Egyptian Journal of Medical Human Genetics

RESEARCH **Open Access**

The potential anticancer effect of bee venom in combination with sorafenib against HepG2 cell lines via induction of apoptosis and autophagy candidate genes

Sara A. Nusair[1], Gehan Galal[1] and Sara M. Radwan[2*]

Abstract

Background Hepatocellular carcinoma (HCC) is a severe threat and a main reason for cancer-related deaths around the world. Drug resistance to sorafenib (Sorf), the effective HCC first-line therapy, is very common. A number of natural compounds, notably bee venom (BV), have been claimed to show a great impact against cancer when administered on its own or in conjunction with chemotherapy. Thus, this study aimed to investigate the anticancer effect of BV alone and/or combined with Sorf on HepG2 liver cancer cell lines.

Methods Both mRNA and protein expressions of Bax, Bcl-2 and Beclin-1 were investigated by quantitative real-time PCR (qPCR) and western blot respectively, to examine the apoptotic and autophagic regulatory effects of BV and Sorf single treatments plus BV/Sorf combination on HepG2 cell lines.

Results Our findings showed that BV and Sorf had considerable dose-dependent anti-proliferative effects on HepG2 cells whether administered alone or in combination, with the greatest impact for the combined therapies. Single BV and Sorf treatments showed IC_{50} of 93.21 and 7.28 µg/ml respectively, while combined treatment showed IC_{50} of 6.73 µg/ml BV + 6.73 µg/ml Sorf. Moreover, both the pro-apoptotic gene Bax and the autophagy-related gene Beclin-1 showed significant up-regulation in their mRNA expression, while the anti-apoptotic Bcl-2 mRNA gene expression showed significant down-regulation after BV/Sorf treatment as compared to either BV or Sorf single treatment. These qPCR results were further confirmed by western blot.

Conclusions These findings indicate that BV synergistically potentiates the anticancer effect of Sorf on HepG2 cells through induction of apoptotic and autophagic machineries.

Keywords Hepatocellular carcinoma, Sorafenib, Bee venom, Apoptosis, Autophagy, Bax, Beclin-1

Introduction

The prevalence of hepatocellular carcinoma (HCC), the third reason of cancer mortality around the world, continues to rise [1, 2]. HCC risk factors include chronic hepatitis B and hepatitis C virus infections, autoimmune hepatitis, obesity as well as chronic alcohol use. Viral hepatitis and liver cirrhosis persuade inflammation and oxidative stress cascades, leading to cytokines, chemokines and free radicals production, finally causing cellular injury, proliferation and malignant

*Correspondence:
Sara M. Radwan
sara.mostafa@pharma.asu.edu.eg
[1] Egyptian Company for Blood Transfusion Services Affiliate of the Holding Company for Biological Products and Vaccines "VACSERA", Giza, Egypt
[2] Biochemistry Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt

© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

transformation [3]. HCC treatment options include the anticancer drug, sorafenib (Sorf), surgical resection and liver transplantation. Although surgical treatment may be the best option for HCC, unfortunately only 20% of cases are suitable for surgical resection [4].

Sorf, a multiple tyrosine kinase inhibitor, is a well-accepted chemotherapeutic drug for advanced HCC due to its anti-angiogenic and anti-proliferative effect [5]. Patients on Sorf treatment suffers several side effects such as anorexia, weight loss, nausea, vomiting, diarrhea, hypertension, and risk of cardiac events and drug resistance [6]. Consequently, it is compulsory to find an effective and safe alternative or adjuvants for Sorf.

Arthropod extracts, such as bee, spider, snake and scorpion venom, as well as plant and marine products have all lately demonstrated significant value as natural compounds in the treatment of cancer. Bee venom (BV), a unique multi-component complex, has been known as a traditional medicine for its wide antibacterial, antiviral and anti-inflammatory effects. It is rich in peptides, counting melittin, phospholipase A2 and apamin, as well as non-peptide components such as free amino acids, lipids and carbohydrates [7]. It has been widely applied as a treatment for a wide diversity of diseases such as back pain, musculoskeletal pain, arthritis, rheumatism and cancerous tumors. The antitumor activity of BV has been attributed to its ability to inhibit tumor cell growth, proliferation of cancer cells and metastasis [8], thus suggesting its promising rising application as an adjuvant for cancer chemotherapy or as an alternative medicine treatment for a wide variety of tumors.

Both apoptosis and autophagy are processes of programmed cell death that play vital roles in different cancers. Thus, their regulation in normal and cancer cells has become an essential topic in cancer research [8]. There are two identified discrete pathways of apoptosis: the extrinsic pathway, which functions independently of mitochondria [9], and the intrinsic mitochondrial pathway that is regulated by the Bcl-2 family of proteins [10]. Anti-apoptotic proteins and pro-apoptotic proteins are considered two functionally separate groups within the Bcl-2 family. Whereas Bax, a pro-apoptotic protein, is abundantly expressed through apoptosis promoting cell death, Bcl-2, an anti-apoptotic protein, defends against cell death [11]. Bcl-2 prevents apoptosis by maintaining the mitochondrial membrane. Additionally, it interacts with and deactivates Bax and other pro-apoptotic proteins, preventing apoptosis [12]. It is generally known that a high Bax-to-Bcl-2 ratio causes release of cytochrome c from the mitochondria, which in turn results in apoptosis. As a result, it is believed that cells' propensity for apoptosis is influenced by Bax/Bcl-2 ratio [13].

