ScientificScholar®
Knowledge is power
Publisher of Scientific Journals

https://asjo.in

Asian Journal of Oncology

Original Article

Action of ultra-diluted ethanol extract of *Bryonia alba* on HepG2 liver cancer cells

Sanket Bandyopadhyay[1], MSc, Debasmita Chatterjee[2], MSc, PhD, Banhishikha Singh[3], MSc, Krishnendu Paira[2], BHMS, MBA, Satadal Das[4], MBBS, MD, DCP

[1]Department of Biotechnology, MS Ramaiah College of Arts, Science, and Commerce, Shyambazar, Kolkata, [2]Department of Genetic Research & Tissue Culture Unit, Heritage Institute of Technology, Kolkata, [3]Department of Biotechnology, Heritage Institute of Technology, Kolkata, [4]Department of Tissue Culture Unit, Heritage Institute of Technology, Chowbhaga, Kolkata, India.

ABSTRACT

Objectives: In this study we explored Bryonia alba as a new anti-cancer agent against liver cancer cells.

Material and Methods: The Bryonia 6C - an alternative medicine was applied on HepG2 liver cancer cell line to find out its effectiveness indicating morphological and cytokine changes by observing cytopathic effect and RT-PCR study.

Results: The result showed a possible anti-cancer effect of Bryonia 6C with degenerative changes on the cancer cells. A cytokine imbalance was also noticed within 24 hours detrimental to the malignant environment of these cells.

Conclusion: The alternative medicine Bryonia 6C is an effective anticancer agent against liver cancer cells.

Keywords: HepG2 cells, Bryonia 6C, Cytokines

INTRODUCTION

Alternative medicine is a broad term that includes an array of medical methods. These practices are typically supported by tradition and rarely taught in Western medical schools. Such practices range from the well-known ancient Eastern practices of acupuncture and Tai-chi to herbal medicine, chiropractic manipulation, Reiki, aromatherapy, massage therapy, folk healing, etc., and are generally used interchangeably with the word "alternative medicine," a designation created in the 19th century that distinguished these practices as "alternative" to allopathic medicine.[1] As a medicinal plant, Bryonia is a prominent name that is being used primarily in homeopathy as an anti-inflammatory agent. According to Mert Ilhan *et al.*, *B.alba* roots exhibited statistically significant anti-inflammatory, antioxidant, and antinociceptive activities that can serve as an alternative treatment for rheumatic disorders. It has also been widely used as a laxative and diuretic agent.[2,3] The primary active components found in the *B.alba* extract are glucosides, cucurbitacin, and trihydroxyoctadecadienoic acids (THODA).[2]

The biological activities of the active compounds are linked with the biosynthesis of corticosteroids and eicosanoids, which are important intermediary factors in the endocrine, immune, and nervous systems. For instance, after the devastating Chernobyl nuclear plant disaster, the extract of Bryonia was used to treat the workers who had experienced vegetovessel dystonia and other associated illnesses.[2] It showed impressive results in preventing cytostatic side effects and radiation-induced disorders in cancer therapy.[2] Most cucurbitacins have anti-tumor, cytotoxic, anthelmintic, hepatoprotective, anti-inflammatory, antimicrobial, cardiovascular, and anti-diabetic properties. The effects of these properties depend on the target cells.[4] For example, the free-radical scavenging activities and antioxidant capabilities of cucurbitacin E and cucurbitacin B glucosides have shown effective results in averting human diseases associated with free radical and oxidative stress.[4,5] Considering their anti-inflammatory actions, it has been established that they are responsible for inhibiting the expression of tumor necrosis factor-alpha (TNFα) in

Corresponding author: Dr. Satadal Das, Department of Tissue Culture Unit, Heritage Institute of Technology, Chowbhaga, Kolkata, India. satadal.das@heritageit.edu

