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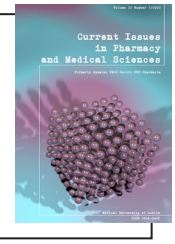

Bromelain-based chemo-herbal combination effect on human cancer cells: in-vitro study on AGS and MCF7 proliferation and apoptosis

Elham Raeisi[1,2], Mathias Hossain Aazami[3], Seyed Mahmud Reza Aghamiri[4], Atefeh Satari[5], Safoura Hosseinzadeh[6], Yves Lemoigne[7], Esfandiar Heidarian[1]*

[1] Clinical Biochemistry Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran
[2] Department of Medical Physics and Radiology, School of Allied Medical Sciences, Shahrekord University of Medical Sciences, Shahrekord, Iran
[3] Cardiology and Cardiac Surgery Department, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran
[4] Department of Radiation Medicine Engineering, School of Nuclear Engineering, Shahid Beheshti University, Tehran, Iran
[5] Student Research Committee, Shahrekord University of Medical Sciences, Shahrekord, Iran
[6] Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran
[7] Institute for Medical Physics, Ambilly, France

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ABSTRACT

Aim. Chemo-herbal combinations promise new clinical anticancer therapeutic modalities. The current study investigated and compared the *in vitro* effects of a bromelain-based chemo-herbal combination to/with cisplatin or 5-FU, with regard to the proliferation and apoptosis of human gastric AGS and breast MCF7 cell lines.

Material and methods. AGS and MCF7 cells were either treated with different concentrations of bromelain, cisplatin or 5-FU; or with bromelain-cisplatin and bromelain-5-FU combinations for 48h. Cell proliferative inhibition and inductive apoptosis were appraised using MTT assay and flowcytometry, respectively. Kruskal-Wallis and Dunn's tests were used to analyze differences in cell groups' means.

Results. AGS proliferation was adversely affected by single treatments of bromelain and cisplatin ($p < 0.003$) or 5-FU ($p < 0.05$). The anti-proliferative impact of single treatments was more pronounced on MCF7 cells. The bromelain-cisplatin combinations displayed synergistic effect on MCF7 cells (CIs ≤1), while being additive or antagonistic with cisplatin IC_{30} and IC_{40} to AGS cell proliferation, respectively. In addition, bromelian-5-FU combinations showed synergistic effect on AGS cells, while antagonistic to MCF7 cells. In terms of cell apoptosis induction, bromelain (IC_{30})-cisplatin (IC_{20}) displayed additive effect on MCF7, compared to cisplatin single treatment ($p < 0.04$), while bromelain (IC_{40})-5-FU (IC_{10}) and bromelain (IC_{30})-5-FU (IC_{20}) afforded additive apoptotic effects on AGS ($p < 0.04$) and MCF7 ($p < 0.05$), respectively, in comparison to 5-FU single treatment.

Conclusion. A bromelain-based combination using cisplatin showed concordant effects on cell proliferation impediment and apoptotic induction on MCF7, while the same results were noticed with a bromelain-5-FU combination to AGS cells. The bromelain-based chemo-herbal pathways should further be investigated in the frame of multi-chemotherapeutic drugs researches.

INTRODUCTION

As anarchic cellular proliferation, cancer ranges amongst the leading causes of mortality worldwide. Chemotherapy, radiotherapy, surgery, immunotherapy and electrochemotherapy are the main established anticancer therapeutic remedies that can be used in combination and according to the cancer type and stage of disease [1,2].

Chemotherapy as a pillar to anti-cancer therapeutic protocols destroys and impedes cell proliferation. Additionally, chemotherapy is known to block cancer cell mitotic cycles

* **Corresponding author**
e-mail: heidarian46@yahoo.com

as to engender vulnerability when exposed to adjuvant therapies such as radiotherapy [2,3].