It is important to know that there is a long debate over the connection between autophagy and apoptosis. Beclin-1, one of many genes involved in autophagy, is crucial in the coordination of autophagy, indicating that triggering its expression also triggers the autophagy pathway. Beclin-1 was found to directly interact with Bcl-2 proteins family members [14].

Nevertheless, whether BV may impact the malignant behavior of HCC by modifying Bax, Bcl-2 and Beclin-1 expression or regulating the interaction between apoptosis and autophagy is still not known. Therefore, this study was designed to explore the anticancer effect of BV alone and combined with Sorf on HepG2 liver cancer cell lines as well as to understand the impact of BV on molecular pathways of coordination between autophagy and apoptosis and to determine the potentiality of using it as an adjuvant for Sorf in HCC cell lines to improve the therapeutic outcome.

Materials and methods

Preparation of Bee venom and Sorafenib

Bee venom from the Egyptian strain of honey bee Apis mellifera was bought from Vacsera sera plant Unit (Giza, Egypt) and prepared in phosphate buffer saline (PBS). Sorafenib was provided by Cipla (Mumbai, India) in the form of tablets with Strength: 200 mg., Batch no: GJ90426 and prepared in dimethyl sulfoxide (DMSO).

Cell lines and cell culture

Hepatocellular carcinoma cell line (HepG2) was purchased from (Vacsera Tissue Culture Unit, Giza, Egypt). HepG2 cells were cultured and maintained in RPMI 1640 medium (Biowest, France; Cat.no.Ms00LZ) enhanced with 10% FBS and 1% penicillin-streptomycin and was incubated (37 °C and 5% CO_2). Cells were regularly rinsed by PBS (pH 7.4), detached using 0.25% Trypsin-EDTA and sub-cultured in RPMI 1640 after reaching 80-90% confluence.

The study was approved by the Research Ethics Committee, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt (ENREC-ASU. 2019-278).

Cytotoxicity analysis

HepG2 cells were seeded separately in a 96-well plate [1×10^4 cells/well, 100 µl/well]. The treatments with various concentrations (0, 1.95, 3.9, 7.81, 15.62, 31.25, 62.5, 125 and 250 µg/ml) were added to HepG2 and divided into vehicle-untreated, BV-treated, Sorf-treated and BV/Sorf (equal concentrations of each combined in a ratio of 1:1)-treated cells and incubated for 24 h. Then, cells were treated for 4 hours with MTT (5 mg/ml, Sigma). After the incubation period was up, the media was removed, and formazan crystals (MTT metabolic product) were

dissolved using 100 µL DMSO (Sigma) and vortexed for 20 minutes. An optical microplate reader was used to measure absorbance at 570 nm. The assay was performed in triplicate. From the dose–response sigmoidal curve, the concentration of BV, Sorf and BV/Sorf inhibiting 50% of cells (IC_{50}) was estimated via GraphPad Prism 7 statistic software.

Morphology study

HepG2 cells were stained with crystal violet stain after being treated with different doses of BV, Sorf and BV/Sorf. By using inverted light microscopy, the treated cells were photographed and compared to untreated cells.

Beclin-1, Bax and Bcl-2 genes expression analysis

Qiagen RNA extraction kit was used for extracting the total RNA from the cells. RNA concentration and purity were verified by Nanodrop. cDNA synthesis was carried out using Maxima first-strand cDNA synthesis Kit. Primer sequences of Beclin-1, Bax and Bcl-2 genes were supplied from Thermo Scientific, USA. Quantitative real-time PCR (qPCR) reactions were carried out using Bio-Rad SYBR Green PCR master mix according to the manufacturer's instruction and run on a real-time Rotor-Gene 1.7.87 system. Each gene expression was normalized to the housekeeping gene glyceraldehyde3-phosphate dehydrogenase (GAPDH). All reactions were performed in triplicate. Fold changes in gene expression were calculated using $2^{-\Delta\Delta CT}$ method.

Beclin-1, Bax and Bcl-2 proteins expression analysis

Protein expression levels of Beclin-1, Bax and Bcl-2 were estimated by western blotting according to standard techniques [15] using the following primary antibodies supplied from Thermo Fisher Scientific, USA: Beclin-1 Monoclonal Antibody (Product # MA5-15825), Bax monoclonal antibody (Product # MA5-14003) and Bcl-2 monoclonal antibody (Product # MA5-11757). Band density was quantified using ImageJ software. The relative levels of proteins were normalized to β-actin.

Statistical analysis

Calculation of IC_{50} was done using the dose–response sigmoidal curve via GraphPad Prism 7 statistic software. Data analysis was done using IBM SPSS Statistics version 23 (IBM© Corp., Armonk, NY). Data were presented as the mean ± SE of replicates from all independent experiments. The statistical significance was calculated by one-way analysis of variance (ANOVA) followed by the post Hoc Tukey test. When $p < 0.05$, the values were considered statistically significant.

Results

Effects of bee venom, sorafenib and bee venom/sorafenib on HepG2 cell viability

Analysis of cytotoxicity using MTT assay confirmed dose-dependent anti-proliferative activity of BV and Sorf as single treatments on HepG2 cells when compared to untreated cells. Single BV (Fig. 1A) and Sorf (Fig. 1B) treatments showed IC_{50} of 93.21 and 7.28 µg/ml respectively, on HepG2 cells. Likewise, combined treatment of BV/Sorf presented a dose-dependent anti-proliferative effect on HepG2 cells with IC_{50} of 6.73 µg/ml, showing a higher cytotoxic effect on HepG2 cells than single treatments (Fig. 1C).