Received: 18 April 2022 **Accepted:** 11 January 2023 **Published:** 26 September 2023 **DOI** 10.25259/ASJO-2022-28-(372)

lymphocytes and macrophages and interference with the activity of nuclear factor-kappa-B (NF-κB).[6–9] Various cucurbitacin compounds have shown antiproliferative effects on different human tumor xenografts and cancer cell lines, which include skin, prostate, breast, liver, lung, uterine cervix, and brain cancers.[4]

MATERIAL AND METHODS

Chemicals and Reagents

DMEM (1X) + GlutaMAX-1 (Dulbecco's Modified Eagle Medium, DMEM), penicillin/streptomycin/amphotericin B solution (100×), F12 (1×), tissue culture grade ethylenediaminetetraacetic acid disodium salt (EDTA), bovine serum albumin (BSA), fetal bovine serum (FBS), iTaqUniversal SYBR Green Supermix, and PBS all were purchased from Gibco-ThermoFisher, RNAiso Plus from Takara, and Bryonia 6C from a government authorized alternative medicine company named "HAPCO," India.

Cell culturing and Proliferation

In this experiment, HepG2 (Human liver carcinoma) cells were cultured in DMEM supplemented with FBS (10%) at 37°C under a humidified atmosphere of 5% CO_2. Cells were cultivated for a minimum of two passages in new media before experiments were initiated.[10] When cell confluence reached ~80%, the cells were seeded in a six well plate and incubated for 24 h. On the day of the experiment, all the plates are observed under a microscope to check optimal cell growth of 3×10^5 cells using a hemocytometer and a photograph is taken. After that, the cells were washed carefully with PBS and a fresh medium was added. Two wells were left intact as a control set, while the other two sets were treated with 100 µL of Alcohol and Bryonia 6C solution respectively. The plate is then incubated for 24 hours at 37°c. After incubation, the cells were extracted from the six wells plate using 1 mL of RNAisoplus and incubated for 5 minutes.

Molecular Biology Study

All samples were analyzed in biological duplicates. Total RNA isolation was done by TRIzol™ Reagent according to the manufacturer's instructions. After extraction, the RNA samples were estimated for purity, and cDNA synthesis was accomplished using a cDNA synthesis kit (BioRad, USA). The resulting cDNA was divided into aliquots for PCR amplification of the cDNA products using various sets of sense-antisense primers and iTaq Universal SYBR Green Super-mix (Gibco-BRL).[11] The sequences for the primers were as follows:

IFN-γ (sense: GAGTGTGGAGACCATCAAGGAAG, antisense: TGCTTTGCGTTGGACATTCAAGTC) IL-6 (sense: AGACAGCCACTCACCTCTTCAG, antisense: TTCTGCCAGTGCCTCTTTGCTG), IL-8 (sense: GAGAGTGATTGAGAGTGGACCAC, antisense: CACAACCCTCTGCACCCAGTTT), IL-10 (sense: TCTCCGAGATGCCTTCAGCAGA, antisense: TCAGACAAGGCTTGGCAACCCA), IL-1β (sense: CCACAGACCTTCCAGGAGAATG, antisense: GTGCAGTTCAGTGATCGTACAGG), TGF-β1 (sense: TACCTGAACCCGTGTTGCTCTC, antisense: GTTGCTGAGGTATCGCCAGGAA) TGF-β3 (sense: CTAAGCGGAATGAGCAGAGGATC, antisense: TCTCAACAGCCACTCACGCACA), TNF-α (sense: CTCTTCTGCCTGCTGCACTTTG, antisense: ATGGGCTACAGGCTTGTCACTC)

Estimation of INFγ, IL-6, IL-8, IL-10, IL-1β, TGF- β1, TGF- β3, and TNF-alpha expressions

Comparative gene expression analysis was done using real-time PCR (Bio-Rad, CFX96) with Taq polymerase enzyme tagged with SYBR Green dye, dNTPs, Taq polymerase, MgCl2, buffer, etc. The changes of gene expressions were recorded and analyzed as fold increase or decrease from the value of the normal control and compared with a house-keeping gene β-actin.

