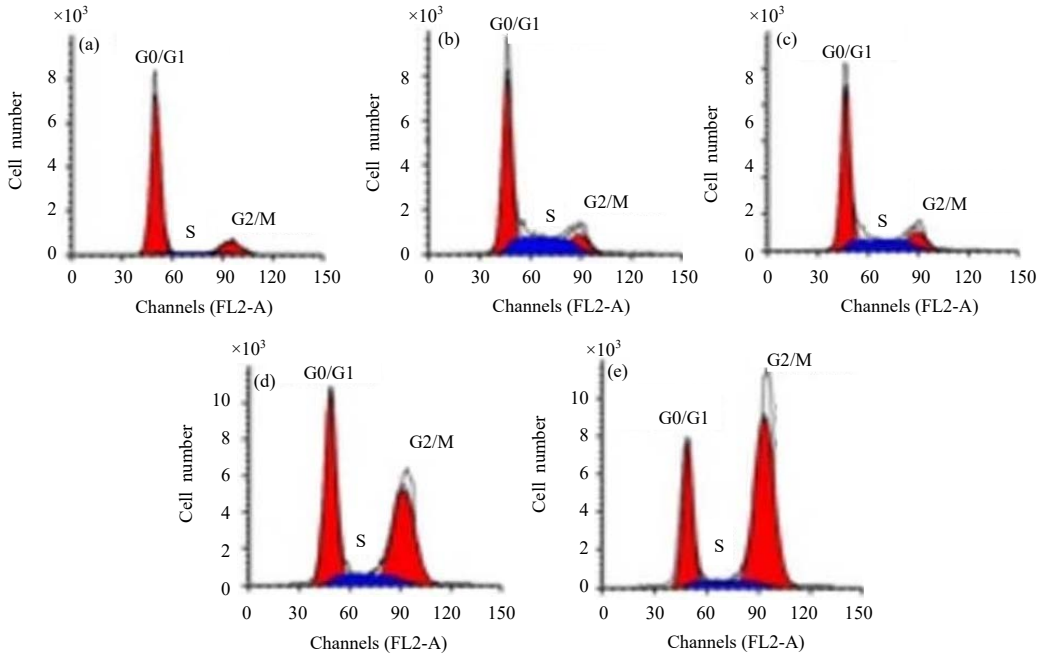

Fig. 4(a-e): Baicalein exposure changes distribution of cells in cell cycle. The U87 cells exposed to various concentration of baicalein was analyzed by flow cytometry for cell cycle, (a) Control, (b) Cells treated with 2 μM (c) Cells treated with 3 μM (d) Cells treated with 4 μM and (e) Cells treated with 5 μM concentrations of baicalein

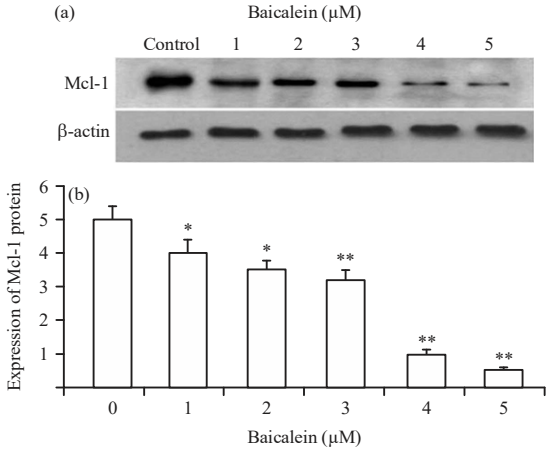

Fig. 5(a-b): Baicalein decreases Mcl-1 protein expression in U87 cells, (a) Cells exposed to various concentrations of baicalein or DMSO for 24 h were subjected to western blotting for determination of Mcl-1 protein expression and (b) Changes in Mcl-1 protein expression by baicalein treatment

Values are represented as average of three measurements, *p<0.05, **p<0.01 vs. control cells

Baicalein exposure of U87 cells causes G0/G1 phase cell cycle arrest: Baicalein exposure of U87 cells increased percentage of cells in G2/M phase with the reduction of cell population from G0/G1 phase (Fig. 4). Exposure to 2, 3, 4 and 5 μM concentrations of baicalein decreased G0/G1 phase cell percentage significantly in comparison to the control cell cultures. The cell population in G2/M phase was increased significantly on exposure to 2, 3, 4 and 5 μM concentrations of baicalein in comparison to that in the control cultures (Fig. 4).