Effect of bee venom, sorafenib and bee venom/sorafenib on HepG2 cell morphology

As shown in Fig. 2, untreated cells showed normal morphology, being closely arranged and well adherent in large numbers. Following treatment with different concentrations of BV, Sorf and BV/Sorf for 24 hrs, the density of cells diminished and morphology altered showing fragmentation, cell shrinkage, poor adherence and rounding in dose-dependent manner.

Effect of bee venom, sorafenib and bee venom/sorafenib on apoptosis and autophagy-related genes expression levels in HepG2 cells

As shown in Table 1, both the autophagy-related gene Beclin-1 and pro-apoptotic gene Bax showed significant up-regulation in their gene expression after BV/Sorf treatment by 38.19% and 93.06 %, respectively, as compared to BV treatment group and by 151.56% and 41.84%, respectively, as compared to Sorf treatment group at $p < 0.05$. On the other hand, the anti-apoptotic Bcl-2 gene showed significant down-regulation in its expression after BV/Sorf treatment by 76 % and 76.92 % as compared to both BV treatment group and Sorf treatment group, respectively, at $p < 0.05$. Concerning Bax/Bcl-2 ratio after BV/Sorf treatment, it was elevated by 8.7 and 6.6 folds as compared to both BV treatment group and Sorf treatment group, respectively, at $p < 0.05$.

Effect of bee venom, sorafenib and bee venom/sorafenib on apoptosis and autophagy-related proteins expression levels in HepG2 cells

As shown in Fig. 3, treatment with BV/Sorf showed significantly up-regulated Beclin-1 and Bax protein expression as well as significant decrease of anti-apoptotic Bcl-2 protein expression by 172.72%, 146.58% and 36.25%, respectively, as compared to BV treatment group ($p < 0.05$) and by 36.36%, 159.48% and 34.62%,

