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Short Communications

Cytotoxicity and Apoptosis-Induction Potential of Fraction F3 from Fruits of *Bersama engleriana* (Melanthaceae) against Human Cervical Cancer Cells

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Abstract: Cervical cancer is one of the most life-threatening malignancies worldwide, despite intensive research for more efficacious chemotherapeutic agents. Recent efforts geared towards eradicating the scourge of cancer have embodied a major surge of interest in natural products as promising lead molecules in pharmaceutical development and therapeutic translation. Consequently, this study aimed to assess the cytotoxicity of a previously identified plant Fraction (F3) from *Bersama engleriana* against cervical cancer (HeLa) cells and non-cancerous normal human foreskin fibroblasts (HFF cells). Flow cytometric analysis via Annexin V/PI in HeLa cells was carried out to assess apoptosis induction. We report here the 50% Inhibitory Concentration (IC_{50}) of fraction F3 to be 96.15 µg/mL and a 50% Cytotoxic Concentration (CC_{50}) of 527.4 µg/mL, as well as a Selectivity Index (SI) of 5.48, thus confirming that fraction F3 is relatively non-toxic to the normal cells compared to the cancer cells evaluated. In addition, microscopic observations showed a higher reduction of cells, aggregated with dark color in HeLa cells treated with fraction F3 compared to control (cells only). The Annexin V/PI analysis showed the percentages of apoptotic cells were 4.6±0.05, 7.9±0.9 and 7.6±0.15, respectively, for the control and treatments of cells with 120 and 150 µg/mL fraction F3 in early apoptosis (Annexin V+/PI-) 48 h post-treatment. Percentages of apoptotic cells were 2.1±0.1, 8.9±0.2 and 7.7±0.2, respectively, for control, 120 and 150 µg/mL fraction F3 treatments in late apoptosis (Annexin V+/PI+) after 48 h posttreatment. Nevertheless, a comparison of the lowest percentage inhibition of apoptosis with the highest number of reduced cells observed with fluorescence microscopy suggests that fraction F3 may exert its growth inhibitory action on HeLa cells via another mechanism. The results established that fraction F3 from *B. engleriana* is a promising source for exploring antitumor activity.

Keywords: *Bersama engleriana*, Cytotoxicity, Apoptosis, Flow Cytometry, HeLa Cells Subjects: Drug Discovery, Cancer, Natural Products

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Introduction

It is estimated that 604,000 new cases of cervical cancer were attributed to women in 2020, resulting in 342,000 deaths worldwide. Second, only to breast cancer, the highest incidence of cervical cancer in women is particularly rampant in sub-Saharan Africa (Ferlay *et al*., 2020; Sung *et al*., 2021). Currently, cervical cancer is ranked fourth in terms of both incidence and mortality among women (Bray *et al*., 2018). Cancer-causing infections such as hepatitis and Human Papillomavirus (HPV) are responsible for up to 25% of cancer cases in low- and middle-income countries (Plummer *et al*., 2016). More than 85% of these cases and deaths occur in developing countries. This disproportionately high burden of disease is disturbingly worrisome due to causative factors such as population aging, eating habits, poverty, the absence of preventive and screening programs, intrauterine device use, chlamydia infection, multiple full-term pregnancies, multiple sexual partners, smoking, and some hormonal drugs as Diethylstilbestrol (DES), long-term use of oral contraceptives and Human Papillomavirus (HPV) infections (Alberg *et al*., 2013). HPV infection (particularly types 16 and 18) is present in more than 99.7% of cervical cancer cases (Bosch *et al*., 1995; Zhai and Tumban, 2016). In 2018, the U.S. Food and Drug Administration (FDA) approved Gardasil as an HPV vaccine for men and women between the ages of 27-45 years old, expanding on the previously approved age range of 9-26 years old (Alber *et al*., 2021). While the Gardasil vaccine can significantly reduce risks associated with HPV, many individuals are not receiving them as adolescents. The low Gardasil vaccination rate has been attributed to a lack of financial resources, high cost of vaccine and vaccine delivery, low cervical cancer screening levels, poor health system capabilities, low awareness and knowledge of HPV and cervical cancer, poor health system capabilities and inaccessibility to medical care (Dutta *et al*., 2018). Lack of basic information on the cause, prevention, and risk factors of the disease (Dozie *et al*., 2021) may also be a contributing factor.