Cisplatin (*cis*-diamminedichloroplatinum (II) or CISPLATIN) sits in the anti-cancer therapeutic armamentarium as one of most resorted chemotherapeutic drug in the setting of different solid neoplasms (ovarian, testicular, stomach, prostate, breast, neck and head, cervix, small-cell and non-small-cell lung cancer) [4]. Two major clinical concerns with cisplatin consist of primary tumor resistance and drug side-effects [5]. Exhibited drug resistance by certain neoplasms, emerging after initial clinical response, further clouds cisplatin anticancer efficacy. The prevalent cisplatin side effects include nephrotoxicity, myelosuppression, ototoxicity, anaphylactic reactions, peripheral neuropathies and hypomagnesaemia. 5-Fluorouracil (5-FU) is one of the widely used chemotherapy drugs (especially in breast and prostatic cancers). The main therapeutic side-effects of 5-FU are nausea, vomiting, mucositis, stomatitis, and diarrhea [6].

Overcoming tumor and drug resistance in parallel with attempts to undermine clinical side-effects of chemotherapeutic agents has motivated researches on chemo-herbal combinations. Chemo-herbal combinations also increase the effectiveness of chemotherapeutic drugs, while lessening their toxicity [7]. Aqueous extract of pineapple (bromelain), widely used in certain traditional medicine (South America and Asia), has shown permissive effects in many physiological and pathophysiological processes such as digestion, wound healing, burnt debris, and optimizing antibiotic absorption. *Immunomodulatory, anti-inflammatory* and anticancer properties were reported as being within the range of bromelain therapeutic effects. Indeed, *in vitro* anticancer effects of bromelain were reported in the case of different human cancer cells [8]. This study was conducted as to investigate the *in-vitro* impact of bromelain on proliferative and apoptotic aptitude to two different human cancer cells, gastric carcinoma (AGS) and breast adenocarcinoma (MCF7) cells in the frame of chemo-herbal combination treatment with either cisplatin or 5-FU.

MATERIALS AND METHODS

Procurement of human cancer cells

Human gastric carcinoma (AGS) and human breast adenocarcinoma (MCF7) cell lines were sourced from the Pasteur Institute (Tehran, Iran).

Cell culture

Roswell Park Memorial Institute (RPMI) -1640 supplemented with fetal bovine serum (FBS) at 10% volume concentration and penicillin/streptomycin 1% volume concentration (Life Technologies, California, USA) was used as cell culture medium. Trypsin/EDTA (Life Technologies) was applied to enable cell detachment. Cells were counted and seeded in new flasks following recommended standard protocols (RSP) consisting of maintaining 98% humidity, a temperature of 37°C, and a CO_2 partial pressure of 5%.

Preparation of stock solutions

Bromelain and cisplatin were purchased from Merck (Merck CO., Darmstadt, Germany), and 5-FU was sourced from Haupt Pharma (Wolfratshausen GmbH Co, Germany). The bromelain, cisplatin, and 5-FU stock solutions were prepared using dilution with RPMI-1640 as to obtain respective concentrations of 30 μM, 250 μM, and 30 μM. Stock solutions were kept at 4°C and protected from the light. The different target concentrations were obtained by subsequent dilution with RPMI-1640.

MTT assay

The bromelain, cisplatin, and 5-FU inhibitory effect on AGS and MCF7 cells proliferation was assessed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [9]. In brief, 10 μL of MTT solution at a concentration of 5 mg/ml (Life Technologies, California, USA) was added to each well that was incubated for 4 hours. After dissolving cells in 200 μL of dimethyl sulfoxide (DMSO) (Merck, Darmstadt, Germany) and having incubated for 20 min, the optical density (OD) of each well was measured using a microplate reader (Stat Fax-2100, Awarness Inc., Florida, USA) on the light wave-length range of 490-570 nm. The percentage of cancer cells was assessed based on the absorbance of treated cells, as opposed to the untreated control cells (viability = A (sample)/A (control) × 100) [9].

Annexin V-PI staining to apoptosis assay

AGS and MCF7 cells were seeded in 6-well plates (2×10^5/well) and incubated for 24 hours. Consequently, the incubated cells were treated as to reach 75% cells confluence, either singly with bromelain, cisplatin, and 5-FU; or with the two chemo-herbal combination types (bromelain-cisplatin and bromelain 5-FU). The wells were kept incubated for the following 48h under RSP. The treated cells, dead or viable, were collected by trypsination, washed with PBS that incorporated the stain, annexin V (BD Bioscience California, USA) at the ambient temperature for 35 min. Cell analyzing was performed by flowcytometry (CYFLOW space, Patrick, Germany) in observance to manufacture's operational guidelines.

