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.2022.746.752

Research Article
Goniothalamin Suppressed Glioblastoma Cell Proliferation Through p38 MAPK Phosphorylation Mediated Apoptosis

[1]Rentao Li, [2]Lei Zhao, [3]Sandhanasamy Devanesan, [4]Murali Kannan Maruthamuthu and [5]Yu Yin

[1]Department of Neurosurgery, Jiyang People's Hospital of Jinan, No.17, Xinyuan Street, Jinan, Shandong, 251400, China
[2]Public Security Clinic, Jiaozhou Peoples Hospital, Jiaozhou City, Shandong, 266300, China
[3]Department of Physics and Astronomy, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Kingdom of Saudi Arabia
[4]Division of Pharmacoengineering and Molecular Pharmaceutics Therapeutic Biomaterials Laboratory, Eshelman School of Pharmacy, The University of North Carolina at Chapel Hill, North Carolina
[5]Department of Medical Insurance, Central Hospital Affiliated to Shandong First Medical University, No. 105, Jiefang Road, Jinan City, Shandong Province, 250013, China

Abstract

Background and Objective: Malignant gliomas, particularly Glioblastoma (GBM) are a primary cause of mortality and morbidity in brain malignancy. The p38 MAPK signalling reduction leads to the tumorigenesis of GBM. Recognizing novel agents that can regulate p38 MAPK signalling is essential for GBM treatment. Goniothalamin (GTN), a naturally arising styryl-lactone compound with accepted anticancer properties. To explore the anticancer and apoptosis efficacy of GTN on GBM human cancer cells. **Materials and Methods:** The U251 malignant cells were treated with various GTN dosages to fix its molecular action on cells viability, apoptosis, cell cycle regulation and p38 MAPK/JNK/ERK protein expressions. The anti-proliferative activity of GTN on U-251 cells has been exposed by CCK-8 assay and live/dead assay. **Results:** Apoptotic death contributed by GTN on the reduction of U251 cell viability was demonstrated by DAPI staining, Bax and Bcl-2 protein expressions. The G2/M cell cycle regulation was also confirmed by reducing the protein expression of cyclin B1, CDK1 and enhanced p21 level. Additionally, GTN induced apoptosis via p-p38 upregulation and little effect on p-JNK1 and p-ERK1 levels. **Conclusion:** In this study, GTN treatment induces proapoptotic factors and inhibits proliferative markers by inhibiting p38 MAPK expression in glioblastoma cells. Our findings recommend that GTN would be beneficial for GBM treatment.

Key words: Goniothalamin, glioblastoma, U-251 cells, apoptosis, proliferation, p38 MAPK, CDK1

Citation: Li, R., L. Zhao, S. Devanesan, M.K. Maruthamuthu and Y. Yin, 2022. Goniothalamin suppressed glioblastoma cell proliferation through p38 MAPK phosphorylation mediated apoptosis. Int. J. Pharmacol., 18: 746-752.

Corresponding Author: Yu Yin, Department of Medical insurance, Central Hospital Affiliated to Shandong First Medical University, No. 105, Jiefang Road, Jinan City, Shandong Province, 250013, China

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

Glioblastoma (GBM) is the utmost destructive brain malignancy and is greatly invasive, encompassing nearly 50-60% of cerebral gliomas with the uppermost mortality ratio[1,2]. Even though wide-ranging treatment regimens such as surgical intervention with a combination of surgery, chemotherapy and radiotherapy. Still, the main treatment strategy for GBM relapse remains problematic as resulting in a poor prognosis[3,4]. The normal survival time of GBM is 12-15 months only and almost <6% is the 5 years survival rate[5]. Moreover, amplified drug resistance and the blood-brain barrier frequently reduce the chemotherapy drugs efficacy[6]. Hence, novel effective drugs are instantly needed to recover for GBM patients.

Numerous accepted bioactive agents with strong cytotoxic properties had been employed as anticancer remedies. The bioactive styryl-lactone Goniothalamin (GTN) is isolated from the family *Annonaceae* and largely found in the genus *Goniothalamus* with potent antiproliferative activity[7]. GTN has been shown to subdued cell viability and encouraged cytotoxicity in an assortment of malignant cells, for instance, gastric, kidney[8], cervical[9], leukemia[10], ovarian, melanoma, colon[11], breast[12], liver[13] and lung[14] cancer cells. Besides, GTN has been exposed to its own anticancer and apoptosis-persuading activities in several cancers. However, the apoptotic and molecular mechanism of GTN on human GBM has not yet been explored. Hence, we assessed the anti-cancer and apoptotic action of GTN on human GBM U251 cells.