Several therapeutic approaches are needed to control and eradicate the disease. Among the standard treatment options such as surgery, radiation therapy, targeted therapy, and immunotherapy, chemotherapy is the most promising modality. Currently, intravenous cisplatin with concurrent radiation therapy is the standard of care for patients with local and regional cervical cancer. However, cisplatin induces serious dose-limiting systemic toxicities and recurrence frequently occurs (Federico *et al*., 2021). To date, the specific treatment and efficiency of cervical cancer remain unsatisfactory, with more than 30% of the initially treated patients diagnosed with relapse and metastasis within 2 years and a 5-year survival rate of less than 10% (Wentzensen *et al*., 2021; Zhou *et al*., 2021). Therefore, developing novel strategies in the search for newer, more efficacious anti-cervical cancer drugs is the impetus for the current study. Current protocols to achieve this objective include the study of the anticancer properties of plant-derived products. Presently, ~60% of approved anticancer treatment drugs are of natural origin (Newman *et al*., 2003). Drugs like Vinca alkaloids, the taxanes, and the camptothecins derived from the Madagascar periwinkle plant, *Catharanthus roseus*, the Pacific yew, *Taxus brevifolia*, and the Chinese tree, *Camptotheca acuminate*, have significantly improved the chemotherapy of some cancers (Kuete and Efferth, 2015; Talib and Mahasneh, 2010). *Bersama engleriana* (Melianthaceae) is a tree indigenous to forests and forest margins of tropical and subtropical Africa. This plant, as well as other plants of the genus *Bersama*, is commonly used in the treatment of many diseases by the local populations (Kuete *et al*., 2008; Kupchan *et al*., 1971). Nonetheless, most cancer chemotherapeutics currently in use were developed mainly by screening for cell growth inhibition without a clear understanding of their modes of action. Therefore, understanding the oncolytic mechanisms of action of lead and investigative translational chemotherapeutics would not only accelerate their fine-tuning in terms of potency and efficacy but also limit unwanted side effects and high attrition rates in drug development and preclinical studies. Past reports demonstrated that the inhibition of cancer cells by apoptosis is an efficient strategy for tumor chemotherapy and is considered an indicator for the prevention and treatment of tumors (Gu *et al*., 2013). In a previous study, the authors (Majoumouo *et al*., 2020a) showed that F3 (a fraction from the methanolic extracts of *B. engleriana* fruits, BEfr) possesses anticancer activity with IC_{50} (µg/mL) estimates of 50.11±1.63, 60±0.91, 53.73±0.79 and 50.91±0.46 µg/mL on human lung carcinoma epithelial cells (A549), human glioma cells (U-87MG), human osteosarcoma cells (MG-63) and pancreatic ductal adenocarcinoma cells (MIAPaca-2), respectively, but the mechanism of cell death was not delineated. Thus, this study aimed to evaluate the *in-vitro* antiproliferative potential and pro-apoptotic activity of the F3 fraction derived from *B. engleriana* against the human cervical cancer cell line (HeLa). Additionally, we evaluated the effects of the F3 fraction on the non-tumor normal Human Foreskin Fibroblast (HFF) cell line to ascertain whether there is a difference in response to the extract between tumor and non-tumor cells.

Materials and Methods

Plant Materials

Various parts of *Bersama engleriana* were freshly harvested in Bamendjou, Cameroon, in December 2015. A voucher specimen number (reference: 24725/HNC)

was linked to the plant, to identify the plant at the National Herbarium in Yaoundé, Cameroon. The plant parts were carefully washed with fresh water to remove any contaminants, after which the various parts of the plant were allowed to shade-dry for three weeks. When a constant weight was attained, the dry plant was grounded to a fine powder which was stored at room temperature until further use.

Preparation of the Plant Fraction

The preparation of the plant extracts, the phytochemical screening analysis, and the fractionation of the active crude extracts was performed as previously described (Majoumouo *et al*., 2020b).