Mcl-1 expression is inhibited by baicalein: The cells were exposed to 1, 2, 3, 4 and 5 μM concentration of baicalein for 24 h and then analyzed for Mcl-1 protein expression (Fig. 5). Baicalein treatment caused concentration dependent decrease in Mcl-1 protein expression in U87 cells. The decrease of Mcl-1 protein expression in U87 cells was significant from 2 μM concentration of baicalein. The inhibitory effect of baicalein on Mcl-1 protein expression was maximum at 5 μM concentration.

DISCUSSION

The present study was designed to investigate the effect of baicalein a compound present in *Scutellaria baicalensis* on glioma cell proliferation. The results demonstrated that baicalein treatment inhibits glioma cell viability, induces apoptosis and arrests cell cycle. It inhibited Mcl-1 and

anti-apoptotic protein expression. The inhibitory effect of baicalein on the viability of C6 and U87 glioma cells was investigated. The results from MTT assay demonstrated that baicalein exposure inhibits C6 and U87 glioma cell viability in concentration based manner. Flow cytometry showed that increasing the concentration of baicalein significantly enhanced apoptotic cell proportion in U87 cell cultures. In cancer cells, exposure to therapeutic agents leads to arrest of cell cycle in G2/M phase and causes cells to undergo apoptosis[22]. Apoptosis of carcinoma cells is linked with the cytochrome c release from the mitochondria leading to activation and cleavage[21] of caspase-3. Arrest of the cell cycle is accompanied by Bcl-2 activation, its subsequent degradation and cyclin protein A and B1 aggregation[23]. In the present study, baicalein exposure of U87 cells caused arrest of cell cycle in G0/G1 phase and increased cell population in G2/M phase. The results from present study showed that baicalein exposure of U87 cells caused release of cytochrome c from the mitochondria and lead to activation of caspase-3. Treatment of the U87 cells with baicalein promoted cleavage of PARP markedly compared to the untreated cell cultures. Resistance of cancer cells to therapeutic agents and progression of tumor growth is associated with the over expression of proteins belonging to Bcl-2 family and down-regulation of pro-apoptotic proteins[24,25]. In the current study, baicalein exposure of U87 cells significantly up-regulated the expression of pro-apoptotic proteins. It is well established that Mcl-1 which is an anti-apoptotic protein plays important role in enabling cells to escape apoptosis[26,27]. Suppression of Mcl-1 protein expression in cancer cells used siRNA inhibits proliferation and caused apoptosis induction[28]. The characteristic feature of apoptosis induction is the translocation of cytochrome c into cell cytosol and up-regulation of caspase-3 and PARP cleavage[29]. Down-regulation of Mcl-1 protein expression has been shown to sensitize the multiple myeloma cells towards disruption of mitochondria by BH3-only proteins[30,31]. Inhibition of Mcl-1 down-regulates expression of anti-apoptotic proteins belonging to the Bcl-2 family and enhances Bax/Bak ratio. Up-regulation of Bax and Bak catalyses activation of caspases and release of cytochrome c by increasing mitochondrial membrane permeability[30-34]. It has been reported that down-regulation of Mcl-1 produces Bim free from Mcl-1 that is directly associated with the induction of apoptosis events[33]. In the present study expression of Mcl-1 protein in U87 cells was decreased significantly on exposure to baicalein. The inhibitory effect of baicalein on Mcl-1 protein expression was maximum at 5 µM concentration.

CONCLUSION

Present study concluded that baicalein treatment promotes pro-apoptotic and suppresses anti-apoptotic protein especially, Mcl-1 expression in glioma cells. It arrests cell cycle in G0/G1 phase and causes apoptosis induction in the glioma cells. Therefore, baicalein can be of therapeutic significance for the treatment of glioma. Thus more studies need to be performed to investigate the role of baicalein in the treatment of glioma either alone or as combination therapy.

SIGNIFICANCE STATEMENT

The present study discovered that baicalein can be beneficial for the treatment of glioma. Therefore, the study will help the researchers to develop a novel and effective treatment strategy for the treatment of glioma.