Single treatment

Each well of a 96-well plate was filled with 5×10^3 cells per 200 μL issued from the prepared AGS and MCF7 cells culture medium. Following an overnight incubation under RSP, each well was singly treated with different target concentrations of bromelain in the range of 0, 0.5, 1, 2, 3.5, 7, 14, and 28 μM [10], cisplatin in the range of 0, 0.1, 1, 5, 10, 20, 40, 50, and 100 μM [10], and 5-FU in the range of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 μM [9]. Then, wells were kept incubated at RSP for 48h. A control well was confined to each singled treatment. The experiment for each single treatment was repeated 3 times as three independent experiments as to MTT assay and annexin V-PI staining. Cell viability percentage was plotted against different concentrations of bromelain, cisplatin, or 5-FU. Then, regression probit was used to calculate inhibitory concentration of bromelain,

cisplatin, and 5-FU corresponding to 10, 20, 30, 40 and 50% of cancer cell viability (IC_{10}, IC_{20}, IC_{30}, IC_{40} and IC_{50}).

Chemo-herbal combinational treatment

Having determined ICs by single treatments, wells incorporating AGS or MCF7 were treated with four different chemo-herbal combinations targeting an additive combinational IC_{50} of either bromelain-cisplatin (IC_{10} bromelain plus IC_{40} cisplatin, IC_{20} bromelain plus IC_{30} cisplatin, IC_{30} bromelain plus IC_{20} cisplatin and IC_{40} bromelain plus IC_{10} cisplatin); or bromelain-5-FU (IC_{10} bromelain plus IC_{40} 5-FU, IC_{20} bromelain plus IC_{30} 5-FU, IC_{30} bromelain plus IC_{20} 5-FU and IC_{40} bromelain plus IC_{10} 5-FU). Then, wells were kept incubated at RSP for 48h. A control well was confined to each chemo-herbal combination type. The experiment for each chemo-herbal combination type was repeated 3 times in three independent experiments. CI values were measured by applying CompuSyn software (Combo SynInc, City, State, USA). Combination index (CI) values indicated synergistic (CI <1), additive effect (CI =1) or antagonism (CI >1).

Data processing

Data were collected and inputted into SPSS 20 statistical software (SPSS, Chicago, IL, USA) and Graph Pad Prism 6 (GraphPad *Software* Inc., San. Diego, CA, USA). Results are presented as the percentage of cell viability after treating with different concentrations of bromelain, cisplatin, or 5-FU (Cell viability % = OD of treated groups/OD of untreated groups)[9]. Kruskal-Wallis and Dunn's tests were used to analyze differences in cell group means. A level of p-value <0.05 was considered significant.

RESULTS

Inhibitory effect on cell proliferation

Single treatments

Single treatment on AGS cells

AGS cell viability was significantly decreased in respect to single treatment with bromelain (p <0.003), cisplatin (p<0.003) and 5-FU (p<0.003), when compared to the control in a dose-dependent manner (Fig. 1 A-C). Different levels of IC were estimated accordingly (Tab. 1). IC_{50} was obtained as 1.2 µM, 6 µM, and 40 µM with regard to bromelain, cisplatin and 5-FU, respectively. The inhibitory effect on AGS cells with bromelain showed a similar pattern to that displayed by cisplatin (Fig. 1 A-B).

Single treatment on MCF7 cells

Significant inhibitory effect on MCF7 proliferation was detected for the complete range of concentration to bromelain (p <0.007), cisplatin (p <0.003) and 5-FU (p <0.007) in a dose dependent manner (Fig. 1 D-F), with bromelain displaying the most noticeable impact at lower range of concentrations. Different levels of ICs were estimated accordingly (Tab. 1). IC_{50} was obtained as 0.17 µM, 11 µM, and 9 µM to bromelain, cisplatin and 5-FU, respectively.