Cell Lines and Culture Conditions

The Human cervical cancer (HeLa) cell line was selected for measuring cell proliferation activity, while Human Foreskin Fibroblasts (HFF) cell lines were used for the cell toxicity activity of the plant fraction. Both cell lines were purchased from the American Type Culture Collection (ATCC) (Manassas, Virginia, USA). Both cells lines were grown in DMEM (Sigma, New Delhi, India) which was supplemented with 10% foetal bovine serum (Sigma-Aldrich St. Louis, MO), phenol red (Thermo Scientific, New Delhi, India) and 1% penicillin-streptomycin (Sigma, New Delhi, India). The cell lines were grown in an incubator maintained at 37ºC, containing 5% CO_2 and 95% air, in a humidified atmosphere.

Cell Proliferation Assay

The cell proliferation assay was performed using the 3-[4, 5-dimethylthiazol-2-yl]-2, 5diphenyl tetrazolium (MTT) dye (Majoumouo *et al*., 2020a). To summarize, the HeLa cell lines in the exponential phase (at 80% confluence) of growth were selected and harvested by treatment with 2X Trypsin-EDTA. For this experiment, the cell density was adjusted to 7×10^3 cells/well/100-µL cell suspension after trypsinization. A cell suspension of 100 µL was added to each well of a 96-well plate and the plate was incubated for 24 h at 37°C in a 5% CO_2 incubator. Serial dilutions (0-150 µg/mL) of the plant fraction were prepared with the complete growth medium from the stock solution of the extract (10 mg/mL dissolved in 10% Dimethyl Sulfoxide (DMSO)) and 80 µL of the F3 plant fractions were added to microplate wells containing the HeLa cell lines. Subsequently, the 96-well plate with the treated HeLa cell lines was incubated at 37°C in an atmosphere of 5% CO_2 for 48 h. The control wells contained only cells and all experiments were executed in triplicate. Following the 48-h incubation, 100 µL of MTT was then added to each well containing fresh cell culture medium, and the plate was further incubated at 37ºC for 3 h in a humidified incubator (5% CO_2: 95% air). Finally, the medium was removed after incubation, and the formazan crystals formed were dissolved in DMSO (100 µL per well) and the plate was incubated on a shaker at 37°C in an incubator (5% CO_2: 95% air) for 10 min. The optical density was measured using an ELISA plate reader (Thermo Scientific, USA) at 550 nm. Control cells were exposed to the highest concentration of the vehicle.

The percentages inhibition was determined using the formula:

$$Viability(\%) = \frac{Average\,absorbance\,of\,test\,(treated\,cell)}{Average\,absorbance\,of\,control\,(untreated\,cells)} \times 100\% \quad (1)$$

The 50% Inhibition Concentration (IC_{50}) value for the HeLa cell lines was determined using.

GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, California USA, 167 www.graphpad.com).

Cell Viability Assay of Fraction F3 Against Normal HFF Cells

The Promega 3-[4,5-dimethylthiazol-2-yl]-5-[3-carboxymethoxyphenyl]-2-[4-sulfophenyl]170 2H-Tetrazolium (MTS) cell proliferation assay was utilized to evaluate the cytotoxic activity of F3 on mammalian Human Foreskin Fibroblasts (HFF) cells as previously described (Majoumouo *et al*., 2020b). Briefly, cultured HFF cell lines at 80% confluence were trypsinized with 2X Trypsin-EDTA, and cells were reconstituted in a neat culture medium. Afterward, 7×10^3 cells/100 µL/well were seeded into 96-well flat-bottom tissue culture plates in complete medium and the plated was incubated at 37°C in an atmosphere of 5% CO_2 for 24 h. Following the 24 h incubation, various dilution concentrations of the plant extract solutions were prepared with complete growth medium from the stock solutions of the extract (10 mg/mL prepared in 10% Dimethyl Sulfoxide (DMSO)) and 80 µL of the dilutions of extract solution were added to each well of the microplate well-containing HFF cells and the plate was incubated for 48 h at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air. DMSO (0.4% v/v) was tested as the positive control of the experiment. Thereafter, 100 µL of MTS was added to each well and the plate was further incubated for another 3 h at 37°C in a humidified incubator containing 5% CO_2 and 95% air. Finally, 100 µL DMSO was added to each well to dissolve the formazan crystals formed, after carefully removing the supernatant of the treatment. The plate was incubated with agitation on a shaker at 37°C in an incubator (5% CO_2: 95% air) for 10 min. The optical density was measured using an ELISA plate reader 187 (Thermo Scientific, USA) at 490 nm.

