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The Effects of Bitter Melon Extract on Three Human Cancer Cell Lines: BT549, A549 and PC3

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Abstract

Bitter melon is a plant that has been found to exhibit beneficial medicinal properties against human diseases. Studies have revealed that plant extracts are capable of not only reducing lipid peroxidase but also inducing apoptosis in cancer cell lines. The present study aims to evaluate the anticancer properties of bitter melon (*Momordica charantia*) extract (BME) in a dose-dependent manner against human breast cancer cell line BT549, lung cancer cell line A549 and prostate cancer cell line PC3. **Method:** The anti-tumor properties of the BME were evaluated *in vitro* by the exposure of the cancer cells to serially diluted concentrations of BME for 24 hours. Alamar Blue was used to determine the cell viability, and ELISA (Enzyme-linked Immunosorbent Assay) assay for caspase-3 activity. Furthermore, levels of Lipid Peroxidation measured as the thiobarbituric reactive substances (TBARS) were assayed following exposure to BME. **Results:** The results indicate that a crude extract of bitter melon stimulated cell proliferation at lower concentrations (*i.e.* 0.079 µg/ml and 0.157 µg/ml), whereas a gradual but significant decrease (<0.05) in cell proliferation, and was observed at the highest concentration of 5.03 µg/ml compared to the control (*i.e.*, cells treated with DMSO). The lipid peroxidase assay showed a significant decrease only in TBARS for the A549 at the respective doses of 2.52 µg/ml and 5.03 µg/ml. That of BT549 and PC3 cells had significant decrease in TBARS only at the respective doses of 5.03 µg/ml compared to the controls. Caspase-3 activity showed a gradual increase in apoptosis in all three cell lines at the lower doses. However, significant increase in apoptosis was observed for all three cell lines treated with BME at the 5.03 µg/ml dose. **Conclusion:** The findings from this study indicate that BME contains anti-oxidative as well as anti-tumor properties which could be beneficial and promote health. Future studies would be aimed at isolating and characterizing the various metabolites in bitter melon to find out which compound has the most potential to inhibit cancer growth and promote healthy living.

Keywords

Cancer Cell Lines, Caspase-3, Lipid Peroxides, Bitter Melon Extract (BME), Anti-Tumor

1. Introduction

Plant resources have been a major part of human culture throughout history. World Health Organization (WHO) projected that 80% of the developing world's population uses traditional herbal medicines [1]. The growth of public interest in and use of traditional medicine and complementary and alternative medicine (T/CAM) has been well documented. Almost half of the population in many industrialized countries now use some form of T/CAM (United States, 42%) [2]. Plant-based compounds are very well known to regress colon cancer in many ways, like delaying tumor growth, managing chemotherapy and radiation therapy side-effects, and working at the molecular levels. Medicinal plants contain many bioactive phytochemicals [3]. The certification of ancestral knowledge in ethnobotanical surveys could cover the present gap in uncovering effective drugs [4].

Polyphenols are found ubiquitously in plants, and their regular consumption has been associated with a reduced risk of several chronic diseases, including cancer, cardiovascular disease (CVD), and neurodegenerative disorders [5]. Phytochemicals are organic compounds produced by plants or fungi. They do not directly regulate the growth, development, or reproduction of vegetables, fruits, or mushrooms, and are classified as secondary metabolites [6]. The health benefits of plant food-based diets could be related to both integrated antioxidant and anti-inflammatory mechanisms exerted by a wide array of phytochemicals present in fruit, vegetables, herbs, and spices [7]. Pomegranate seeds are a rich source of phytochemicals and have demonstrated health promoting effects [8].

Natural Bioactive or phytochemicals, which may be used as nutraceuticals, are referred to as compounds that lack essential nutrients and have specific biological activity in humans. There are estimated to be over 10,000 different varieties of phytochemicals, and each may potentially affect some type of disease or physiology. Numerous bioactive and phytochemicals derived from vegetative foods have been demonstrated to be effective in preventing metastasis [9]. Phytochemicals and their derivatives are biologically active compounds and have shown anti-cancer effects [10]. Although modern and easy chemotherapeutic drugs offer first-line treatment, the problem associated with them is their various side effects. Therefore, researchers are interested in treatments with minimal side effects [11]. The phytochemicals effectively target different cancers and minimize various hallmarks of cancer, reducing their intensity. The chemo-protective roles of the phytochemical are exerted by modulating the signaling pathways involved in cancer. This is found to have connections with the apoptosis induction and suppression of the epithelial to mesenchymal EMT, thereby resulting in the blockage of the metastatic behavior of cancer cells [12]. The phytochemicals interfere with various signaling cascades such as MAPK pathway, nuclear factor kappa B (NF-fB) signaling, and the PI3K-mTOR pathway [13].