Table 1. The viability percentage, the value of CI (combination index) and DRI (dose reduction index) of AGS and MCF7 cell lines treated with combination of bromelain and cisplatin after 48h incubation. CI <1: synergistic effect, CI = 1: additive effects and CI >1: antagonistic effect

Cells	Combination Number	Dose Combination		Cell Viability±SD %	CI values	DRI values	
		Bromelain (IC value)	Cisplatin (IC value)			Bromelain	Cisplatin
AGS	No. 1	0.25 µM (IC_{10})	3.4 µM (IC_{40})	83±4.89	4.52	1.62	0.25
	No. 2	0.5 µM (IC_{20})	2 µM (IC_{30})	59±4.08	1.01	2.07	1.8
	No. 3	0.7 µM (IC_{30})	1.26 µM (IC_{20})	41±2.6	0.52	2.59	6.99
	No. 4	1 µM (IC_{40})	0.4 µM (IC_{10})	46±3.2	0.70	1.5	17.2
MCF7	No. 1	0.04 µM (IC_{10})	4.36 µM (IC_{40})	22±1.2	0.12	11.96	22.06
	No. 2	0.09 µM (IC_{20})	1.77 µM (IC_{30})	28±3.5	0.26	4.24	29.74
	No. 3	0.11 µM (IC_{30})	0.63 µM (IC_{20})	21±6.74	0.22	4.53	170.7
	No. 4	0.13 µM (IC_{40})	0.15 µM (IC_{10})	41±2.4	0.51	1.9	118.04

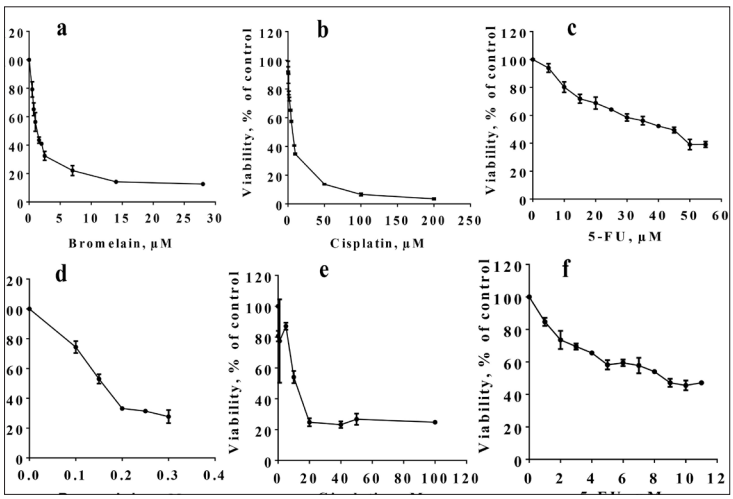
Chemo-herbal combinational treatment on AGS and MCF 7 cell viability

Bromelain-cisplatin combinational treatment on AGS cells

AGS cells were treated with 4 different bromelain-cisplatin concentrations targeting an additive IC_{50} (Tab. 1). Cell viability was significantly lessened in all 4 different combined concentrations (Tab. 1). Combination index (CI) was measured for each of 4 combined concentrations. The reported combined concentrations number 3 and 4 resulted in CI less than one, indicating a synergistic effect, while a CI of more than one was obtained with combined concentrations number 1 and 2 which reflected an additive and antagonistic effects, respectively.

Bromelain- 5-FU combinational treatment on AGS cells

AGS cells were treated with 4 different combined concentrations of bromelain-5-FU targeting an additive IC_{50} (Tab. 2). Cell viability was significantly lessened in all 4 different



Data are presented as means ± SD of at least three independent experiments

Figure 1. Changes in the percentage of cell viability in AGS cells (a-c), and MCF7 cells (d-f) after treatment with different concentrations of bromelain (a, d), cisplatin (b, e), and 5-FU (c, f)

combined concentrations (Tab. 2). When CI was measured for each of 4 combined concentrations, the 4 combined concentrations resulted in CI less than one, indicating a synergistic effect.