Apoptosis is an apparent form of involuntary cell death, in concert a significant part for the modulation of homeostasis, diseases and development, comprising innumerable cancers and neurodegenerative illnesses[15,16]. The anticancer agents have induced apoptosis into the cancer cells, which act as a key molecular mechanism for the anti-cancer drugs investigation[17]. The Mitogen-Activated Protein Kinases (MAPKs) are the class of protein kinases, which comprises ERK1/2, JNKs and p38 MAPKs have been associated with the development and progress of GBM[18]. Numerous stresses, such as cytokines, growth factors and various cellular signalling, have been shown to stimulate p38 MAPKs overexpression[19,20]. However, the regulation of p38 MAPK signalling in GBM remains unknown. Thus, p38 MAPK regulation of GTN may provide a new perspective on the cell death process as well as potential treatments for GBM.

Therefore, In this study, we investigated the Goniothalamin inhibits the growth of glioblastoma through p38 MAPK phosphorylation mediated apoptosis.

MATERIALS AND METHODS

Study area: This study project was performed from 15th August, 2018-31st January, 2021 in the Digestive Endoscopy Room in the Department of Neurosurgery, Jiyang People's Hospital of Jinan, No.17, Xinyuan Street, Jinan, Shandong, 251400, China

Chemicals: Goniothalamin, FBS, antibiotics and other biochemicals were obtained from Merck, Germany. The antibodies against p38 MAPK, JNK, ERK, Bax, Bcl-2, cyclin B1, CDK1, p21 and GAPDH were procured from Labome, USA.

Cell culture: The GBM human cancer cell line U251 was obtained from the Peking Union Cell Resource Center (Beijing, China) and expanded growth in culture through DMEM complete medium combined with 10% FBS, penicillin (100 U mL^{-1}) and streptomycin (100 g mL^{-1}) at 37°C in a humidified CO_2 (5%) atmosphere.

CCK-8 cell viability test: The cell counting Kit-8 (Dojindo, Kumamoto, Japan) has been employed to investigate cell viability. GBM cells were sowed into a 96-well plate at 1×10^4 cells/well and various doses of GTN were added. To 10 µL of CCK-8 solution were added to each well after incubation. The optical density at 450 nm wavelength was determined by using a microplate reader 1 hr later.

Cell viability (live/dead) assay: The cell viability analysis has been achieved for GTN (25 and 30 µM) treated cells performing the cell viability assay kit (Gibco, USA) executed to the company's guidelines. Live cells are permitted to enter live-cell dye and hydrolyzed with intracellular esterase and to release a fluorescent hydrophilic compound which is measured at 485/530 nm. The injured cells membrane permitted to infiltrate dead cell dye, binding of the DNA to discharged a red fluorescent signal, measured at 495/635 nm. To, investigate the live/dead cells by employing Leica DMi8 fluorescence microscopy.

Apoptosis analysis by staining with DAPI: The U-251 cells were sowed 1×10^5 cells in each well of 6 and supplemented to GTN (25 and 30 µM). These cells were stained with DAPI to investigate the nuclear alterations allied with apoptosis. Afterwards, the sections were fixed on a glass slide, and the imageries were observed through a fluorescence microscope.

Western blotting analysis: The GTN (25 and 30 µM) were treated to the U251 cells and the lysates were done by ice-cold

lysis buffer involving protease inhibitors and western blotting analysis has performed. Shortly, electrophoretically dispersed proteins were shifted to a PVDF membrane. Then, it was bunged with the probe for 1 hr and added primary antibodies dilution of 1:1.000 and kept overnight at 4°C. Successively secondary HRP-conjugated antibodies were added. The protein bands with immune-stained were visualized for protein detection. The density of the band was explored by the software imageJ and GAPDH was used as a reference to normalize the expression.

Statistical analysis: Results were stated as Mean±SD by assessing the SPSS 18.0 software for multiple comparisons one-way ANOVA afterwards Dunnett's test. The data of $p<0.05$ has been reflected as significant.