Momordica charantia (*M. charantia*), also known as bitter melon, Karela, bitter gourd or Balsam pear, is a medicinal plant from the Cucurbitaceae family: it is cultivated in Africa, Asia, and South America [14] [15]. The name bitter guard or melon is given to it due to the fruit's bitter flavor, which becomes more pronounced as it ripens. Bitter melon is a medicinal plant with diverse beneficial effects [16]. It is a rich source of different vitamins, potassium, zinc, and other nutrients. The main pharmaceutical benefits of bitter melon are "anti-inflammatory", "antioxidant activity", "antimicrobial characteristics", "anticancer activity", and "anthelmintic activity", "antidiabetic effects", "antiinflammation activity" and "treat skin conditions" [17]. Bitter gourd supplementation had benefits in lowering elevated fasting plasma glucose in prediabetes [18]. Type 2 diabetes is a growing health problem worldwide that is particularly severe in India and China. In these areas, bitter gourd (*Momordica charantia*) is a popular vegetable which is traditionally known to have beneficial health effects not only on diabetes. Bitter gourd could be a cheap possibility to help the poor in these and other countries to control their blood glucose levels [19]. Juices, powders, extracts, and isolated compounds have been evaluated *in vitro* and *in vivo*. Bitter gourd increases insulin secretion of the pancreas, decreases intestinal glucose uptake, and increases uptake and utilization of glucose in peripheral tissues [19]. *M. charantia* is a reliable source of primary metabolites such as carbohydrates, fibers and proteins, minerals, and vitamins. The most important chemical constituents from *M. charantia* include heteropolysaccharides (e.g., arabinose, galactose, glucose, mannose and rhamnose); proteins and peptides (e.g., momordins, momorcharins, etc); terpenoids and saponins (e.g., cucurbitanes and cucurbitacines); flavonoids and phenolic compounds [20]. For centuries, Ayurveda (Indian traditional medicine) has recommended the use of bitter melon (*Momordica charantia*) as a functional food to prevent and treat diabetes and associated complications. It is noteworthy to mention that bitter melon extract has no-to-low side effects in animals as well as in humans [21]. *M. charantia* seeds may induce favism in humans with glucose-6 phosphate dehydrogenase deficiency [22]. Some studies have reported that *M. charantia* leaf powder inhibited adipocyte hypertrophy in diet-induced obese rats due to the process of decreasing lipogenic genes including fatty acid synthase, acetyl CoA carboxylase, lipoprotein lipase and adipocyte fatty acid and protein in epidimymis fat [23]. *M. charantia* reduced the accumulation of visceral fat that was fed to rats with high fat diet and thus help reducing insulin sensitizing and glucose due to its anti-adiposity effect [20]. While the anti-diabetic effects of BM have been studied, studies of BME on cancer cells are limited in literature and few experiments have been done to elevate their effects on cancer cells. Thus, the focus of the current studies was to evaluate some of the mechanistic roles and beneficial effects of BME on three cancer cell lines, namely, breast (BT549), lung (A549), and prostate (PC3) cells with the following three specific aims. 1) To investigate the antiproliferative effects of BME in the three cancer cell lines, 2) The effects of the BME on the lipid peroxide levels on the three cell lines, and finally 3) the BME effects on caspase-3 activation in the said cell lines.

2. Materials and Methods

Sterile Dimethyl Sulfoxide (DMSO) was from Sigma Aldrich, Atlanta GA. Breast (BT549, lung (A549) and prostate (PC3) cancer cell lines were from American Type Culture Collection (ATCC) (Rockville, MD), Fetal calf serum (FCS) was from Atlanta Biologicals (Atlanta GA).