Viability assays

HepG2 cells were seeded at a density of 3×10^5 cells in 96-well plates with a medium and then incubated for 48 hours at 37°C.[12] After incubation, 10 µL of the Bryonia sample is added. For performing this assay, three types of sets were taken: (a) media with cells (control), (b) media with cells and alcohol, and (c) media with cells and drug. The plates are incubated for 1 hour after inoculation. Ten microliters of the MTT reagent was added per well and incubated for another 4 hours. The supernatant was removed and 200 µL of stop solution was added. The absorbance (A) was measured with a Read well TouchMicroplate reader at 570 nm.[13]

RESULTS

When Bryonia 6C was applied on HepG2 cells, all the cells became smaller in size, they were mildly degenerated, and methylene blue staining of the cells also indicated few dead cells. This was not the picture when the experiment was done with control alcohol; most cells were good looking and after methylene blue staining, many cells were viable [Figure 1].

MTT assay indicated that toxicity of Bryonia 6C was comparable to the toxicity of the vehicle alcohol by absorbance study [Figure 2]; however with Bryonia cells are smaller in

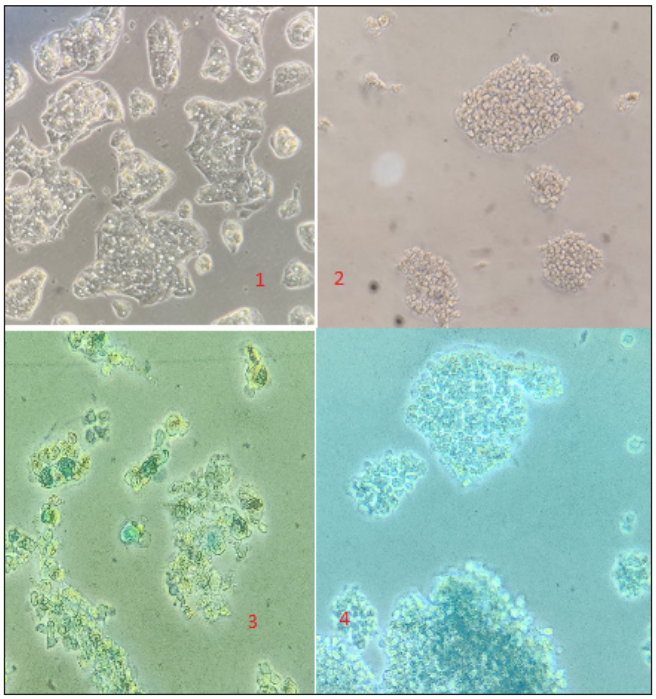

Figure 1: 1 Hep G2 cells (24 h), 2 Bryonia 6C treated HepG2 oells (24 h), 3 Methylene blue stained Bryonia 6C treated HepG2 cells (24 h), 4 Methylene blue stained control alcohol treated HepG2 cells (24 h).

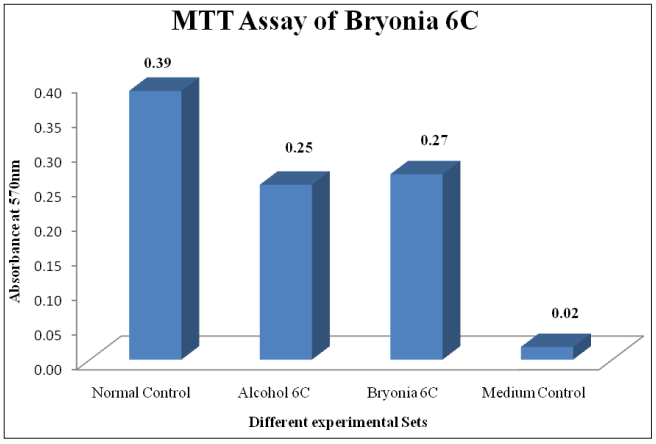

Figure 2: Results of MTT assay of Bryonia 6C on HepG2 cells.

size, degenerated which was not occuring with alcohol. Where the cells were intact although many of them were dead. Thus, the absorbance with Bryonia 6C was mildly increased .