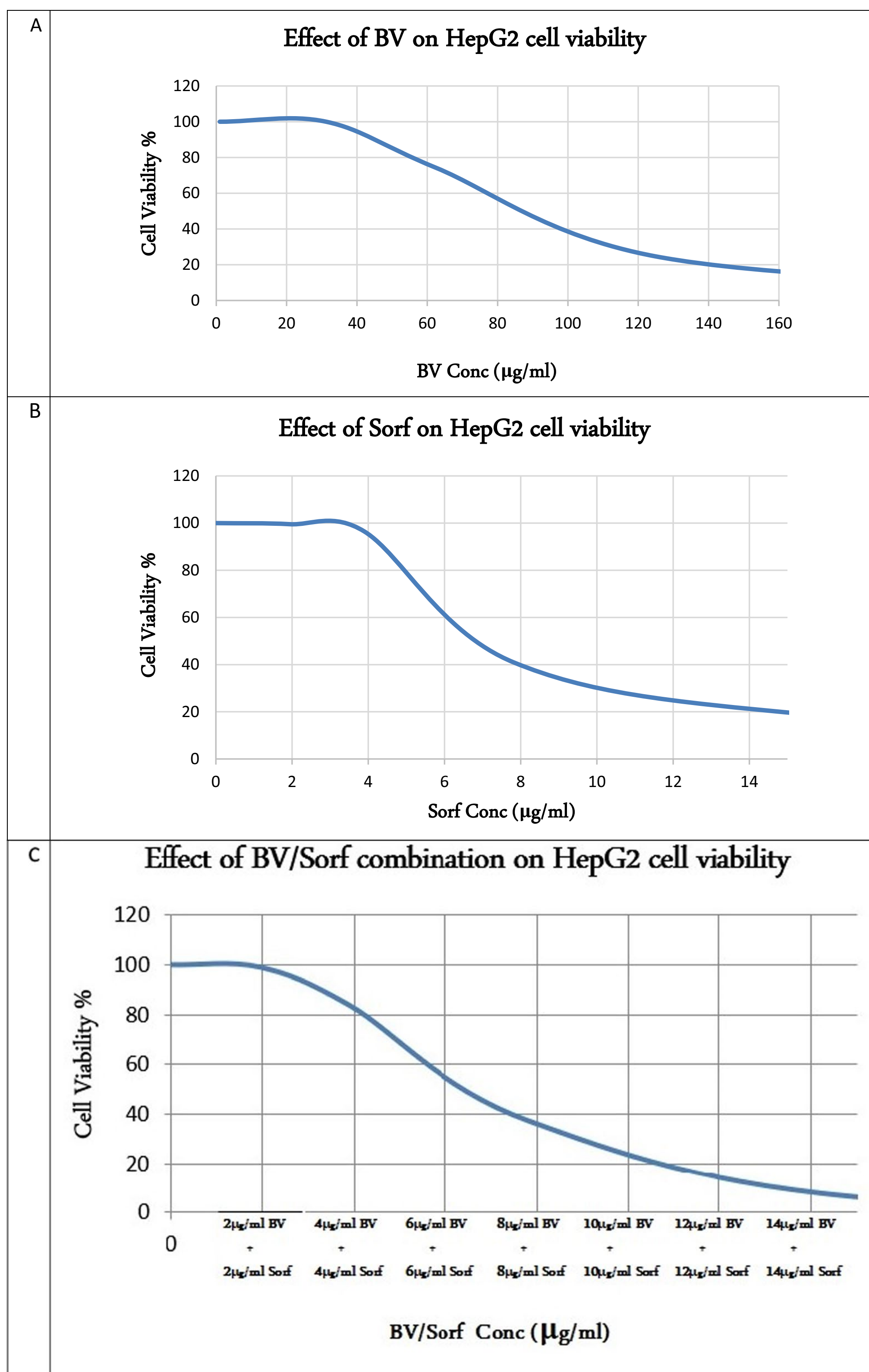

Fig. 1 The sigmoidal dose–response curve for MTT assay showing IC_{50} values after 24-h treatment with **A** BV, **B** Sorf and **C** BV/Sorf (equal concentrations of each combined in a ratio of 1:1) on HepG2 cells. Data were normalized to untreated control cells and expressed as the mean ± SE. Samples ran in triplicate in 3 independent experiments

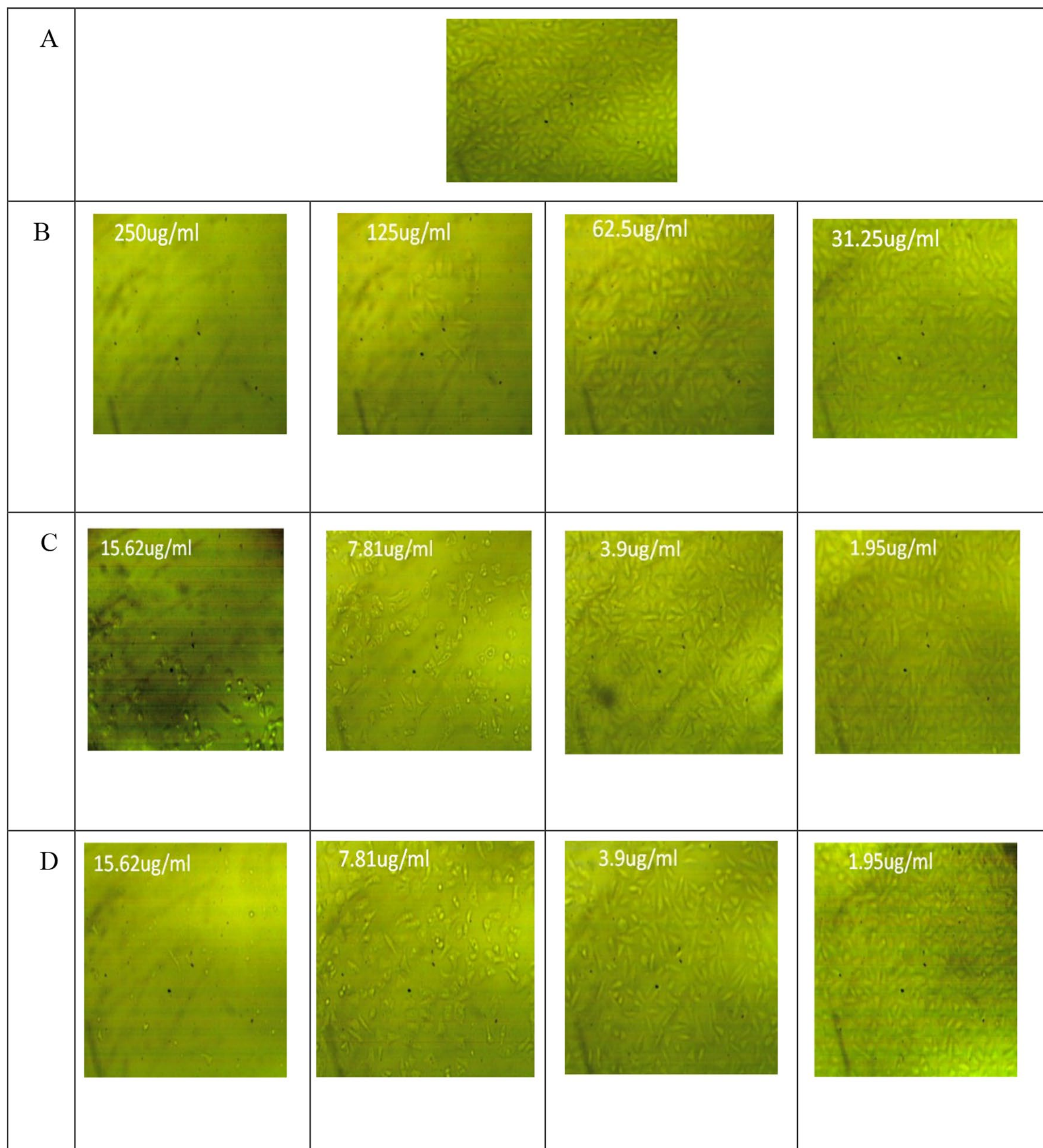

Fig. 2 Morphological alterations of HepG2 cells after 24-h treatment with different concentrations of BV, Sorf and BV/Sorf observed by inverted light microscopy. **A** Untreated cells, **B** BV-treated cells, **C** Sorf-treated cells and **D** BV/Sorf-treated cells. The results present one representative experiment of three independently performed that showed similar patterns

respectively, as compared to Sorf treatment group ($p < 0.05$). For Bax/Bcl-2 ratio it was significantly elevated by 3.82 and 3.98 folds after BV/Sorf treatment as compared to both BV treatment group and Sorf treatment group, respectively, at $p < 0.05$.