2.1. Preparation for Bitter Melon Extracts

Bitter melons were from Dr. Zena Clarda, Department of Agriculture, at Tennessee State University (TSU).

The bitter melons were immediately stored at −20˚C until used. The frozen bitter melons were cut into slices (~1 to 2 mm), cooled further in liquid nitrogen, and then dried using a FD3 freeze dryer (Sigma, St. Louis). The freeze-dried bitter melon slices were ground using a commercial Waring blender (Fisher Scientific, GA). Then, the powder was passed through a 1 mm EFL 2000 stainless steel sieve (Endecotts, London, England). The ground freeze-dried bitter melon powder was then sealed in resealable storage plastic bags (Fisher Scientific, GA) and stored at −80˚C until used.

Bitter melon powder samples (1 g) were added to 100 ml of methanol and incubated for 1 h at different temperatures using a shaking water bath as previously reported [24]. After extraction, the samples were allowed to cool down and settle for 10 min on ice. The extracts were then centrifuged in 50 ml tubes at 4350 × g for 10 min at 10˚C using a Beckman J2-MC Centrifuge and a JA-20 rotor (Beckman Instruments Inc, Palo Alto, CA, USA) and the supernatant from each sample was filtered through a 0.45 μm syringe filter (Phenomenex, CA).

2.2. Total BME Content

The total extract content (TEC), known as the bitter melon extract (BME), was determined as described [25]. The absorption of the solution was measured at 510 nm against a reagent blank. Rutin was used as a standard, and the BME was expressed as mg of Rutin Equivalents (RE) per g of bitter melon powder on a dry basis (36 mg RE/g). Stock solutions (10 μg/ml) were prepared by reconstituting the BME in the proper volume and concentration of dimethyl sulfoxide (DMSO). The reconstituted samples are then aliquoted into 5 ml cryogenic vials and stored at −20˚C until use.

2.3. Serial Dilution

Before the respective cell lines were treated with BME, aliquoted extract was 50% serially diluted into the desired concentrations. The above approach resulted in a geometric progression of the masses and in a logarithmic fashion.

2.4. Cell Culture

Cells were cultured in T75 flasks with F12K supplemented with 10% heat-inactivated FBS, 2mM L-glutamine, and 1% penicillin-streptomycin, except for a low glucose variant (Gibco) having 2mM L-glutamine, non-essential amino acid, penicillin-streptomycin, 10% fetal calf serum supplemented with 0.01 mg/ml insulin and 1mM sodium pyruvate. Cells were grown at 37˚C in a 5% CO_2 humidified incubator. At 80% confluence the cells were trypsinized and counted using a hemocytometer. Cytotoxicity and antiproliferative activity of BME were evaluated by exposing the respective cell lines with the respective doses of BME.

2.5. Cell Viability Studies

Cells were plated in 12 and 24 well tissue culture plates at about 1×10^6 cells/well and in 2 ml of medium in the twelve well plates and 1×10^{10} cells/well and in 2.5 ml of medium in the twenty-four well plate, then incubated at 37 degrees ˚C and 5% CO_2. The medium was removed after 24 hours, and phosphate-buffered saline was used to wash the cells. The cells were treated for 2-3 minutes at 37 degrees with a solution of 0.25% trypan-EDTA. The live cells were counted using a hemocytometer after being stained with trypan blue for 2 minutes. Then the cells were diluted to 1×10^4 cells/well in a total volume of 150 µl in 96 well plates.

2.6. Alamar Blue

Cytotoxic effects of the respective BME extract concentrations on cells membrane integrity and viability were evaluated using the Alamar Blue Assay [26] with some modifications. Briefly, 20 µL of 0.15 mg/ml resazurin were added to each well. After three hours of incubation, the plate was read at 570 and 595 nm on a microplate reader. Viable cells were quantified by subtracting the absorbance reading at 595 nm from the reading at 570 nm, and normalizing to the untreated control cells, set at 100% viability. Alamar Blue Assay was chosen because it provides a rapid and sensitive way to measure cell proliferation and cytotoxicity in various human cell lines, bacteria, and fungi [26].