Mean ODs of the repeated experiments were used to calculate the percentage growth inhibition of HFF cells of the extract by using the following formula below:

$$Growth\,inhibition(\%) = \frac{OD_c - OD_t}{OD_c} \times 100\% \quad (2)$$

where, OD = Optical Density; C = Control (cells only); T = Test (cells + extract).

The 50% Cytotoxic Concentration (CC_{50}) value of the plant extract was determined as described above, using GraphPad Prism.

Selectivity Index (SI) of F3 fraction

The extent to which the F3 fraction can be selectively toxic towards HeLa cancer cell lines, relative to the non-cancerous cell line (HFF) was expressed as the Selectivity Index (SI) (Boyom *et al*., 2014):

$$Selectivity\,index(SI) = \frac{CC_{50}\,in\,non-cancer\,cell\,line(HFF)}{IC_{50}\,in\,cancer\,cell\,line(HeLa)} \times 100\% \quad (3)$$

The Mechanisms of Action of F3 Extract Via Annexin V/PI

The Annexin-V/PI experiment was performed according to the manufacturer's instructions from the Alexa Fluor 488 Annexin V/Dead Cell Apoptosis Kit (Thermo Fisher Scientific Inc., Germany), using flow cytometry. The mechanism of action assay was performed as previously published (Majoumouo *et al*., 2020a). The HeLa cervical cancer cells were detached with 2X Trypsin-EDTA until 80% confluence was achieved and resuspended in a fresh culture medium. The cell suspensions at 1×10^6 cells/mL were plated into a 6-well microplate and incubated at 37°C in an atmosphere of 5% CO_2 for 24 h. Following incubation, the cell lines were then treated with the F3 plant fraction at different concentrations (120 and 150 µg/mL) and the plate was incubated at 37°C in an atmosphere of 5% CO_2 for 36 h. After the incubation period, microscopic observations were made to compare the changes in the treated cells relative to the untreated control cell lines. Following microscopy, the cells were harvested after treatment with Trypsin-EDTA (300 µL) and centrifuged at 4000 rpm for 10 min. The cells were washed using ice-cold annexin binding buffer and kept in the dark for 30 min. Thereafter, the Annexin V dye (3.5 µL) was introduced to each well, excluding the unstained cell lines, and the plate was incubated in the dark for another 30 min at 4ºC. Propidium iodide (5 µL) was then added to each well and the plate was incubated again for 30 min; after which a 100 µL/mL of annexin binding buffer was added into each well and the mixture was transferred to a FACS tube, to measure fluorescence by flow cytometry. Finally, the percentage of apoptotic and necrotic cells was determined using GraphPad Prism (version 8.00).

Statistics Analysis

A two-way ANOVA using GraphPad Prism was used to analyze the data generated during these experiments and the data were expressed as mean ± SD of experiments performed in triplicate. Error bars represent the SD and *p<0.05, which denotes significant differences between the means of the untreated and treated cells or two test groups.

Results

Antiproliferative Potential of F3 Fraction Against HeLa cells

The antiproliferative potential of fraction F3 in HeLa cells were evaluated using the MTT assay. Fraction F3 decreased HeLa cell proliferation at a 50% inhibitory concentration (IC_{50}) of 96.15 µg/mL (Table 1). A previous study by the authors reported that the F3 extract was most active against human lung carcinoma epithelial cells (A549), Human Glioma cells (U-87MG), human osteosarcoma cells (MG-63), and pancreatic ductal adenocarcinoma cells (MIAPaca-2) (Majoumouo, *et al*., 2020b), thus corroborating the antiproliferative activity of the F3 fraction on a broad range of cancer cell lines.