RESULTS

Impact of GTN on U251 glioma cells proliferation: To examine the activity of GTN on U251 cell growth, the CCK-8 was employed to determine the GBM cancer cells viability. The U251 glioma cells viability is analyzed with different doses of GTN (control, 5, 10, 15, 20, 25, 30 and 35) for 24 hrs. Treatment GTN presented the inhibition of cell viability in a dosage-dependent mode. The GTN IC_{50} value was calculated and the concentration of 25 and 30 μM were selected for further studies (Fig. 1).

Influence of GTN on the viability of U251 cells: To scrutinize the cell sustainability of control and GTN (25 and 30 μM) was accomplished by live/dead assay. These were stained with calcein-AM and PI to recognize green live cells and red dead cells. As Fig. 2a showed there is no significant cell death was observed. The dead cells percentage was increased in 25 and 30 μM of GTN against U251 glioma cells (Fig. 2b-c). GTN at a dose of 30 μM exhibited a remarkably elevated level of dead cells (Fig. 2c).

The upshot of GTN on apoptosis induction: Control U251cells stained with DAPI displayed the presence of viable cells. The

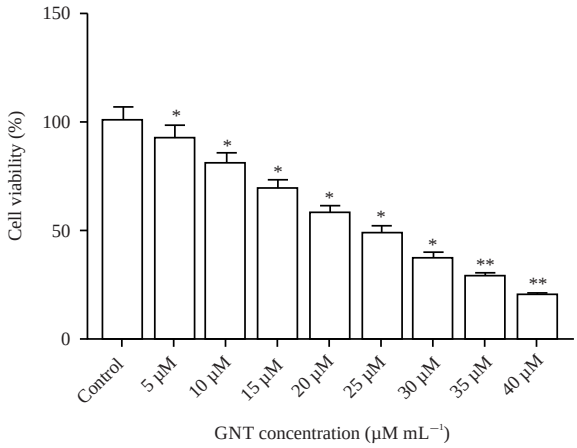

Fig. 1: Influence of GTN on U251 GBM cell proliferation

Human GBM cells U251 were added with different dosages of GTN for 24 hrs, cell proliferation was assessed by a CCK-8 assay, results were noted as Mean±SD for triplicate tests, *$p<0.05$ and **$p<0.01$ versus control

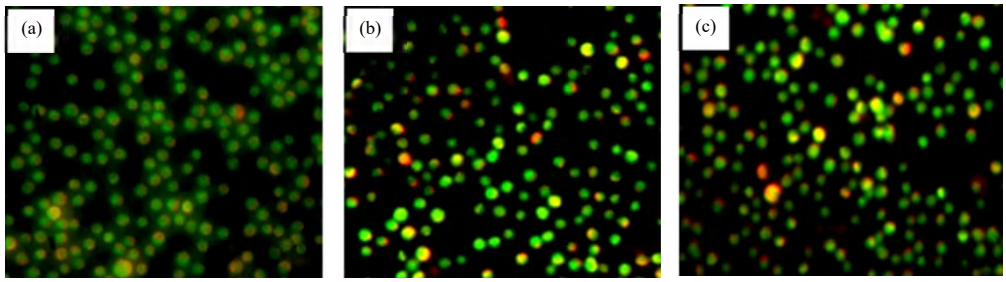

Fig. 2(a-c): Influence of GTN on U251 GBM cell viability, (a) Control, (b) GTN (25 μM) and (c) GTN (30 μM)

Human GBM cells U251 were added with 0, 25 and 30 μM concentrations of GTN for 24 hrs, live and dead cells were analyzed by live/dead cell imaging kit, observed under a fluorescence microscope