2.7. Lipid Peroxidation Assay

Lipid peroxidation assay is used to determine the levels of malondialdehyde (MDA). The formation of lipid peroxidation products leads to the spread of free radical reactions which leads to cell damage. MD Biosciences Inc. provided the kit for the MDA assay.

2.8. Apoptotic Effect

Apoptosis is a genetically programmed cell death mechanism that can be activated by various stimuli. The protein is probed with a combination of antibodies: one antibody is specific to the protein of interest (primary antibody), and another antibody specific to the host species of the primary antibody (secondary antibody). The secondary antibody is complexed with an enzyme that is combined with a substrate that will produce a detectable signal.

2.9. Protein Content in Cell Lysates

The soluble protein content of all cell lysates will be determined by the Coomassie blue protein assay method [27] using bovine serum albumin (BSA) as standard. Absorbance of the samples together with standards will be read in a Synergy Microplate Reader (Fisher Scientific, GA) at 595 nm.

2.10. Statistical Analysis

The results are expressed as the mean + standard deviation (SD). One-way ANOVA analysis was applied to determine the statistical difference and Stat View 5.0.1. (SAS Institute Inc., Cary, N). $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. Cell Proliferation in Cell Lines Following Treatment with BME

The cytotoxicity of the bitter melon extract was determined on the breast cancer cell line BT549 and the lung cancer cell line A549 as well as the prostate cancer cell line PC3. Using the cell viability assay the proliferation was evaluated. To study the effect of cell viability in a dose-response manner, the three cancer cell lines were incubated with various concentrations of BME (0.079, 0.157, 0.315, 0.629, 1.26, 2.52, and 5.03 μg/ml) for 24 hours in 96 well plates. The dose response experiments were executed in triplets with cancer cells treated with BME (from three separate batches) and the analysis revealed that bitter melon has therapeutic potential to prevent the incidence of cancer. The concentration of DMSO in all treatment groups was less than 0.5% [24].

3.2. The Effect of BME on the Proliferation of Breast Cancer Cell Line BT549

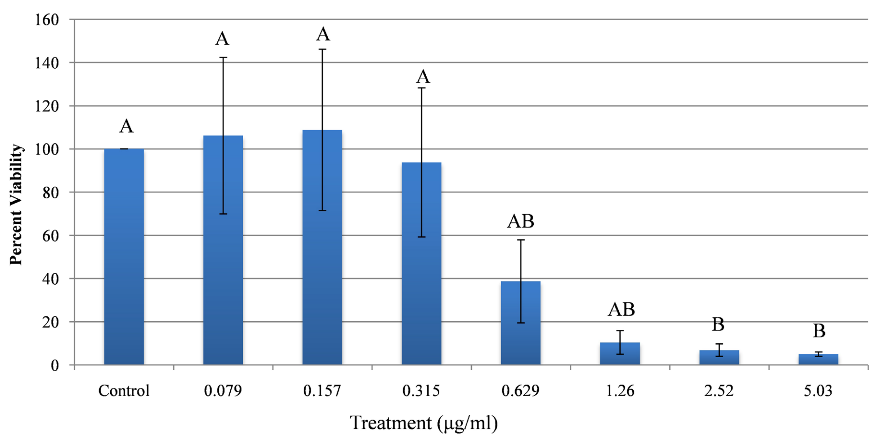

Figure 1. Viability analysis of breast cancer cell line BT549 after 24 hours exposure to bitter melon extract.

In breast cancer cell line BT549 the lowest concentration of BME (0.079 μg/ml) stimulated proliferation by about 6% (**Figure 1**) and the second lowest (0.157 μg/ml) increased cell viability nearly 22%, while on the contrary the next three lowest concentrations (0.313, 0.629 and 1.26 μg/ml) resulted in the decrease of cell viability with the increase in concentration approximately 6%, 61% and 90% respectively. In the second highest concentration (2.52 μg/ml) around 98% of the cancer cells died, while the highest concentration (5.03 μg/ml) resulted in 100% cell death. The results were compared to the cells treated with DMSO, which was the control in the experiment (and no BME).