Cytotoxicity of Fraction F3 Against the Normal HFF Cells

The cytotoxic concentration of this fraction was tested against the non-cancerous foreskin fibroblast cell line (HFF cells) using the MTS assay and an electron coupling reagent (Phenazine Methosulfate; PMS) (Promega CellTiter 96® AQueous Nonradioactive Cell Proliferation Assay). It was determined that the fraction showed a cytotoxicity concentration (CC_{50}) of 527.4 µg/mL against the normal human foreskin fibroblast cells, coupled with a recorded IC_{50} of 96.15 µg/mL against the HeLa cell line and a selectivity index of 5.48 was obtained with HeLa (Table 2). This demonstrates that fraction F3 is safe and less toxic to the non-cancerous foreskin fibroblast cell line and will prevent the proliferation of the HeLa cervical cancer cell line to a greater extent than that of the HFF cells.

Table 1: The antiproliferative activity of F3 against the HeLa cervical cancer cell line

Code	HeLa cells IC_{50} (µg/mL)
F3	96.15

The IC_{50} value was determined using GraphPad Prism. *p<0.05, **p<0.001, ***p<0.0001, significantly different from control. Samples were compared using One-Way ANOVA and Tukey comparison of all pairs of columns. IC_{50}: Extract concentration required to reduce HeLa cancer growth by 50%. F3: A fraction from the methanolic extracts of fruits, BEfr

Table 2: Cytotoxic effect of F3 against HFF cells and its selectivity index

	Non-cancerous fibroblast HFF cells	
Code	CC_{50} (µg/mL)	SI
F3	527.1	5.48

CC_{50}: Extract concentration required to reduce HFF cell viability by 50%; SI: (Selectivity Index) = CC_{50}/IC_{50} CC_{50} was determined using GraphPad Prism. *p<0.05, **p<0.001, ***p<0.0001, significantly different compared to control. Samples were compared using One-Way ANOVA and Tukey comparisons of all pairs of columns. F3: A fraction from the methanolic extracts of fruits, BEfr

Morphology of HeLa Cells after 36 and 48 h of Incubation with F3

We assessed the morphology of the HeLa cell line after treatment with different concentrations

of fraction F3 derived from fruits of *B. engleriana*, followed by the Annexin/PI treatment.

HeLa cells treated with different concentrations of fraction F3 clearly showed a reduction in the cell population for both 36 h (Fig. 1a) and 48 h (Fig. 1b) post-treatment.

Furthermore, HeLa cells exhibited morphological changes such as a reduction in size and cell volume, cell shrinkage, and aggregation in the F3-treated cultures compared to the untreated controls. Also, cells treated with various concentrations of the F3 fraction, have a reduced cell population as compared to the untreated controls for both times after incubation (Fig. 1).

This reduction in HeLa cell density could be due to the cells undergoing apoptosis and/or necrosis.

Apoptosis or Necrosis Evaluation by Annexin V/PI

With the measurement of the antiproliferative activity of fraction F3, the induction of apoptosis is an important parameter to evaluate the anticancer properties of the plant extracts. Therefore, the Annexin/PI analysis was performed to determine the early apoptosis/late apoptosis by analyzing the flow cytometry results and the data were recapitulated in (Fig. 2a and b). The proapoptotic activity of fraction F3 from fruits of *B. engleriana* was investigated after the cellular morphological changes were recorded. The apoptotic results of the treatment of HeLa cell lines with different concentrations of fraction F3 from fruits of *B. engleriana* by Annexin/PI clearly showed the formation of different apoptotic bodies, confirming the antiapoptotic activity of the plant fraction.

A time-dependent increase in the induction of apoptosis was also observed when the cells were treated with the F3 fraction for different periods. The percentages of viable, apoptotic, and necrotic cells were noted for different incubation periods with fraction F3 (Fig. 2a and 2b). The cell viability of HeLa cell lines was found to decrease to 77.1 and 79.6% for 36 and 48 h at 150 and 120 µg/mL, respectively. When observing cell viability after 36 h, 150 µg/mL F3 treatment significantly decreased cell survival by 22.9% (Fig. 2a). Moreover, an increase in cell death population at the late apoptotic stage was observed with an F3 concentration at 150 µg/mL after 36 h (Fig. 2a). Similarly, after 48 h incubation, an increase of cell population at the early apoptotic stage was demonstrated with a fraction F3 concentration of 120 µg/mL (Fig. 2b).