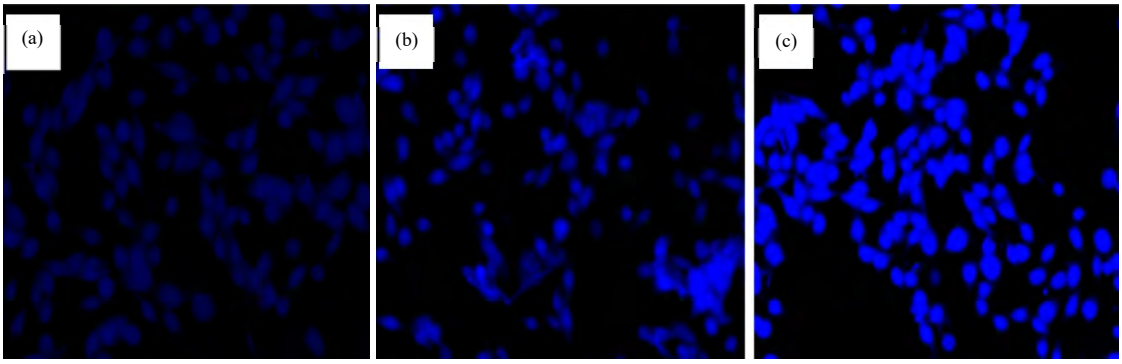

Fig. 3(a-c): Impact of GTN on U251 GBM cells apoptosis, (a) Control, (b) GTN (25 µM) and (c) GTN (30 µM)
Human GBM cells U251 were added with 0, 25 and 30 µM concentrations of GTN for 24 hrs, cell apoptosis was determined by exhausting DAPI staining

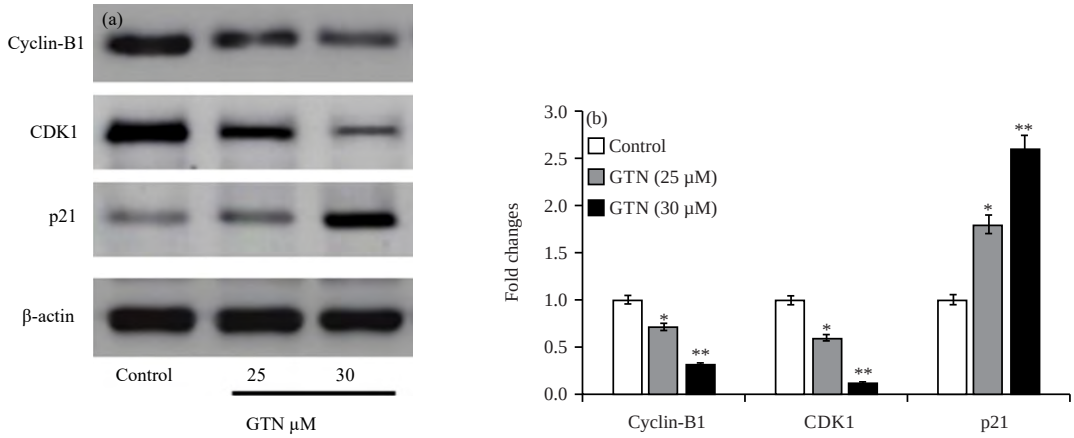

Fig. 4(a-b): Upshot of GTN on the protein expression of Cyclin B1, CDK1 and p21
Human GBM cells U251 were added with 0, 25 and 30 µM of GTN for 24 hrs, protein expression of cyclin B1, CDK1 and p21 was analyzed by western blot, values that do not share a common marking differ significantly at $p<0.05$ (Duncan's multiple-range test), *Significantly dissimilar from control ($p<0.05$), **Significantly dissimilar from control and GTN (25 µM) ($p<0.05$)

GTN (25 and 30 µM) treated with U251 glioma cells exposed the induction of apoptosis. As Fig. 3a exhibits there is no nuclear fragmentation was observed. The cytoplasm shrinkage, loss of nuclear envelopes and fragmentation of the nucleus were also detected in GTN treatment (Fig. 3b-c). The apoptotic effect was high in 30 µM GTN treated in the cells U251 (Fig. 3c).

Western blot analysis of cyclin B1/CDK1/p21: The western blot analysis exhibited that GTN (25 and 30 µM) treatment depleted cyclin B1 and CDK1, whereas p21 was elevated in a concentration mode. These results revealed that GTN regulates cell-cycle in GBM cells, adding to its effects on apoptotic (Fig. 4a). The data in Fig. 4b exhibited the densitometric analysis of cyclin B1 and CDK1 and p21which was normalized by β-actin expression.

Exploration of Bax/Bcl-2 protein expression: On treatment with GTN (25 and 30 µM), the pro-apoptotic Bax expression has enhanced while anti-apoptotic Bcl-2 reduced its protein expression (Fig. 5a). The data in Fig. 5b exhibited the densitometric analysis of Bax and Bcl-2 was normalized by β-actin expression. The results established the apoptotic action of GTN in a concentration-dependent mode.