3.3. The Effect of BME on the Proliferation of Lung Cancer Cell Line A549

In the lung cancer cell line A549 proliferation increased approximately 15% and 21% in the two lowest concentrations 0.079 and 0.157 μg/ml respectively (**Figure 2**). However, in the next concentrations 0.35, 0.629 and 1.26 μg/ml the proliferation decreased with increasing BME concentrations 11%, 51% and 65% respectively, and in the two highest concentrations 2.52 and 5.03 μg/ml there was significant decrease in the proliferation of 98% and 100%, respectively. The results were compared to the cells treated with DMSO, which was used as the control in the experiments (and no BME).

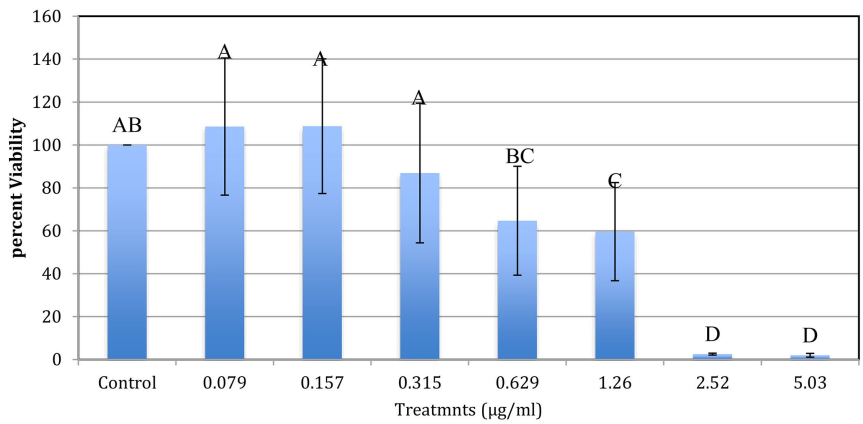

Figure 2. Viability analysis of lung cancer cell line A549 after 24-hour exposure to bitter melon extract.

3.4. The Effect of BME on the Proliferation of Prostate Cancer Cell Line PC3

In prostate cancer cell line PC3 the lowest three concentrations 0.079, 0.157 and 0.315 μg/ml there was an increase in the proliferation of approximately 41%, 33% and 28% respectively (**Figure 3**). Conversely, in the higher concentrations 0.629, 1.26, 2.52 and 5.03 μg/ml the proliferation decreased by 5%, 27%, 79% and 94%, respectively. The results were compared to the cells treated with DMSO, which was used as the control in the experiments. The control was not treated with BME.

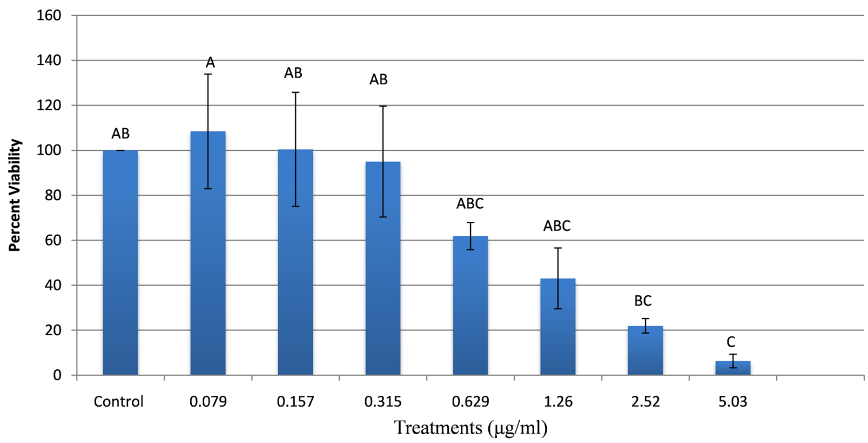

Figure 3. Viability analysis of prostate cancer cell Line PC3 after 24-hour Exposure to bitter melon extract.

3.5. The Effects of BME on Lipid Peroxides (Measured as Malondialdehyde (MDA) on Three Cancer Cell Lines, BT549 (Breast), A549 (Lung) and PC3 (Prostate)

Reactive oxygen Species (ROS) are a normal product of cellular function. However, excessive amounts of ROS can be harmful and leads to the formation of reactive carbonyl compounds that are most abundant in Malondialdehyde (MDA). MDA is the product of lipid peroxide, and it is reported as being the highest in cancer cells. Consequently, the measurement of MDA is used as an indicator of lipid peroxidation and to detect oxidative change. In these experiments we assessed the effects BME has on the cells' ability to assemble lipid peroxidase, which is measured by the production of cellular malondialdehyde.