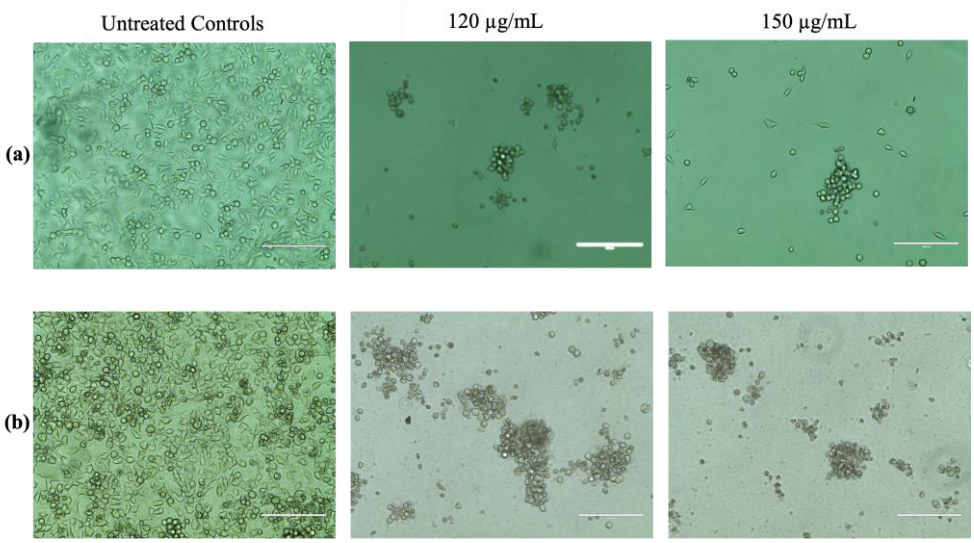

Fig. 1: Representative photomicrographs of the cellular morphological signatures of HeLa cells after treatment with 120 and 150 µg/mL of F3 during various treatment periods. (a) HeLa cells were treated with the F3 extract for 36 h; (b): HeLa cells were treated with the F3 extract for 48 h

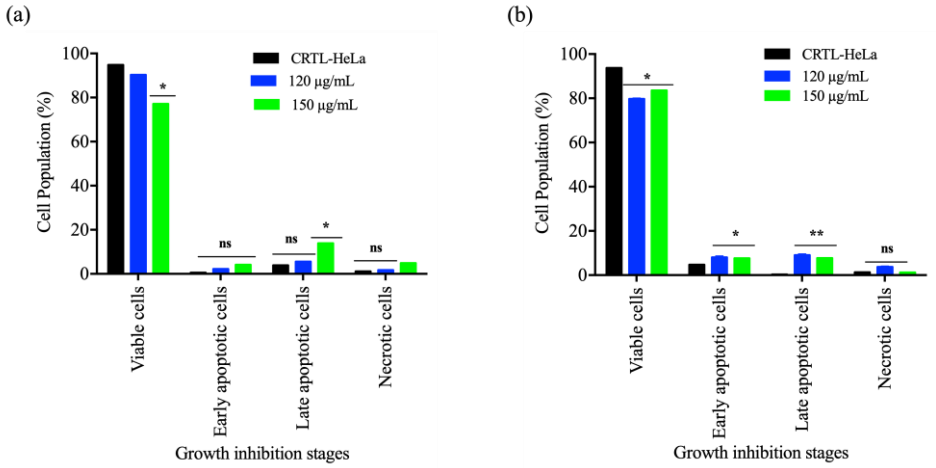

Fig. 2: Apoptotic effect of fraction F3 against HeLa cells. The proportions of Annexin V+/PI− and Annexin V+/PI + cells indicate the percentage of viable cells, early and late stages of apoptosis and necrosis. (a) apoptotic percentage of HeLa treated with F3 and Annexin V/PI 36 h post-treatment; (b) apoptotic percentage of HeLa treated with F3 and Annexin V/PI 48 h post-treatment. A two-tailed, unpaired t-test was used to analyze the significance. **p<0.05*, *** p<0.001*, ****p<0.0001*, significant difference compared to untreated sample