Discussion

Chemotherapeutics are still the major option for cancer therapy, although providing inadequate outcome as well as affecting normal cells. Another main obstacle is chemo-resistance developed following initial treatment.

Table 1 Gene expression levels of Beclin-1, Bax, Bcl-2 and Bax/Bcl-2 ratio in untreated HepG2 cells, BV-treated cells, Sorf-treated cells and BV/Sorf-treated cells

	Untreated cells	BV-treated cells	Sorf-treated cells	BV/Sorf-treated cells
Beclin-1	1.01 ± 0.09	2.33 ± 0.27[a]	1.28 ± 0.15	3.22 ± 0.23[a,b,c]
Bax	1.03 ± 0.14	3.60 ± 0.79[a]	4.90 ± 0.24[a]	6.95 ± 0.09[a,b,c]
Bcl-2	1.03 ± 0.16	0.50 ± 0.02[a]	0.52 ± 0.04[a]	0.12 ± 0.02[a,b,c]
Bax/Bcl-2	1.07 ± 0.22	7.24 ± 1.66[a]	9.58 ± 0.74[a]	63.25 ± 9.69[a,b,c]

Samples ran in triplicates in 3 independent experiments. The used treatment concentrations are those of IC_{50}. Data expressed as the mean fold change ± SE

[a] Significantly different from untreated cells at $p < 0.05$

[b] Significantly different from BV-treated cells at $p < 0.05$

[c] Significantly different from Sorf-treated cells at $p < 0.05$

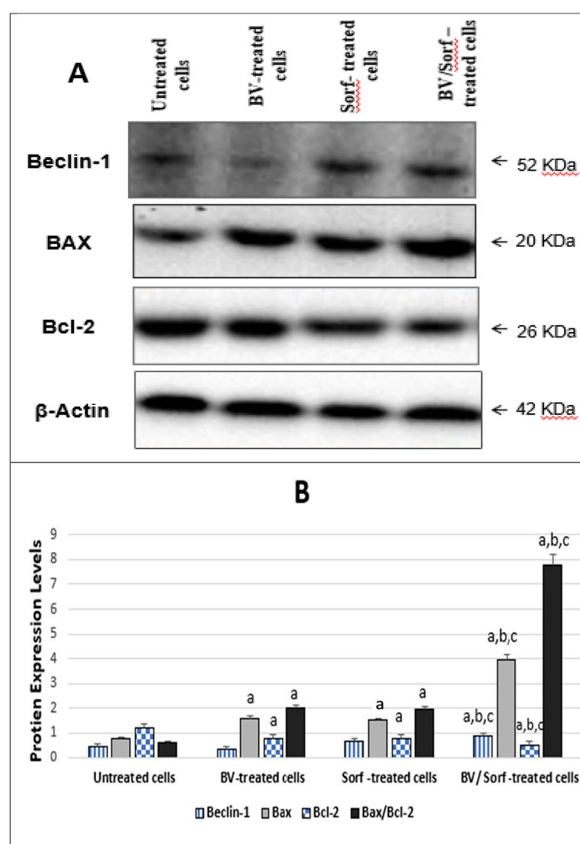

Fig. 3 The protein expressions of Beclin-1, Bax and Bcl-2 in untreated HepG2 cells, BV-treated cells, Sorf-treated cells and BV/Sorf-treated cells. **A** Representative blots **B** quantitative analysis. Samples ran in triplicates in three independent experiments. Data expressed as the mean ± SE. **a** significantly different from untreated cells at $p < 0.05$. **b** significantly different from BV-treated cells at $p < 0.05$. **c** significantly different from Sorf-treated cells at $p < 0.05$

This has encouraged the extensive research on using safe natural anticancer drugs. The massive diversity in venoms and toxins made them an incomparable source for developing novel therapeutics. Recently, studies have been directed to finding new strategies for treatment of HCC, either by looking for Sorf substitutes having less side effects and resistance or by combining it with natural compounds [16]. We hypothesized that combining BV with Sorf might have a positive synergistic impact. However, different biochemical and biological factors may affect this interaction. Thus, this study was concerned with investigating the antitumor impact of BV as well as its prospective interactive mechanism when being combined with Sorafenib in HCC treatment.

We used the well-differentiated hepatocyte cell line, HepG2, which closely resembles the human hepatocyte in culture to study the cytotoxic effect of BV and Sorf alone along with BV/Sorf combination. Our results provided evidence that BV/Sorf combination leads to potentiated cytotoxicity in the HepG2 cell lines with IC_{50} of 6.73 μg/ml BV + 6.73 μg/ml Sorf as compared to bee venom treatment alone (IC_{50} of 93.21 μg/ml) or sorafenib treatment alone (IC_{50} of 7.28 μg/ml). These findings are supported by earlier studies which showed that the IC_{50} values of Sorf on HepG2 cells were in range of 7.0 to 19.5 μg/ml after 24 hrs and 3.4 to 12.0 μg/ml after 48 h [5, 17]. Our results also support many studies that established BV anticancer effect [18–20]. For instance, the study of Gajski et al. demonstrated the anti-proliferative effects of BV on cervical cancer cell lines [21] and Jang et al. study revealed its anticancer effect on the human lung cancer cell line NCI-H1299 [11]. Different several mechanisms have been reported concerning BV cytotoxicity against a diverse number of cancers including altering cell cycle, affecting cell growth and inducing apoptosis or autophagy [13, 22, 23].