3.6. The Effect of BME on the MDA Levels in the BT549 Breast Cancer Cell Line

The cells were plated at 1 × 10 and then treated with BME concentrations of (0, 2.52 and 5.03 μg/ml) for 24 hours. We found that the BME reduced the MDA levels in BT549 cancer cell lines. The results were compared to the control (DMSO) that had MDA levels of 5.09 μM/mg. The MDA levels decreased at the higher concentrations of 5.03 μg/ml (**Figure 4**).

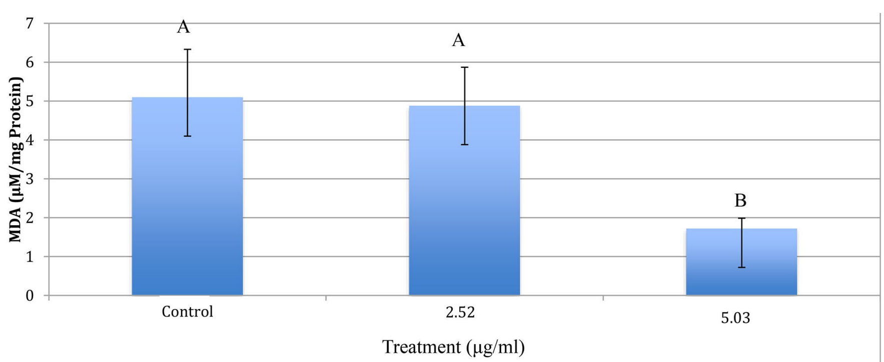

Figure 4. The effect of bitter melon extract on MDA level in BT549 cancer cell line.

3.7. The Effect of BME on the MDA Levels in the A549 Lung Cancer Cell Line

The cells were plated at 1 × 10 then treated with BME concentrations of (0, 2.52 and 5.03 μg/ml) for 24 hours. We found that the BME reduced the MDA in the A549 cancer cell line. The results were compared to the control (DMSO) that had MDA levels of 10.63 μM/mg. The MDA level decreased to 6.90 μM/mg in the lower concentration of 2.52 μg/ml (Figure 5) and reduced even further to 5.82 M/mg in the highest concentration of 5.03 μg/ml.

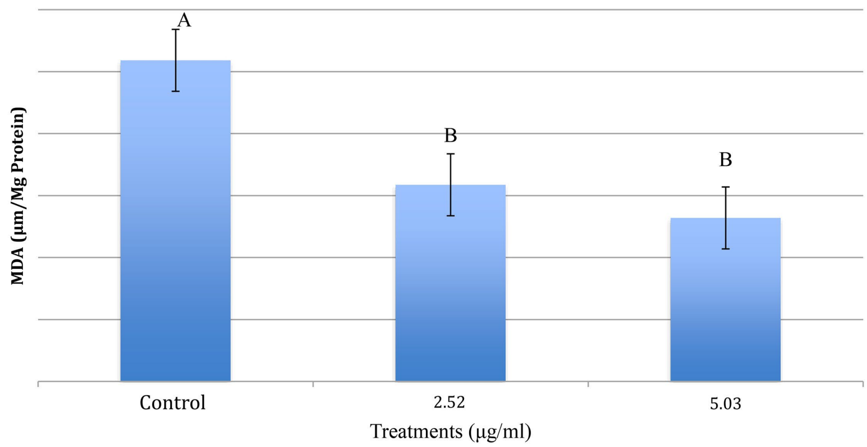

Figure 5. The effect of bitter melon extract on MDA level in A549 cancer cell line.

3.8. The Effect of BME on the MDA Levels in the PC3 Prostate Cancer Cell Line

The cells were plated at 1 × 10 then the cells were treated with the following concentrations of BME (0, 2.52 and 5.03 μg/ml) for 24 hours. The results were compared to the control DMSO and no BME and MDA levels of 5.41 μM/mg. There was a slight decrease in the MDA levels of concentration 2.52 μg/ml to 5.06 μM/mg (Figure 6), however, the concentration 5.03 μg/ml the MDA level decreased significantly to 2.86 μM/mg.