Table 2. The viability percentage, the value of CI (combination index) and DRI (dose reduction index) of AGS and MCF7 cell lines treated with combination of bromelain and 5-FU after 48h incubation. CI < 1: synergistic effect, CI = 1: additive effects and CI >1: antagonistic effect

Cells	Combination Number	Dose Combination		Cell Viability±SD %	CI values	DRI values	
		Bromelain (IC value)	5-FU (IC value)			Bromelain	5-FU
AGS	No. 1	0.25 µM (IC_{10})	30 µM (IC_{40})	26±15	0.30	12.26	4.43
	No. 2	0.5 µM (IC_{20})	20 µM (IC_{30})	18±2.8	0.20	8.79	10.78
	No. 3	0.7 µM (IC_{30})	10 µM (IC_{20})	14±1.6	0.16	7.9	29.3
	No. 4	1 µM (IC_{40})	5 µM (IC_{10})	9±1.92	0.13	8.1	97.7
MCF7	No. 1	0.04 µM (IC_{10})	4.46 µM (IC_{40})	92±0.98	16.5	0.89	0.06
	No. 2	0.09 µM (IC_{20})	2.39 µM (IC_{30})	87±1.5	5.7	0.57	0.24
	No. 3	0.11 µM (IC_{30})	1.14 µM (IC_{20})	84±2.12	3.1	0.56	0.72
	No. 4	0.13 µM (IC_{40})	0.41 µM (IC_{10})	97±2.83	13.11	0.13	0.17

Bromelain - Cisplatin combinational treatment on MCF7 cells

MCF7 cells were treated with 4 different combined concentrations of bromelain-cisplatin targeting an additive IC_{50} (Tab. 1). Cell viability was significantly lessened in all 4 different combined concentrations (Tab. 1). When CI was measured for each of 4 combined concentrations, the 4 combined concentrations resulted in CI less than one, indicating a synergistic effect.

Bromelain - 5-FU combinational treatment on MCF7 cells

All four tested combined concentrations targeting an additive IC_{50} did not result in any significant inhibitory effect on MCF7 cell viability (Tab. 2). Attendant calculated CIs displayed an antagonistic effect with 4 combinational concentrations (CI >1).

Effect of chemo-herbal combinational treatment on cell apoptosis induction

Bromelain - Cisplatin combinational treatment on AGS cells

AGS cells were treated with single bromelain (IC_{30}), cisplatin (IC_{20}), or their combination with an additive IC_{50} (Fig. 2). The bromelain (IC_{30})-cisplatin (IC_{20}) combination did not result in any additive apoptotic effect on AGS cells when compared to single treatments with bromelain (IC_{30}) or cisplatin (IC_{20}) (p >0.3) (Fig. 2).

Bromelain - 5-FU combinational treatment on AGS cells

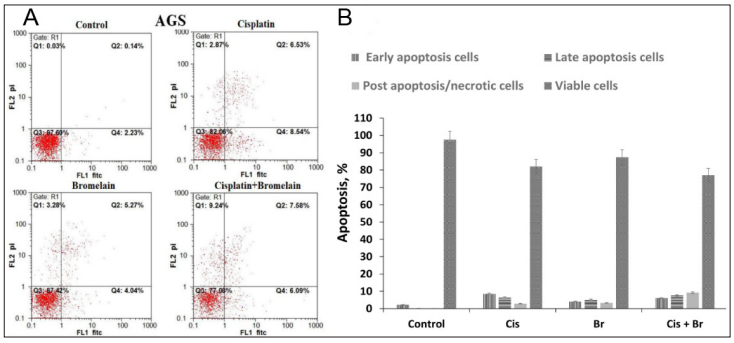
AGS cells were treated with single bromelain (IC_{40}), 5-FU (IC_{10}), or their combination with an additive IC_{50} (Fig. 3A-B). Herein, significant additive apoptotic effect on AGS cells was detected using bromelain (IC_{40}) 5-FU (IC_{10}) when compared to single 5-FU (IC_{10}) treatment (p <0.04). No additive apoptotic effect was detected in comparison to single bromelain (IC_{40}); (p <0.3) (Fig. 3B).

Bromelain - Cisplatin combinational treatment on MCF7 cells

MCF7 cells were treated with either single bromelain (IC_{30}), Cisplatin (IC_{20}), or their combination with an additive IC_{50} (Fig. 4A-B). Significant additive apoptotic effect on MCF7 cells was detected using bromelain (IC_{30})-cisplatin (IC_{10}) combination, when compared to single cisplatin (IC_{20}) treatment (p<0.04). No additive apoptotic effect was detected in comparison to single bromelain (IC_{30}); (p >0.3).