Exploration of p38 MAPK/JNK/ERK protein expression: The phosphorylated p38 MAPK protein expression has upregulated to the treatment with GTN on U-251 cells, but the pERK and pJNK exhibited only little variation against control glioma cells (Fig. 6a). The data in Fig. 6b exhibited the densitometric analysis of p-p38, p-ERK and p-JNK which was normalized by β-actin expression.

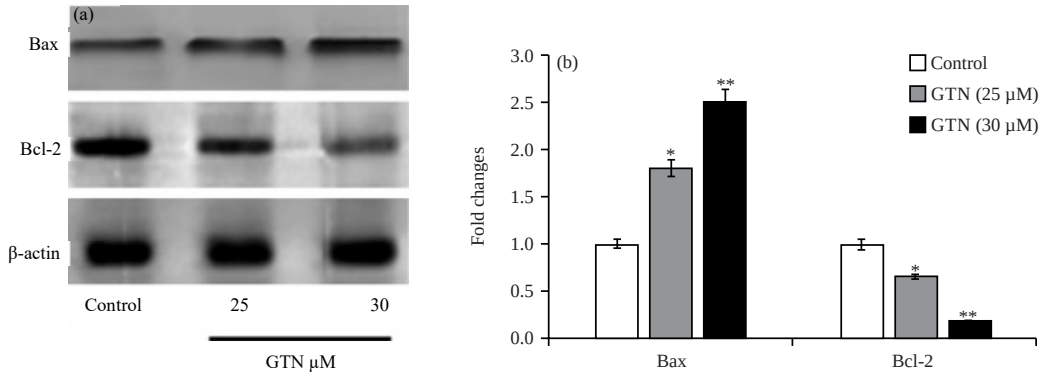

Fig. 5 (a-b): Impact of GTN on the Bax and Bcl-2protein levels

Human GBM cells U251 were treated with 0, 25 and 30 µM concentrations of GTN for 24 hrs, protein level of Bax and Bcl-2 was scrutinized by western blotting, values that do not share a common marking differ significantly at p<0.05 (Duncan's multiple-range test), *Significantly dissimilar from control (p<0.05), **Significantly dissimilar from control and GTN (25 µM) (p<0.05)

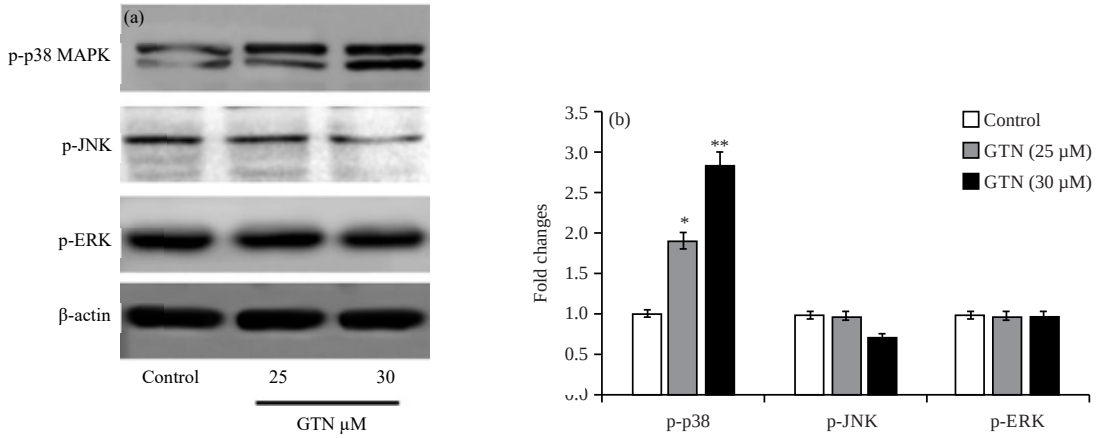

Fig. 6 (a-b): Influence of GTN on p38 MAPK, JNK and ERK protein expression on U251 GBM cells

Human U251 cells were added with 0, 25 and 30 µM of GTN for 24 hrs, level of p38 MAPK, JNK and ERK protein was analyzed by western blotting, values that do not share a common marking differ significantly at p<0.05 (Duncan's multiple-range test), *Significantly dissimilar from control (p<0.05), **Significantly dissimilar from control and GTN (25 µM) (p<0.05)

DISCUSSION

In this study, we investigated that natural phytochemical Goniothalamin (GTN) inhibits proliferative markers on GBM through p38 MAPK regulation. GBM is the deadliest brain malignancy that affects the central nervous system even with the years of basic and clinical research[21]. Although GBM in the early stages can be succeeded with surgery and several therapeutic agents, the inherent resistance is leading to aggressive clinical sequences and deprived prognosis[22]. It has been reported that elevation of GBM associated morbidity and mortality contributes to numerous cancer-causing signalling abnormalities with extreme cell viability. Currently, we investigated that GTN repressed the U251 glioma cells proliferation through the induction of apoptosis and cell cycle regulation.