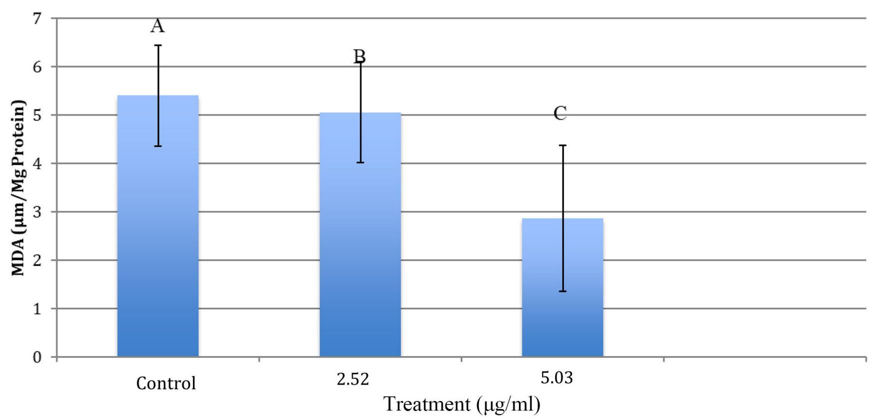

Figure 6. The effect of bitter melon extract on MDA level in PC3 cancer cell line.

The Assessment of the Activation of Caspase-3 by BME on the Three Human Cancer Cell Lines, BT549 (breast), A549 (lung) and PC3 (prostate).

In these experiments we determined whether the cancer cells exposed to BME would activate Caspase-3, and it was assessed using the enzyme-linked immunosorbent assay (ELISA) using anti-caspase-3. Cancer cells from the three human cell lines, BT549, A549 and PC3 were treated with BME and DMSO as the control. There was a significant increase in the apoptosis levels in the three cancer cell lines.

3.9. Analysis of the Activation of Caspase-3 in the BT549 Cancer Cell Line

In the breast cancer cell line BT549 the results from the treatment of BME (0, 2.52, 3.02, 3.52, 4.03, 4.53 and 5.03 µg/ml) with the cancer cells revealed a substantial gradual increase in the apoptosis levels in the cells with the higher concentrations of 3.52, 4.03, 4.53 and 5.03 µg/ml when compared to the cells treated with DMSO the control (Figure 7). However, there was not a significant increase observed in apoptosis levels in the lower concentrations of 2.52 and 3.02 µg/ml.

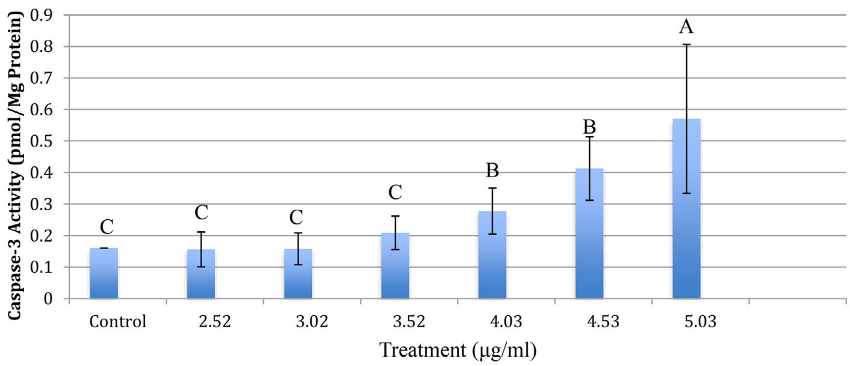

Figure 7. The effect of bitter melon extract on the Caspase-3 activity in BT549 cancer cell line expressed as (pmol/mg protein).