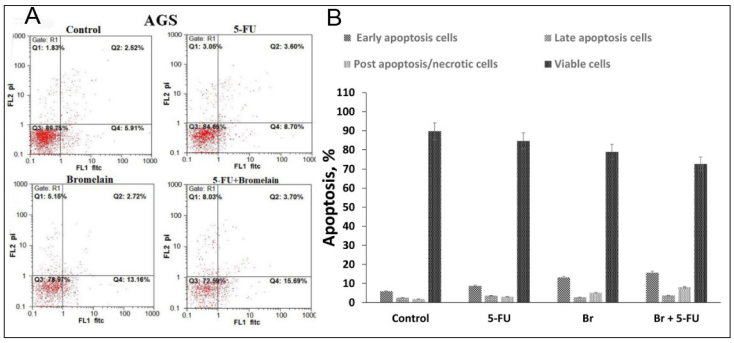
Bromelain - 5-FU combinational treatment on MCF7 cells

MCF7 cells were treated with either single bromelain (IC_{30}), 5-FU (IC_{20}), or their combination with an additive IC_{50} (Fig. 5 A-B). Significant additive apoptotic effect on MCF7 cells was detected using bromelain (IC_{30})-5-FU (IC_{20}) when compared to single 5-FU (IC_{20}) treatment (p<0.05). No additive apoptotic effect was detected in comparison to single bromelain (IC_{30}); (p >0.3) (Fig. 5).



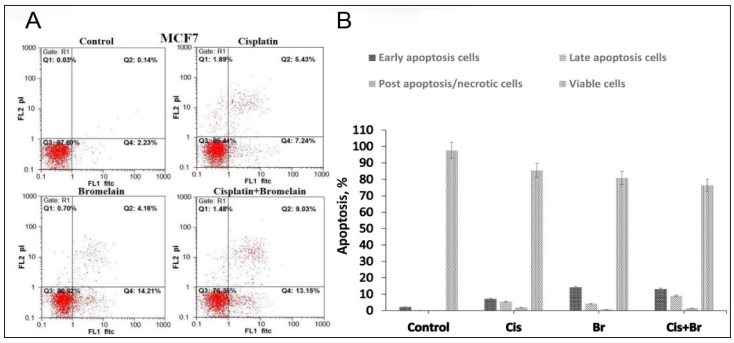
All data were expressed as the mean ± SD

Figure 2. Bromelain and cisplatin effect on the apoptosis of AGS. (A) Dot-plots from flowcytometric illustrating apoptotic status in AGS cells. (B) Total percentage of apoptosis in AGS cells treated with the indicated concentrations of bromelain (0.7 µM) and cisplatin (1.26 µM) or a combination of bromelain with cisplatin (0.7 µM and 1.26 µM, respectively) for 48h



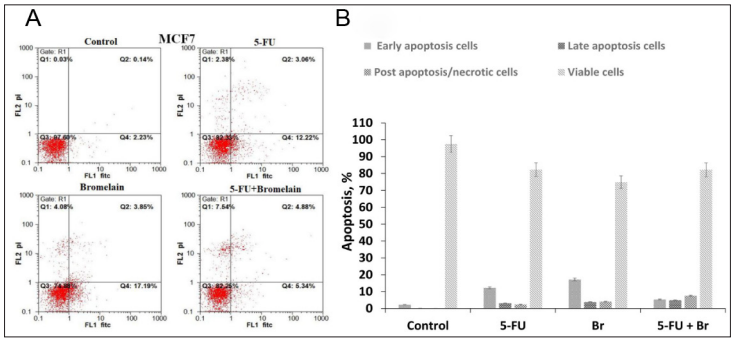
All data were expressed as the mean ± SD

Figure 3. Bromelain and 5-FU effect on the apoptosis of AGS. (A) Dot-plots from flowcytometric illustrating apoptotic status in AGS cells. (B) Total percentage of apoptosis in AGS cells treated with the indicated concentrations of bromelain (1 µM) and 5-FU (5 µM) or a combination of bromelain with 5-FU (1 µM and 5 µM, respectively) for 48h