The plant-derived bioactive molecules have exerts unique properties possible to impede the growth and expansion of numerous cancers[23]. They prevent the cancer cells proliferation at various mechanisms which comprise the apoptosis induction, autophagy and cancer cell cycle arrest at different phases[24]. Besides, plant-derived compounds are supposed to be safe for consumption owing to their less toxic effects[25]. Reports suggest that GTN might have therapeutic potential for cancer treatment based on previous *in vitro*[26,27] and *in vivo*[28] findings. The antiproliferative action of GTN was very noticeable in cancer cells. Also, current research has proposed possible discrimination of GTN counter to malignant cells with slight action in contrast to normal cells, which has gained more importance recently[29,30]. In the current study, we perceived the antiproliferative and cytotoxic action of GTN against U251 in a concentration-dependent mode.

The CCK-8 and live/dead assays revealed that GTN could expressively impede GBM cells viability, specifying that GTN exhibited a strong anti-viability effect on U251. Apoptosis is a natively regulated cell death practice that preserves tissue homeostasis[31]. Indeed, certain anticancer bioactive compounds could abolish cancer cells by renovating faulty apoptosis[32]. In western blot analysis exposed that GTN diminished Bcl-2 and enhanced Bax protein levels in U-251 cells. These findings demonstrated that GTN stimulated apoptosis in U251 cells.

The checkpoints of the cell cycle have profound actions in the growth of cells and apoptosis[33] and GTN was reported that encourage cancer cell growth to halt at the G2/M phase[34]. The shift from the S phase into the G2/M phase is generally driven by the CDK1-cyclin B1 complexes[35]. The upshots of the cell cycle study indicated that GTN persuaded G2/M phase detention in U251. The GTN supplementation augmented p21 expression but reduced the CDK1-cyclin B1 levels. These results suggested that GTN inhibited U251 cells viability via the commencement of apoptosis in cell cycle-specific at G2/M phase arrest are utmost inclined.

The MAPK kinases are regarded as having a vital role in the growth of cells, survival and apoptosis. Conventional MAPKs in mammalian cells consists of p38, ERK and JNK which are triggered over a precise phosphorylation cascade[36]. It is renowned that ERK promotes cell survival, while p38 and JNK induce apoptosis. Administration of GTN upregulated phosphorylated p38 MAPK, while JNK and ERK were not affected. Reports indicated that GTN repressed cancer cells by the MAPK signal modulation[37]. Our results established that GTN regulates MAPK signalling and exerts apoptotic and anti-proliferative effects on U251 glioma cells.

CONCLUSION

Our studies demonstrated that GTN suppressed cell viability and promoted apoptosis on human GBM cells. The apoptosis and cell cycle detention caused by GTN together with p38 MAPK activation could be elaborate in the anticancer mechanism of GTN on these U251 cells. Thus, GTN has a potent natural anti-cancer drug for human GBM treatment. These findings may provide an innovative perception of the GTN as a chemotherapeutic agent.

SIGNIFICANCE STATEMENT

This study discovers the possibility that GTN treatment induces proapoptotic factors and inhibits proliferative markers in U251 glioblastoma cells by regulating p38 MAPK expression. This study will aid the researcher's future research into the role of GTN-mediated regulation of other signalling events in glioblastoma.

ACKNOWLEDGMENT

The authors are grateful to the Researchers Supporting Project Number (RSP-2021/398), King Saud University, Riyadh, Saudi Arabia.