Cytokine studies showed decreased INF-γ (Control alcohol 30.21 , Br 6C 16.06), IL-6 (Control alcohol 8.59, Br 6C 2.23), IL-1β (Control alcohol 15.3, Br 6C 2.47), TNF-α (Control alcohol 5.43, Br 6C 1.57), TGF-β1 (Control alcohol 0.88, Br 6C 0.2), IL-8 (Control alcohol 0.63, Br 6C 0.15), IL-10 (Control alcohol 1.97, Br 6C 0.18), TGF-β3 (Control alcohol 0.95, Br 6C 0.11) when Bryonia 6C was added to HepG2 cells

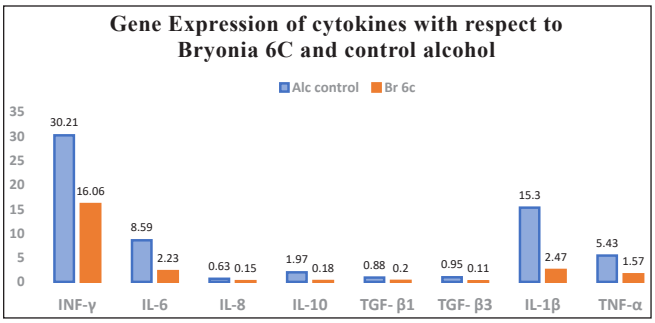

Figure 3: Cytokine gene expressions of HepG2 cells challenged with Bryonia 6C.

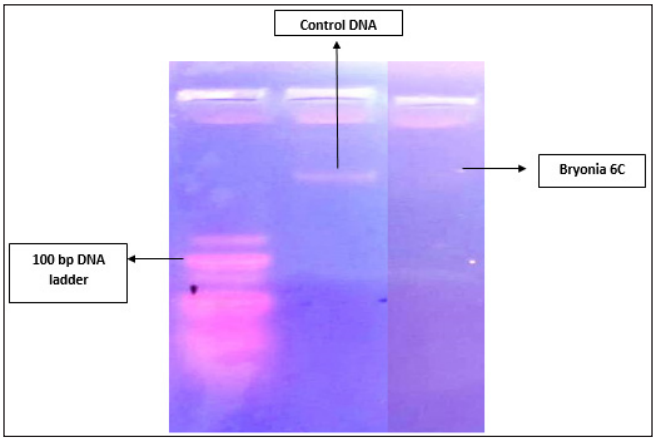

Figure 4: DNA fragmentation analysis of Bryonia 6C challenged HepG2 Cells in comparison to normal control.

[Figure 3]. Thus, there was a cytokine imbalance. Although most of the cells were living after 24 hours, few cells were dead. In general, increased INF-γ usually helps in preventing the spread of cancer cells and it has also antitumorigenic efficacy.[14] Increased IL-6 than IL-10 indicated induction of cytokine imbalance in the malignant cells by Bryonia 6C. The increase of IL-10 was comparatively much less and it cannot counteract IL-6. Thus, all these cytokine changes delineate a probable onset of a storm in the malignant cells in their bioactive chemical environment. This is detrimental and may lead to the later death of the cells. Mild DNA fragmentation was also found [Figure 4].

DISCUSSION

IFN-γ has a unique role in gene regulation as it can exhibit both pro- and anti-inflammatory roles.[15] In the case of human erythroid colony-forming cells (ECFC), INF-γ gene persuades apoptosis and inhibits cell growth.[14,16] In this study, a 1.88-fold decrease of IFN-γ has been observed.

IL-1β acts as a pro-inflammatory cytokine that has been involved in inflammation, pain, and autoimmune conditions.[17] In this study, the expression of IL-1β has been decreased by 6.19-fold,

which indicates that the drug Bryonia has successfully managed to suppress the pro-inflammatory activity of IL-1β.

TNF-α is one of the key pro-inflammatory cytokines.[18] In the case of Bryonia challenged HepG2 cells, the expression was decreased 3.46 times than the control alcohol, which again indicates a positive anti-inflammatory effect of Bryonia.