All data were expressed as the mean ± SD

Figure 4. Bromelain and cisplatin effect on the apoptosis of MCF7. (A) Dot-plots from flowcytometric illustrating apoptotic status in MCF7 cells. (B) Total percentage of apoptosis in MCF7 cells treated with the indicated concentrations of bromelain (0.11 μM) and cisplatin (0.63 μM) or a combination of bromelain with cisplatin (0.11 μM and 0.63 μM, respectively) for 48h



All data were expressed as the mean ± SD

Figure 5. Bromelain and 5-FU effect on the apoptosis of MCF7. (A) Dot-plots from flowcytometric illustrating apoptotic status in 5-FU cells. (B) Total percentage of apoptosis in MCF7 cells treated with the indicated concentrations of bromelain (0.11 μM) and 5-FU (1.14 μM) or a combination of bromelain with 5-FU (0.11 μM and 1.14 μM, respectively) for 48h

DISCUSSION

Anticancer therapy has evolved from a pure chemico-physical approach towards gaining advantages in the ongoing insight of multi-modal disease management. Currently, chemotherapy as a pillar to anticancer therapy, carries drug side-effects and tumor resistance as two major clinical realms. Cisplatin (cis-diamminedichloroplatinum (II) or cisplatin) and 5-FU (5-fluorouracil) are two major chemotherapeutic drugs that are widely integrated in anti-cancer protocols [4]. Cisplatin's mode of action is based on hidden DNA repair mechanisms (crosslinking with the purine bases); thereby damaging DNA and inducing cancer cell death. By replacing thymine in the DNA or uracil in RNA, 5-FU acts as antimetabolite leading to cancer cell apoptosis induction [6].

As part of continuous efforts to overcome chemotherapy clinical limitations, enhance its therapeutic impact, and ameliorate attendant clinical tolerance, chemo-herbal combinations have emerged as promising investigational ways to explore. By coupling the properties of natural herbal products to classical chemotherapeutic drugs, chemo-herbal combination does synergistically boost the anticancer radius of chemotherapy that may lead towards reducing the required doses of chemical drugs [7].

Bromelain, the core component of the pineapple, is noted for having anti-inflammatory and anticancer properties that deserved scientific interest and focus. Immunomodulation, anti-fibrinolysis, and proteolysis represent other bromelain reported clinic benefits. The *in vitro* influence of bromelain on various human cancer cells was reported [8]. Increasing expression of p53 and Bax genes involved in apoptosis induction, and inhibiting the anti-apoptotic regulator genes (such as Akt/Erk genes) were acknowledged as bromelain anticancer modes of action. Certain radio-sensitizing properties of bromelain in murine breast cancer 4T1-cells were also demonstrated [11].

Given the reported anticancer potential, research was oriented towards chemo-herbal combinations that include bromelain. The synergistic impact of bromelain was investigated with N-acetylcysteine on gastrointestinal cancer cells [12,13], cisplatin on MDA-MB-231 human breast cancer cells [9], papain on human cholangiocarcinoma cells [14], as well as with cisplatin or 5-FU on malignant peritoneal mesothelioma [15].

Mirroring the advocated anticancer properties of bromelain, the current study aimed at investigating its impact in combination with chemotherapeutic drugs on cancer cell viability inhibition, as well apoptosis induction. Inhibition to cell viability resulted in the frame of single treatment with bromelain on AGS showing a pattern similar to that displayed by cisplatin (Fig. 1A-B). When it came to MCF7, cisplatin displayed the same pattern of cell viability inhibitory effect, though with a higher IC_{50} (11 μm) when compared to the IC_{50} (6 μm) obtained on AGS. In contrast, bromelain and 5-FU showed higher potency on MCF7 at a lower range of concentrations, as witnessed by their respective IC_{50} of 0.17 and 9 μM (Fig. 1D-F).