REFERENCES

1. Lapointe, S., A. Perry and N.A. Butowski, 2018. Primary brain tumours in adults. Lancet, 392: 432-446.
2. Zhou, Y., W. Wu, H. Bi, D. Yang and C. Zhang, 2020. Glioblastoma precision therapy: From the bench to the clinic. Cancer Lett., 475: 79-91.
3. Bleeker, F.E., R.J. Molenaar and S. Leenstra, 2012. Recent advances in the molecular understanding of glioblastoma. J. Neuro-Oncol., 108: 11-27.
4. Ludwig, K. and H.I. Kornblum, 2017. Molecular markers in glioma. J. Neuro-Oncol., 134: 505-512.
5. Miller, T.E., B.B. Liau, L.C. Wallace, A.R. Morton and Q. Xie *et al*., 2017. Transcription elongation factors represent *in vivo* cancer dependencies in glioblastoma. Nature, 547: 355-359.
6. Corazzari, M., M. Gagliardi, G.M. Fimia and M. Piacentini, 2017. Endoplasmic reticulum stress, unfolded protein response and cancer cell fate. Front. Oncol., Vol. 7. 10.3389/fonc.2017.00078.
7. Wiart, C., 2007. *Goniothalamus* species: A source of drugs for the treatment of cancers and bacterial infections?. Evidence-Based Complementary Altern. Med., 4: 299-311.
8. de Fátima, Â., L.K. Kohn, J.E. de Carvalho and R.A. Pilli, 2006. Cytotoxic activity of (S)-goniothalamin and analogues against human cancer cells. Bioorg. Med. Chem., 14: 622-631.
9. Sophonnithiprasert, T., W. Mahabusarakam, Y. Nakamura and R. Watanapokasin, 2017. Goniothalamin induces mitochondria-mediated apoptosis associated with endoplasmic reticulum stress-induced activation of JNK in HeLa cells. Oncol. Lett., 13: 119-128.
10. Inayat-Hussain, S.H., L.T. Wong, K.M. Chan, N.F. Rajab and L.B. Din *et al*., 2009. RACK-1 overexpression protects against goniothalamin-induced cell death. Toxicol. Lett., 191: 118-122.
11. de Fatima, A., L.K. Kohn, M.A. Antonio, J.E. de Carvalho and R.A. Pilli, 2005. R-goniothalamin: Total syntheses and cytotoxic activity against cancer cell lines. Bioorg. Med. Chem., 13: 2927-2933.
12. Khaw-on, P., W. Pompimon and R. Banjerdpongchai, 2019. Goniothalamin induces necroptosis and anoikis in human invasive breast cancer MDA-MB-231 cells. Int. J. Mol. Sci., Vol. 20. 10.3390/ijms20163953.