IL-6 is one of the prime cytokines found at high concentrations in tumors. It plays an important role, and is known to be deregulated in cancer. The overexpression of IL-6 has been seen in almost all types of tumors. In the case of Bryonia challenged human liver carcinoma cells, it showed a substantial 3.85-fold reduction in IL-6 expression. Thus, inhibiting IL-6 signaling with Bryonia could work as a potential treatment for cancers.[19]

IL-8 signaling is responsible for developing angiogenic responses in endothelial cells. IL-8 increases proliferation and helps endothelial and cancer cells to survive. It enhances the migration of endothelial cells, cancer cells, and infiltrating neutrophils at the tumor site.[20] In this study, Bryonia treated HepG2 cells showed four times lesser IL-8 expression, which again indicates the positive effects of this drug.

The function of IL-10 in the development of the tumor is very controversial. Some scientific studies suggest that IL-10 has a positive contribution to tumor growth and promotion, whereas other studies have shown that it aids in the suppression and eradication of metastasis and angiogenesis necessary for long-term patient survival. In this experiment, the drug Bryonia managed to decrease the gene expression by 11-fold, which makes it hard to interpret the result.[21]

TGF-β1 can be activated to inhibit the progression of tumors in HepG2 cells; then again, with the further development and progression of tumors, tumor cells are tolerant to the TGF-β1, resulting in the rapid growth of tumors.[22] In this experiment, Bryonia made a considerable impact on the TGF-β1 gene by downregulating its expression by 4.4-fold.

According to Seystahl *et al*., a study on human glioblastoma showed high TGF-β3 expression in relation to poor survival of the cells; hence, therapeutic targeting of TGF-β3 might be most effective to fight tumors. Fortunately, in this study, Bryonia showed some promising results on human liver carcinoma cells by reducing the gene expression by 8.6-fold.[23]

MTT assay indicated that toxicity of Bryonia 6C was comparable to the toxicity of the vehicle alcohol by absorbance study. DNA fragmentation was also observed using agarose gel electrophoresis and the result indicates that the cells might have gone through apoptosis.

CONCLUSION

The alternative medicine Bryonia 6C is an effective anti-cancer agent against liver cancer cells.

ACKNOWLEDGMENTS

The authors thank Dr. Sajjan Bhajanka Trustee member of the Kalyan Bharti Trust for his support. The authors also thank the Medical Laboratory/Infrastructure support given by Shri Pradip Agarwal (CEO - Heritage Institute of Technology) for this research work.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Declaration of Patient Consent

The authors certify that they have obtained all appropriate patient consent.

REFERENCES

1. Kisling LA, Stiegmann RA. Alternative Medicine. In: StatPearls. Treasure Island (FL): StatPearls Publishing; July 26, 2021.
2. Panossian A, Gabrielian E, Wagner H. Plant adaptogens. II. Bryonia as an adaptogen. Phytomedicine 1997;4:85-99.
3. Ilhan M, Dereli F, Tümen I, Akkol E. Anti-inflammatory and antinociceptive features of Bryonia alba L.: As a possible alternative in treating rheumatism. Open Chemistry 2019;17:23-30.
4. Alghasham AA. Cucurbitacins - a promising target for cancer therapy. Int J Health Sci (Qassim) 2013;7:77-89.
5. Tannin-Spitz T, Bergman M, Grossman S. Cucurbitacin glucosides: Antioxidant and free-radical scavenging activities. BiochemBiophys Res Commun 2007;364:181-6.
6. Escandell JM, Kaler P, Recio MC, Sasazuki T, Shirasawa S, Augenlicht L, *et al*. Activated kRas protects colon cancer cells from cucurbitacin-induced apoptosis: the role of p53 and p21. Biochem Pharmacol 2008;76:198-207.
7. Park CS, Lim H, Han KJ, Baek SH, Sohn HO, Lee DW, *et al*. Inhibition of nitric oxide generation by 23,24-dihydrocucurbitacin D in mouse peritoneal macrophages. J Pharmacol Exp Ther 2004;309:705-10.
8. Escandell JM, Recio MC, Máñez S, Giner RM, Cerdá-Nicolás M, Ríos JL. Cucurbitacin R reduces the inflammation and bone damage associated with adjuvant arthritis in lewis rats by suppression of tumor necrosis factor-alpha in T lymphocytes and macrophages. J Pharmacol Exp Ther 2007;320:581-90.
9. Escandell JM, Recio MC, Máñez S, Giner RM, Cerdá-Nicolás M, Ríos JL. Dihydrocucurbitacin B, isolated from Cayaponiatayuya, reduces damage in adjuvant-induced arthritis. Eur J Pharmacol 2006;532:145-54.
10. ArodinSelenius L, Wallenberg Lundgren M, Jawad R, Danielsson O, Björnstedt M. The Cell Culture Medium Affects Growth, Phenotype Expression and the Response to Selenium Cytotoxicity in A549 and HepG2 Cells. Antioxidants (Basel) 2019;8:130.