The bromelain-cisplatin effect on AGS cell viability inhibition also showed an additive impact with combined concentrations number 2 (Tab. 1), while combined concentrations number 3 and 4 revealed synergistic effects (Tab. 1). A synergistic effect on MCF7 was noticed with the all four different bromelain-cisplatin combined concentrations, advocating a higher *in-vitro* efficacy of the latter chemo-herbal combinational treatment on MCF7, when compared to that observed with AGS cells (Tab. 1). The latter was in contrast with the bromelain-5-FU chemo-herbal combination, where all the four different combined concentrations displayed synergistic effects on AGS; while being antagonistic on MCF7 with all 4 different combined concentrations (Tab. 2).

The chemo-herbal combination of bromelain-cisplatin on AGS apoptotic induction displayed no additive effects, in comparison to the respective single treatment either with bromelain or cisplatin (Fig. 2). In contrast, bromelain-5-FU chemo-herbal treatment demonstrated an additive effect on AGS cell apoptotic induction when compared to control and 5-FU (Fig. 3). The chemo-herbal combination of bromelain- cisplatin also revealed an additive effect on MCF7 cell apoptotic induction, compared to control and single cisplatin

treatment (Fig. 4). Moreover, the bromelain-5-FU combination exerted additive effect on MCF7 cell apoptotic induction, in comparison to the 5-FU single treatment (Fig. 5). These findings sustained the potent bromelain anticancer effect that can be taken advantageously in enhancing the effect of cisplatin and 5-FU chemotherapeutic drugs.

The driver synergistic or additive role of bromelain in combination with chemotherapeutic drugs was reported in a few previous studies. Pillai *et al*. demonstrated that chemo-herbal bromelain-cisplatin exerted a significant enhanced cytotoxic effect on PET and YOU cells (malignant peritoneal mesothelioma), when compared to cisplatin alone [15]. Similarly, Pauzi *et al*. reported the *in vitro* synergistic enhancing effect of bromelain in combination with cisplatin on MDA-MB-231 breast cancer cells in respect to apoptotic induction [9]. The latter paralleled the observed current findings of the synergistic or additive effect of bromelain-based chemo-herbal combinations on AGS and MCF7 with regard to inhibiting cell viability and apoptotic induction processes. Nevertheless, Pillai *et al*. did not report any anticancer enhancement using bromelain-5-FU combination on PET and YOU cells [15].

In the current study, the latter chemo-herbal combination displayed synergistic anticancer effects on AGS cells, but demonstrated an antagonist effect on MCF7 cell viability inhibition. The synergistic effect of bromelain was also highlighted in association with N-acetylcysteine on a panel of gastrointestinal cancer cells in terms of increasing cytotoxicity, cytophagy and cell apoptotic induction [12]. Compounding the previous reported anticancer effects of bromelain-based chemo-herbal combination in conjunction with the current findings emphasizes the potential benefits of bromelain in future anticancer multi-modal therapy.

CONCLUSIONS

Bromelain is innately dotted with *in vitro* inhibitory properties on human PC3 and MCF7 cell viability, as well as in the induction of cell apoptosis. In our work, we demonstrated that bromelain-based chemo-herbal combination with cisplatin or 5-FU produced various effects on cell viability and apoptosis in AGS or MCF7 cell lines that are positive in terms of anticancer treatment. In addition, current and previous research has revealed that bromelain-based chemo-herbal combinations do yield to clinical perspectives as to lessen chemotherapeutic clinical limitations, while substantially boosting their anticancer radius of action. Nevertheless, it is inventively advised that bromelain anticancer effects be further investigated on the basis of multi- chemotherapeutic drug protocols.

ETHICAL ISSUES

This study did not encompass any human or animal subjects.

CONFLICT OF INTEREST

The authors disclose no conflict of interest.

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ORCID iDs

Elham Raeisi https://orcid.org/0000-0003-3934-0398
Mathias Hossain Aazami https://orcid.org/0000-0002-4787-8676
Seyed Mahmud Reza Aghamiri https://orcid.org/0000-0002-5080-0482
Atefeh Satari https://orcid.org/0000-0001-5669-1072
Safoura Hosseinzadeh https://orcid.org/0000-0001-7898-3113
Yves Lemoigne https://orcid.org/0000-0003-0399-6563
Esfandiar Heidarian https://orcid.org/0000-0002-4701-144X

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