The percentage of apoptotic HeLa cells varied from 0.51±0.01 to 13.9±0.2%. The Annexin/PI showed the percentage of apoptotic cells at two stages, with the values of 2.2±0.04 and 4.1±0.06% obtained at 120 and 150 µg/mL, respectively, in early apoptosis (Annexin V+/PI-), compared to untreated cells which had a percentage of 0.51±0.01%. Furthermore, compared to untreated HeLa cell lines (3.9±0.1%), the values of 5.5±0.15 and 13.9±0.2% were obtained at 120 and 150 µg/mL in late apoptosis (Annexin V+/PI+) after 36 h (Fig. 2a). However, values of 7.9±0.9 and 7.6±0.15% were obtained for HeLa cell lines treated with 120, 150 µg/mL fraction F3, respectively, in early apoptosis (Annexin V+/PI-) when compared to untreated cells (4.6±0.05%) 48 h post-treatment. In addition, in HeLa cell lines, values of 8.9±0.2 and 7.7±0.2% were respectively obtained with control, 120, 150 µg/mL in late apoptosis stage (Annexin V+/PI+), compared to untreated cells (2.1±0.15%) after 48 h post-treatment (Fig. 2b).

These findings emphasize the dose-and time-dependent effects of F3 on HeLa cells. However, compared to the remarkable observation of reduced cells, the percentage of cells undergoing apoptosis was not correlated for both times and F3 concentrations post-treatment. Taken together, these findings indicate that fraction F3 has an apoptotic effect on HeLa cells. However, future investigations will have to explore the underlying mechanisms involved in fractions of F3-induced HeLa cell reduction.

Discussion

Globally, cancer is a leading cause of death and represents a major public health burden. According to the global cancer observatory (GLOBOCAN) report of 2018, there were an estimated 570,000 new cases of cervical cancer, with 311,000 deaths from the disease (Bray *et al*., 2018), mostly caused by the human papillomavirus (Ngcamphalala *et al*., 2021). Traditional treatments of cancer, such as chemotherapy, radiotherapy, and prophylactic vaccines have also been developed to reduce the incidence of HPV-induced cervical cancer. Many reports have demonstrated that the Gardasil vaccine could prevent nearly 2.3 million additional cases of cervical cancer over 80 years in 48 Gavi-eligible countries compared to a bivalent vaccine without cross-protection. However, the failure of prophylactic vaccines to treat existing cases, as well as the high cost of these vaccines have made the development of therapeutic vaccines and tumor-limiting approaches a goal for most gynecology and oncology scientists (Ghanaat *et al*., 2021). Also, chemotherapy is still very important to remove a localized cervix tumor.

The compendium of available drugs for the systemic treatment of cancer encompasses many side effects and drug resistance (Pérez-Ruiz *et al*., 2020). According to (Dehelean *et al*., 2021), the interest in alternative therapies using natural products (especially those derived from plants) is increasing due to the progressively higher number of cancer cases worldwide. For instance, it has been shown that the methanolic extracts of the bark, leaves, root, stem, and wood of *B. engleriana* possess antioxidant, antimicrobial, and antituberculosis activities (Kuete *et al*., 2008). The current study reports the antiproliferative activity of fraction F3, a fruit of *B. engleriana*, on the HeLa cancer cell line using the MTT assay with an IC_{50} of 96.15 µg/mL. Furthermore, the F3 fraction demonstrated low toxicity on the HFF non-