Both apoptosis and autophagy are tumor-suppressive mechanisms. While apoptosis stops cancer cells from surviving, autophagy enables the destruction of oncogenic chemicals, preventing cancer growth. Accordingly, inadequate or defective autophagy or apoptosis may cause cancer [24]. It is established that the optimum method for an antitumor drug to work is to lead cancer cells to undergo apoptosis [13].

In this study we examined the apoptotic regulatory effect of BV and Sorf single treatments in addition to BV/Sorf combination on HepG2 cell lines. Our results revealed significant inhibition of the anti-apoptotic Bcl-2 gene expression along with significant induction of Bax gene expression and Bax/Bcl-2 ratio in both Sorf and BV single treatment groups in comparison to the control untreated group, which was further confirmed by the results of protein expression. Moreover, results of these genes expression showed a superior effect of Sorf over BV single treatments, but it was not statistically significant. The effect of Sorf on apoptosis in the present study is in line with the study of Garten et al., where Sorf treatment tempted apoptosis in HepG2, Hep3B and HUH7 HCC cell lines [25]. Similarly, other investigations demonstrated that Sorf triggers the intrinsic route of apoptosis by cytochrome c release and mitochondrial translocation of Bax [26, 27]. Furthermore, in the study of Ip et al., it was discovered that BV triggered apoptosis in human cervical carcinoma cells by up-regulating pro-apoptotic Bax expression, down-regulating anti-apoptotic Bcl-2 expression and increasing cytochrome c release [13]. Another study found that BV affected Bax and Bcl-2 expression in human breast cancer MCF7 cells, which in turn caused apoptosis and decreased cancer growth [28].

Interestingly, HepG2 cells treated with the combined Sorf/BV treatment showed significantly the lowest Bcl2 expression and the highest Bax expression and Bax/Bcl-2 ratio as compared to each of the single treatment groups, indicating higher apoptotic rate and synergism. This reveals that BV may synergistically potentiate the apoptotic effect of Sorf on HepG2 cells. Likewise, the study of Khamis et al. demonstrated that BV synergistically potentiates the antitumor effect of tamoxifen against breast cancer cells [29].

Autophagy, a cytoprotective process and a quality control mechanism, degrades unnecessary cellular components through lysosomal enzymes [30]. Autophagy means timely preventing the occurrence of cellular abnormalities such as tumorigenesis. Extensive research has shown how closely autophagy and cancer development are related. In cancer research, both autophagy activation and inhibition have frequently been studied [31].

Little is known about the effect of BV on autophagy; thus, we investigated the effect of BV alone or in combination with Sorf on the expression of Beclin-1, a key regulator of autophagy. Interestingly, the present study revealed that administration of BV induced Beclin-1 gene expression in HepG2 cells as compared to untreated cells. Moreover, treatment with BV/Sorf significantly triggered autophagy, which was obvious by significant up-regulation of Beclin-1 gene and protein expressions as compared to both the BV-treated and Sorf-treated cells. The study of Tai et al. demonstrated that activation of Beclin-1 mediates autophagic cell death induced by sorafenib in hepatocellular carcinoma cells [32]. Furthermore, the study of He et al. demonstrated that treatment with Melittin, the major component of BV, induced autophagy of fibroblast like synoviocytes in rheumatoid arthritis patients [33]. To the best of our knowledge, this is the first study revealing the anticancer effect of BV against HepG2 cells through induction of autophagy.

The composition of BV includes a variety of peptides such as Melittin which is the chief BV peptide constituent. The study of Mansour et al. demonstrated that each BV or Melittin may enhance the activity of Sorf against HepG2 cell line by targeting diverse cell transduction

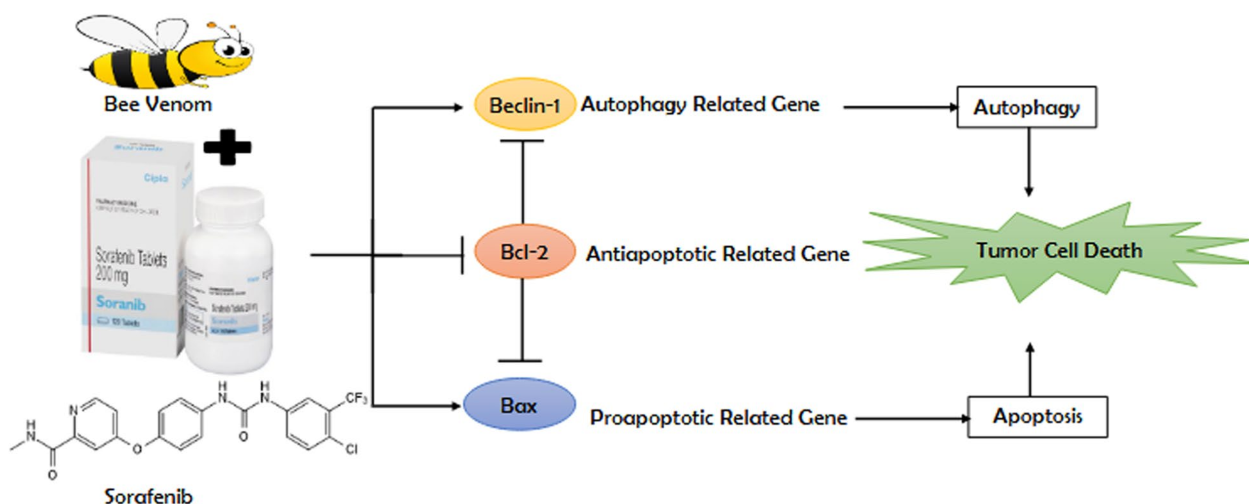

Fig. 4 Graphical representation of the prospective molecular mechanism of BV/Sorf against HCC