11. Gutiérrez-Ruiz MC, Quiroz SC, Souza V, Bucio L, Hernández E, Olivares IP, *et al*. Cytokines, growth factors, and oxidative stress in HepG2 cells treated with ethanol, acetaldehyde, and LPS. Toxicology 1999;134:197-207.
12. Machana S, Weerapreeyakul N, Barusrux S. Anticancer effect of the extracts from Polyalthiaevecta against human hepatoma cell line (HepG2). Asian Pac J Trop Biomed 2012;2:368-74.
13. Duan YM, Jin Y, Guo ML, Duan LX, Wang JG. Differentially expressed genes of HepG2 cells treated with gecko polypeptide mixture. J Cancer 2018;9:2723-33.
14. Jorgovanovic D, Song M, Wang L, Zhang Y. Roles of IFN-γ in tumor progression and regression: a review. Biomark Res 2020;8:49.
15. Markovics A, Rosenthal KS, Mikecz K, Carambula RE, Ciemielewski JC, Zimmerman DH. Restoring the Balance between Pro-Inflammatory and Anti-Inflammatory Cytokines in the Treatment of Rheumatoid Arthritis: New Insights from Animal Models. Biomedicines 2021;10:44. Published 2021 Dec 26.
16. Dai C, Krantz SB. Interferon gamma induces upregulation and activation of caspases 1, 3, and 8 to produce apoptosis in human erythroid progenitor cells. Blood 1999;93:3309-16.
17. Ren K, Torres R. Role of interleukin-1beta during pain and inflammation. Brain Res Rev 2009;60:57-64.
18. Cruceriu D, Baldasici O, Balacescu O, Berindan-Neagoe I. The dual role of tumor necrosis factor-alpha (TNF-α) in breast cancer: molecular insights and therapeutic approaches. Cell Oncol (Dordr) 2020;43:1-18.
19. Kumari N, Dwarakanath BS, Das A, Bhatt AN. Role of interleukin-6 in cancer progression and therapeutic resistance. Tumour Biol 2016;37:11553-72.
20. Waugh DJ, Wilson C. The interleukin-8 pathway in cancer. Clin Cancer Res 2008;14:6735-41.
21. Mannino MH, Zhu Z, Xiao H, Bai Q, Wakefield MR, Fang Y. The paradoxical role of IL-10 in immunity and cancer. Cancer Lett 2015;367:103-7.
22. Sun SL, Wang XY. TGF-β1 promotes proliferation and invasion of hepatocellular carcinoma cell line HepG2 by activating GLI-1 signaling. Eur Rev Med Pharmacol Sci 2018;22:7688-95.
23. Seystahl K, Papachristodoulou A, Burghardt I, Schneider H, Hasenbach K, Janicot M, *et al*. Biological Role and Therapeutic Targeting of TGF-β_3 in Glioblastoma. Mol Cancer Ther 2017;16:1177-86.

How to cite this article: Bandyopadhyay S, Chatterjee D, Singh B, Paira K, Das S. Action of ultra-diluted ethanol extract of *Bryonia alba* on HepG2 liver cancer cells. Asian J Oncol, 2023;9:13.