13. Al-Qubaisi, M., R. Rozita, S.K. Yeap, A.R. Omar, A.M. Ali and N.B. Alitheen, 2011. Selective cytotoxicity of goniothalamin against hepatoblastoma HepG2 cells. Molecules, 16: 2944-2959.
14. Chiu, C.C., P.L. Liu, K.J. Huang, H.M. Wang and K.F. Chang *et al*., 2011. Goniothalamin inhibits growth of human lung cancer cells through DNA damage, apoptosis and reduced migration ability. J. Agric. Food Chem., 59: 4288-4293.
15. Polster, B.M., G. Basañez, A. Etxebarria, J.M. Hardwick and D.G. Nicholls, 2005. Calpain I induces cleavage and release of apoptosis-inducing factor from isolated mitochondria. J. Biol. Chem., 280: 6447-6454.
16. Park, S.Y., H.Y. Kim, J.H. Lee, K.H. Yoon, M.S. Chang and S.K Park, 2010. The age-dependent induction of apoptosis-inducing factor (AIF) in the human semitendinosus skeletal muscle. Cell. Mol. Biol. Lett., 15: 1-12.
17. Díaz, J.G., A.J. Carmona, F. Torres, J. Quintana, F. Estévez and W. Herz, 2008. Cytotoxic activities of flavonoid glycoside acetates from *Consolida oliveriana*. Planta Med., 74: 171-174.
18. Prabhakar, S., S. Asuthkar, W. Lee, S. Chigurupati, E. Zakharian, A.J. Tsung and K.K. Velpula, 2014. Targeting DUSPs in glioblastomas-wielding a double edged sword?. Cell Biol. Int., 38: 145-153.
19. Johnson, G.L. and R. Lapadat, 2002. Mitogen-activated protein kinase pathways mediated by ERK, JNK and p38 protein kinases. Science, 298: 1911-1912.
20. Wagner, E.F. and A.R. Nebreda, 2009. Signal integration by JNK and p38 MAPK pathways in cancer development. Nat. Rev. Cancer, 9: 537-549.
21. Chen, R., A.L. Cohen and H. Colman, 2016. Targeted therapeutics in patients with high-grade gliomas: Past, present and future. Curr. Treat. Options Oncol., Vol. 17. 10.1007/s11864-016-0418-0.
22. Wang, J., X. Huang, K. Zhang, X. Mao and X. Ding *et al*., 2017. Vanadate oxidative and apoptotic effects are mediated by the MAPK-Nrf2 pathway in layer oviduct magnum epithelial cells. Metallomics, 9: 1562-1575.
23. Reddy, L., B. Odhav and K.D. Bhoola, 2003. Natural products for cancer prevention: A global perspective. Pharmacol. Ther., 99: 1-13.
24. Prietsch, R.F., L.G. Monte, F.A. da Silva, F.T. Beira and F.A.B.D. Pino *et al*., 2014. Genistein induces apoptosis and autophagy in human breast MCF-7 cells by modulating the expression of proapoptotic factors and oxidative stress enzymes. Mol. Cell. Biochem., 390: 235-242.
25. Leung, H.W.C., C.J. Lin, M.J. Hour, W.H. Yang, M.Y. Wang and H.Z. Lee, 2007. Kaempferol induces apoptosis in human lung non-small carcinoma cells accompanied by an induction of antioxidant enzymes. Food Chem. Toxicol., 45: 2005-2013.
26. Alabsi, A.M., R. Ali, A.M. Ali, S.A.R. Al-Dubai, H. Harun, N.H.A. Kasim and A. Alsalahi, 2012. Apoptosis induction, cell cycle arrest and *in vitro* anticancer activity of gonothalamin in a cancer cell lines. Asian Pac. J. Cancer Prev., 13: 5131-5136.
27. Seyed, M.A., I. Jantan and S.N.A. Bukhari, 2014. Emerging anticancer potentials of goniothalamin and its molecular mechanisms. BioMed. Res. Int., Vol. 2014. 10.1155/2014/536508.
28. Vendramini-Costa, D.B., I.B.D. de Castro, A.L.T.G. Ruiz, C. Marquissolo, R.A. Pilli and J.E. de Carvalho, 2010. Effect of goniothalamin on the development of ehrlich solid tumor in mice. Bioorg. Med. Chem., 18: 6742-6747.
29. Tian, Z., S. Chen, Y. Zhang, M. Huang and L. Shi *et al*., 2006. The cytotoxicity of naturally occurring styryl lactones. Phytomedicine, 13: 181-186.
30. Wattanapiromsakul, C., B. Wangsintaweekul, P. Sangprapan, A. Itharat and N. Keawpradub, 2005. Goniothalamin, a cytotoxic compound, isolated from *Goniothalamus macrophyllus* (Blume) Hook. f. & Thomson var. *macrophyllus*. Songklanakarin J. Sci. Technol., 27: 479-487.
31. Danial, N.N. and S.J. Korsmeyer, 2004. Cell death: Critical control points. Cell, 116: 205-219.
32. Brown, J.M. and L.D. Attardi, 2005. The role of apoptosis in cancer development and treatment response. Nat. Rev. Cancer, 5: 231-237.
33. Evan, G.I. and K.H. Vousden, 2001. Proliferation, cell cycle and apoptosis in cancer. Nature, 411: 342-348.
34. Inayat-Hussain, S.H., B.O. Annuar, L.B. Din, A.M. Ali and D. Ross, 2003. Loss of mitochondrial transmembrane potential and caspase-9 activation during apoptosis induced by the novel styryl-lactone goniothalamin in HL-60 leukemia cells. Toxicol. *In vitro*, 17: 433-439.
35. Malumbres, M. and M. Barbacid, 2009. Cell cycle, CDKs and cancer: A changing paradigm. Nat. Rev. Cancer, 9: 153-166.
36. Yoon, S. and R. Seger, 2006. The extracellular signal-regulated kinase: Multiple substrates regulate diverse cellular functions. Growth Factors, 24: 21-44.
37. Innajak, S., W. Mahabusrakum and R. Watanapokasin, 2016. Goniothalamin induces apoptosis associated with autophagy activation through MAPK signaling in SK-BR-3 cells. Oncol. Rep., 35: 2851-2858.