machineries leading to cell death, with no significant difference between the effect of BV and Melittin when used either alone or combined with Sorf [16].

All provided in vitro results on HepG2 cells ascertain the efficiency of BV/Sorf drug combination. As shown in Fig. 4, the fundamental mechanism appears to be related with the induction of autophagy concurrently with initiation of the intrinsic apoptotic cascade pathway, ultimately causing diminished cell proliferation.

Finally, it is now clear that new HCC treatment options are necessary due to Sorf resistance, toxicity and loss of efficacy after long-term use. Thus, we investigated the efficacy of BV aiming to provide patients with better outcomes, less systemic toxicity and fewer side effects. From our results, BV may be a promising adjuvant for Sorf but still more future in vitro investigations and in vivo studies on animals HCC model are essential to confirm our results. Moreover, future studies concerning the anticancer efficacy of BV individual components such as melittin, apamin, hyaluronidase and other components needs to be investigated.

Conclusion

The present study hereby reveals that the natural product BV may synergistically enhance the activity of the anticancer drug Sorf against HepG2 cells probably by inducing apoptosis and autophagy. This may possibly provide a prospective tool for creating a novel therapeutic strategy for HCC. However, further future in vivo studies and clinical trials are still crucial to validate the potentiality and safety of this combination.

Abbreviations

HCC	Hepatocellular carcinoma
HepG2	Hepatocellular carcinoma cell line
Sorf	Sorafenib
BV	Bee venom
PBS	Phosphate buffer saline
qPCR	Quantitative real-time PCR

Acknowledgements

Not applicable.

Author contributions

SMR contributed to the study conception, design, methodology, investigation and data analysis. SAN contributed to design, methodology, validation and resources. SMR and SAN wrote the manuscript. GG contributed to methodology, investigation, validation and resources. All authors read and approved the final manuscript.

Funding

No funds, grants or other support was received.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

The study was approved by the Research Ethics Committee, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt (ENREC-ASU. 2019-278).

Consent for publication

Not applicable.

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Received: 7 August 2023 Accepted: 1 April 2024
Published online: 08 April 2024

References

1. Hann HW, Wang M, Hafner J, Long RE, Kim SH, Ahn M et al (2010) Analysis of GP73 in patients with HCC as a function of anti-cancer treatment. Cancer Biomark 7:269–273
2. Couri T, Pillai A (2019) Goals and targets for personalized therapy for HCC. Hepatol Int 13:125–137
3. Wangensteen KJ, Chang KM (2021) Multiple roles for hepatitis B and C viruses and the host in the development of hepatocellular carcinoma. Hepatology 73(Suppl 1):27–37
4. Galle PR, Dufour JF, Peck-Radosavljevic M, Trojan J, Vogel A (2021) Systemic therapy of advanced hepatocellular carcinoma. Future Oncol 17:1237–1251
5. Cervello M, Bachvarov D, Lampiasi N, Cusimano A, Azzolina A, McCubrey JA et al (2012) Molecular mechanisms of sorafenib action in liver cancer cells. Cell Cycle 11:2843–2855
6. Abdel-Rahman O, Lamarca A (2017) Development of sorafenib-related side effects in patients diagnosed with advanced hepatocellular carcinoma treated with sorafenib: a systematic-review and meta-analysis of the impact on survival. Expert Rev Gastroenterol Hepatol 11:75–83
7. Hartmann A, Müllner J, Meier N, Hesekamp H, van Meerbeeck P, Habert MO et al (2016) Bee venom for the treatment of Parkinson disease—a randomized controlled clinical trial. PLoS ONE 11:e0158235
8. Zhou J, Qi Y, Diao Q, Wu L, Du X, Li Y et al (2013) Cytotoxicity of melittin and apamin in human hepatic L02 and HepG2 cells in vitro. Toxin Rev 32:60–67
9. Wang ZZ, Huang TY, Gong YF, Zhang XM, Feng W, Huang XY (2020) Effects of sorafenib on fibroblast-like synoviocyte apoptosis in rats with adjuvant arthritis. Int Immunopharmacol 83:106418
10. Tzifi F, Economopoulou C, Gourgiotis D, Ardavanis A, Papageorgiou S, Scorilas A (2012) The role of BCL2 family of apoptosis regulator proteins in acute and chronic leukemias. Adv Hematol 2012:524308
11. Jang MH, Shin MC, Lim S, Han SM, Park HJ, Shin I et al (2003) Bee venom induces apoptosis and inhibits expression of cyclooxygenase-2 mRNA in human lung cancer cell line NCI-H1299. J Pharmacol Sci 91:95–104
12. Wasfey EF, Shaaban M, Essam M, Ayman Y, Kamar S, Mohasseb T et al (2023) Infliximab ameliorates methotrexate-induced nephrotoxicity in experimental rat model: impact on oxidative stress, mitochondrial biogenesis, apoptotic and autophagic machineries. Cell Biochem Biophys 81:717–726
13. Ip SW, Wei HC, Lin JP, Kuo HM, Liu KC, Hsu SC et al (2008) Bee venom induced cell cycle arrest and apoptosis in human cervical epidermoid carcinoma Ca Ski cells. Anticancer Res 28:833–842
14. Erlich S, Mizrachy L, Segev O, Lindenboim L, Zmira O, Adi-Harel S et al (2007) Differential interactions between Beclin 1 and Bcl-2 family members. Autophagy 3:561–568
15. Burnette WN (1981) "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate–polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal Biochem 112:195–203
16. Mansour GH, El-Magd MA, Mahfouz DH, Abdelhamid IA, Mohamed MF, Ibrahim NS et al (2021) Bee venom and its active component Melittin