REFERENCES

1. Curran, Jr. W.J., C.B. Scott, J. Horton, J.S. Nelson and A.S. Weinstein *et al*., 1993. Recursive partitioning analysis of prognostic factors in three radiation therapy oncology group malignant glioma trials. J. Natl. Cancer Inst., 85: 704-710.
2. Maher, E.A., F.B. Furnari, R.M. Bachoo, D.H. Rowitch, D.N. Louis and W.K. Cavenee, R.A. DePinho, 2001. Malignant glioma: Genetics and biology of a grave matter. Genes Dev., 15: 1311-1333.
3. Chang, S.M., K.R. Lamborn, M. Malec, D. Larson and W. Wara *et al*., 2004. Phase II study of temozolomide and thalidomide with radiation therapy for newly diagnosed glioblastoma multiforme. Int. J. Radiat. Oncol. Biol. Phys., 60: 353-357.
4. Fine, H.A., P.Y. Wen, E.A. Maher, E. Viscosi and T. Batchelor *et al*., 2003. Phase II trial of thalidomide and carmustine for patients with recurrent high-grade gliomas. J. Clin. Oncol., 21: 2299-2304.
5. Marx, G.M., N. Pavlakis, S. McCowatt, F.M. Boyle and J.A. Levi *et al*., 2001. Phase II study of thalidomide in the treatment of recurrent glioblastoma multiforme. J. Neuro-Oncol., 54: 31-38.
6. Eramo, A., L. Ricci-Vitiani, A. Zeuner, R. Pallini and F. Lotti *et al*., 2006. Chemotherapy resistance of glioblastoma stem cells. Cell Death Differ., 13: 1238-1241.
7. Strik, H., M. Deininger, J. Streffer, E. Grote and J. Wickboldt *et al*., 1999. BCL-2 family protein expression in initial and recurrent glioblastomas: Modulation by radiochemotherapy. J. Neurol. Neurosurg. Psychiatry, 67: 763-768.