cancerous normal cell line with a CC_{50} of 527.1 µg/mL and a Selectivity Index (SI) of 5.48 (Table 2). These results confirmed the relative safety of the F3 fraction which can tentatively be classified as non-cytotoxic to normal HFF cells. A previous study has reported the *in-vitro* antiproliferative potential of fraction F3 on resistant cancer cells, with a good selectivity index (Majoumouo, *et al*., 2020a). The broad antiproliferative activity of the F3 fraction on various cancer cell lines is a potential source of alternative anticancer phytotherapy. The antiproliferative potential of the F3 fraction may be due to its rich phytochemical composition of phenolic compounds such as flavonoids, phenols, anthraquinones, and saponins (Kuete *et al*., 2008; Majoumouo, *et al*., 2020b). The antiproliferative ability of F3 could not be completed if the mechanism by which the HeLa cell line growth is inhibited when treated with fraction F3 from fruits of *B. engleriana* is not examined. Apoptosis is a form of programmed cell death that plays an important role in the development and prevention of cancer (Elmore, 2007). Deregulation in the control of apoptosis is often associated with the development of cancer (Afaloniati *et al*., 2021). The activation of apoptosis in cancer cells is, therefore, a counteractive strategy and many anticancer drugs may exert apoptotic effects in cancer cells (Awan *et al*., 2020). The results obtained from our study clearly illustrated the induction of the apoptotic effects by fraction F3 in HeLa cells. On the one hand, microscopic observation after treatment with fraction F3 showed a strong reduction of cells compared to untreated cells (Fig. 1a and 1b). We found that the F3 induced apoptosis in HeLa cells using flow cytometry via Annexin V/PI assays and a significant variation was observed in the responses of the HeLa cells to treatment with the F3. Annexin V/PI showed a very small percentage of HeLa necrotic cells (1.1±0.15, 1.7±0.1 and 4.9±0.2 with control, 120 and 150 µg/mL, respectively, after 36 h of treatment with fraction F3) and (0.1±0.05, 3.6±0.07 and 1.2±0.12 with control, 120 and 150 µg/mL, respectively, after 48 h treatment with fraction F3) (Fig. 2). In addition, there was a slight increase in the cell population at the early and late apoptotic stages that correlated with the incubation time. The increase in the population at the early apoptotic stage could be explained by the presence of alterations in membrane asymmetry and pre-lytic DNA fragmentation (Davenport *et al*., 2021). It is well-known that early apoptosis involves the activation of multiple signal cascades and can be measured by the presence of Phosphatidylserine (PS) in the outer leaflet of the plasma membrane (detected with annexin V); late apoptosis represents the fragmentation of DNA (Elmore, 2007). Interestingly, a significant increase in cell population was observed at the late apoptotic stages and could be explained by the fact that the fraction F3-induced DNA fragmentation content of HeLa cells; also, the activation of mitochondrial pathways mediated by the family of proteins known as caspases (Elmore, 2007). On the other hand, compared to the number of cells that were reduced and also those that were altered, it was surprising that the percentage of apoptotic cells obtained was not very significant. This could be because fraction F3 may stimulate mechanisms of action other than apoptosis to inhibit HeLa cells. Future investigations will be necessary for a proper understanding of the mechanism of action of this extract. Overall, the data suggest that the BEfr extract reported by Majoumouo *et al*. (2020a) and its derived F3 fraction may be good candidates for the development of anticancer agents with high specificity and fewer adverse effects.

Conclusion

We conclusively demonstrated the cytotoxic potential of fraction F3 from fruits of *B. engleriana* against the HeLa cancer cell line. We also showed the ability of the F3 fraction to induce an apoptotic effect on HeLa cells and to be relatively non-toxic on normal HFF cells. To the best of our knowledge, this study is being reported here for the first time. Therefore, the indigenous use of plants as herbal remedies for the treatment of cancer and cancer-related diseases has a scientific basis.

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Author's Contributions

Michele Stella Majoumoua: Collected materials, designed and performed the experiments, analyzed the data, and prepared the paper.

Marius Belmondo Tincho: Designed the experiments, analysed data, prepared and revised the paper.

Thureyah Morris: Provided materials related to the experiment, prepared and revised the paper.

Donavon Charles Hiss: Analysed data and revised the manuscript.

Fabrice Fekam Boyom: Provided materials related to experiment and preparation of the paper.

Data Availability

The data used to support the findings of the present study are available from the corresponding author upon request.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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