synergistically potentiate the anticancer effect of Sorafenib against HepG2 cells. Bioorg Chem 116:105329
17. Chen W, Xiao W, Zhang K, Yin X, Lai J, Liang L et al (2016) Activation of c-Jun predicts a poor response to sorafenib in hepatocellular carcinoma: preliminary clinical evidence. Sci Rep 6:22976
18. Oršolić N (2012) Bee venom in cancer therapy. Cancer Metastasis Rev 31:173–194
19. Zhao J, Hu W, Zhang Z, Zhou Z, Duan J, Dong Z et al (2022) Bee venom protects against pancreatic cancer via inducing cell cycle arrest and apoptosis with suppression of cell migration. J Gastrointest Oncol 13:847–858
20. Liu CC, Hao DJ, Zhang Q, An J, Zhao JJ, Chen B et al (2016) Application of bee venom and its main constituent melittin for cancer treatment. Cancer Chemother Pharmacol 78:1113–1130
21. Gajski G, Čimbora-Zovko T, Rak S, Rožman M, Osmak M, Garaj-Vrhovac V (2014) Combined antitumor effects of bee venom and cisplatin on human cervical and laryngeal carcinoma cells and their drug resistant sublines. J Appl Toxicol 34:1332–1341
22. Liu X, Chen D, Xie L, Zhang R (2002) Effect of honey bee venom on proliferation of K1735M2 mouse melanoma cells in-vitro and growth of murine B16 melanomas in-vivo. J Pharm Pharmacol 54:1083–1089
23. Hu H, Chen D, Li Y, Zhang X (2006) Effect of polypeptides in bee venom on growth inhibition and apoptosis induction of the human hepatoma cell line SMMC-7721 in-vitro and Balb/c nude mice in-vivo. J Pharm Pharmacol 58:83–89
24. Su M, Mei Y, Sinha S (2013) Role of the crosstalk between autophagy and apoptosis in cancer. J Oncol 2013:102735
25. Garten A, Grohmann T, Kluckova K, Lavery GG, Kiess W, Penke M (2019) Sorafenib-induced apoptosis in hepatocellular carcinoma is reversed by SIRT1. Int J Mol Sci 20:4048
26. Rahmani M, Davis EM, Crabtree TR, Habibi JR, Nguyen TK, Dent P et al (2007) The kinase inhibitor sorafenib induces cell death through a process involving induction of endoplasmic reticulum stress. Mol Cell Biol 27:5499–5513
27. Lange M, Abhari BA, Hinrichs TM, Fulda S, Liese J (2016) Identification of a novel oxidative stress induced cell death by Sorafenib and oleanolic acid in human hepatocellular carcinoma cells. Biochem Pharmacol 118:9–17
28. Ip SW, Liao SS, Lin SY, Lin JP, Yang JS, Lin ML et al (2008) The role of mitochondria in bee venom-induced apoptosis in human breast cancer MCF7 cells. In Vivo 22:237–245
29. Khamis AAA, Ali EMM, El-Moneim MAA, Abd-Alhaseeb MM, El-Magd MA, Salim EI (2018) Hesperidin, piperine and bee venom synergistically potentiate the anticancer effect of tamoxifen against breast cancer cells. Biomed Pharmacother 105:1335–1343
30. Radwan SM, Hamdy NM, Hegab HM, El-Mesallamy HO (2016) Beclin-1 and hypoxia-inducible factor-1α genes expression: Potential biomarkers in acute leukemia patients. Cancer Biomark 16:619–626
31. Lv C, Zhang Z, Zhao T, Han MF, Jia DP, Su LZ et al (2019) The anti-tumour effect of Mel and its role in autophagy in human hepatocellular carcinoma cells. Am J Transl Res 11:931–941
32. Tai WT, Shiau CW, Chen HL, Liu CY, Lin CS, Cheng AL et al (2013) Mcl-1-dependent activation of Beclin 1 mediates autophagic cell death induced by sorafenib and SC-59 in hepatocellular carcinoma cells. Cell Death Dis 4:e485
33. He SD, Tan N, Sun CX, Liao KH, Zhu HJ, Luo XG et al (2020) Treatment with melittin induces apoptosis and autophagy of fibroblastlike synoviocytes in patients with rheumatoid arthritis. Curr Pharm Biotechnol 21:734–740

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.