8. Stegh, A.H., H. Kim, R.M. Bachoo, K.L. Forloney and J. Zhang *et al*., 2007. Bcl2L12 inhibits post-mitochondrial apoptosis signaling in glioblastoma. Genes Dev., 21: 98-111.
9. Kozopas, K.M., T. Yang, H.L. Buchan, P. Zhou and R.W. Craig, 1993. MCL1, a gene expressed in programmed myeloid cell differentiation, has sequence similarity to BCL2. Proc. Natl. Acad. Sci. USA., 90: 3516-3520.
10. Zhang, B., I. Gojo and R.G. Fenton, 2002. Myeloid cell factor-1 is a critical survival factor for multiple myeloma. Blood, 99: 1885-1893.
11. Strauss, S.J., K. Higginbottom, S. Juliger, L. Maharaj and P. Allen *et al*., 2007. The proteasome inhibitor bortezomib acts independently of p53 and induces cell death via apoptosis and mitotic catastrophe in B-cell lymphoma cell lines. Cancer Res., 67: 2783-2790.
12. Fribley, A. and C.Y. Wang, 2006. Proteasome inhibitor induces apoptosis through induction of endoplasmic reticulum stress. Cancer Biol. Ther., 5: 745-748.
13. Lioni, M., K. Noma, A. Snyder, A. Klein-Szanto and J.A. Diehl *et al*., 2008. Bortezomib induces apoptosis in esophageal squamous cell carcinoma cells through activation of the p38 mitogen-activated protein kinase pathway. Mol. Cancer Therapeut., 7: 2866-2875.
14. Zhang, L., H. Lopez, N.M. George, X. Liu, X. Pang and X. Luo, 2011. Selective involvement of BH3-only proteins and differential targets of Noxa in diverse apoptotic pathways. Cell Death Differ., 18: 864-873.
15. Chen, Z., K. Nihei, H. Tanaka, Y. Uda and Y. Kabuyama, 2013. Identification of a nitric oxide generation-stimulative principle in *Scutellariae radix*. Biosci. Biotechnol. Biochem., 77: 657-659.
16. Yoon, S.B., Y.J. Lee, S.K. Park, H.C. Kim and H. Bae *et al*., 2009. Anti-inflammatory effects of *Scutellaria baicalensis* water extract on LPS-activated RAW 264.7 macrophages. J. Ethnopharmacol., 125: 286-290.
17. Ho, F.M., Y.H. Liao, A.J. Yang, P.D.L. Chao and Y.C. Hou *et al*., 2012. Anti-atherosclerotic action of Ger-Gen-Chyn-Lian-Tang and AMPK-dependent lipid lowering effect in hepatocytes. J. Ethnopharmacol., 142: 175-187.
18. Lee, J.J., T. Kim, W.K. Cho and J.Y. Ma, 2013. Antithrombotic and antiplatelet activities of Soshiho-tang extract. BMC Complement. Altern. Med., Vol. 13. 10.1186/1472-6882-13-137.
19. Yue, G.G.L., B.C.L. Chan, H.F. Kwok, M.H. To and K.L. Hon *et al*., 2012. Screening for anti-inflammatory and bronchorelaxant activities of 12 commonly used chinese herbal medicines. Phytother. Res., 26: 915-925.
20. Huang, W.H., P.Y. Chien, C.H. Yang and A.R. Lee, 2003. Novel synthesis of flavonoids of *Scutellaria baicalensis* GEORGI. Chem. Pharmaceut. Bull., 51: 339-340.
21. Lee, H.Z., H.W.C. Leung, M.Y. Lai and C.H. Wu, 2005. Baicalein induced cell cycle arrest and apoptosis in human lung squamous carcinoma CH27 cells. Anticancer Res., 25: 959-964.
22. Domina, A.M., J.H. Smith and R.W. Craig, 2000. Myeloid cell leukemia 1 is phosphorylated through two distinct pathways, one associated with extracellular signal-regulated kinase activation and the other with G_2/M accumulation or protein phosphatase 1/2A inhibition. J. Biol. Chem., 275: 21688-21694.
23. Ma, M.H., H.H. Yang, K. Parker, S. Manyak and J.M. Friedman *et al*., 2003. The proteasome inhibitor PS-341 markedly enhances sensitivity of multiple myeloma tumor cells to chemotherapeutic a gents. Clin. Cancer Res., 9: 1136-1144.
24. Gomez-Bougie, P., S. Wuilleme-Toumi, E. Menoret, V. Trichet and N. Robillard *et al*., 2007. Noxa up-regulation and Mcl-1 cleavage are associated to apoptosis induction by bortezomib in multiple myeloma. Cancer Res., 67: 5418-5424.
25. Lesinski, G.B., E.T. Raig, K. Guenterberg, L. Brown and M.R. Go *et al*., 2008. IFN-α and bortezomib overcome Bcl-2 and Mcl-1 overexpression in melanoma cells by stimulating the extrinsic pathway of apoptosis. Cancer Res., 68: 8351-8360.
26. Quinn, B.A., R. Dash, B. Azab, S. Sarkar and S.K. Das *et al*., 2011. Targeting Mcl-1 for the therapy of cancer. Expert Opin. Invest. Drugs, 20: 1397-1411.
27. Le Gouill, S., K. Podar, J.L. Harousseau and K.C. Anderson, 2004. Mcl-1 regulation and its role in multiple myeloma. Cell Cycle, 3: 1259-1262.
28. Derenne, S., B. Monia, N.M. Dean, J.K. Taylor and M.J. Rapp *et al*., 2002. Antisense strategy shows that Mcl-1 rather than Bcl-2 or Bcl-x_L is an essential survival protein of human myeloma cells. Blood, 100: 194-199.
29. Clohessy, J.G., J. Zhuang, J. de Boer, G. Gil-Gomez and H.J.M. Brady, 2006. Mcl-1 interacts with truncated bid and inhibits its induction of cytochrome C release and its role in receptor-mediated apoptosis. J. Biol. Chem., 281: 5750-5759.
30. Craig, R.W., 2002. MCL1 provides a window on the role of the BCL2 family in cell proliferation, differentiation and tumorigenesis. Leukemia, 16: 444-454.
31. Willis, S.N., L. Chen, G. Dewson, A. Wei and E. Naik *et al*., 2005. Proapoptotic Bak is sequestered by Mcl-1 and Bcl-x_L, but not Bcl-2, until displaced by BH3-only proteins. Genes Dev., 19: 1294-1305.
32. Chen, L., S.N. Willis, A. Wei, B.J. Smith and J.I. Fletcher *et al*., 2005. Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. Mol. Cell, 17: 393-403.
33. Han, J., L.A. Goldstein, B.R. Gastman and H. Rabinowich, 2006. Interrelated roles for Mcl-1 and BIM in regulation of TRAIL-mediated mitochondrial apoptosis. J. Biol. Chem., 281: 10153-10163.
34. Gomez-Bougie, P., R. Bataille and M. Amiot, 2004. The imbalance between Bim and Mcl-1 expression controls the survival of human myeloma cells. Eur. J. Immunol., 34: 3156-3164.