3.10. Analysis of Caspase-3 Activation in the A549 Lung Cancer Cell Line

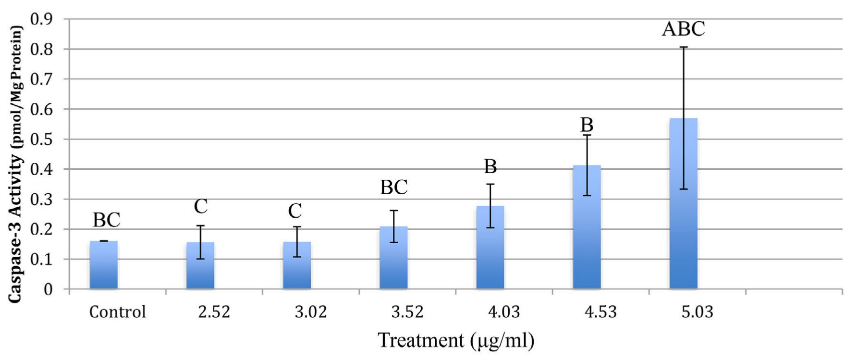

Figure 8. The effect of bitter melon extract on the Caspase-3 activity in the A549 cancer cell line expressed as (pmol/mg protein).

In the lung cancer cell line A549 the results from the treatment of BME (at 0, 2.52, 3.02, 3.52, 4.03, 4.53 and 5.03 µg/ml) with the cancer cells show a gradual increase in the apoptosis levels in the cells with the highest concentrations of 4.03, 4.53 and 5.03 µg/ml, when compared to the cells treated with DMSO the control (**Figure 8**). However, in the lowest concentration of 2.52 µg/ml, there was not an increase in the apoptosis level.

3.11. Analysis of Caspase-3 Activation in the PC3 Prostate Cancer Cell Line

In the prostate cancer cell line PC3 the results from the treatment of BME (at 0, 2.52, 3.02, 3.52, 4.03, 4.53 and 5.03 µg/ml) with the cancer cells show an increase in all the concentrations. The lower three concentrations 2.52, 3.02 and 3.52 µg/ml increased about the same (**Figure 9**), however, in the three higher concentrations 4.03, 4.53 and 5.03 µg/ml there was a substantial gradual increase in the apoptosis levels, when compared to the cell treated with DMSO control.

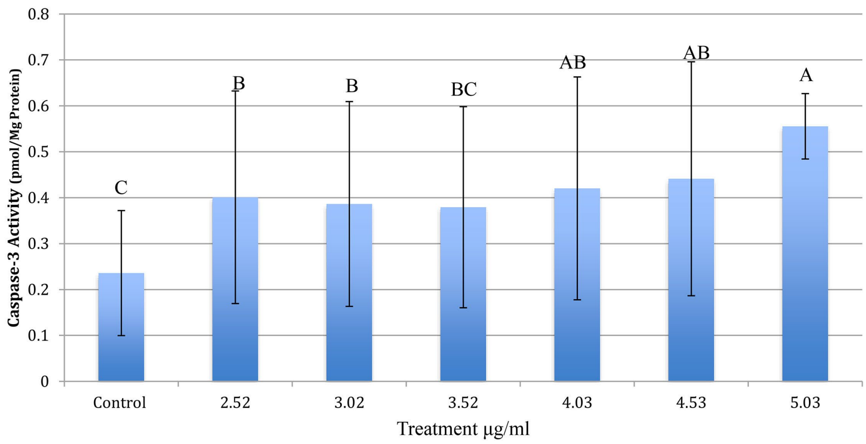

Figure 9. The effect of bitter melon extract on the Caspase-3 activity in the PC3 cancer cell line expressed as (pmol/mg protein).

4. Discussion

The results of the first aim of study suggest that the crude extract of bitter melon decreases the growth of cancer cells in the highest concentrations in all three cell lines BT549 breast, A549 lung and PC3 prostate in comparison to the DMSO control cells. The dose range of the crude extract was 0.079 to 5.03 µg/ml for 24 hours. The crude extract of bitter melon started decreasing the proliferation of BT549 and A549 cells at 0.315 µg/ml (**Figure 1** and **Figure 2**) and in the PC3 cells at 0.157 µg/ml (**Figure 3**) and continued to decrease the proliferation of all three cell lines as the concentration of the extract increased. The proliferation of BT549 cells, however, started to decrease significantly at 1.26 µg/ml, and in A549 cells at 2.52 µg/ml, and in PC3 at 5.03 µg/ml.

Since the crude extract of bitter melon had a significant rate of antitumor activity in all three cell lines, we proceeded to the second aim which was to study the effect of the crude extract on the lipid peroxides levels on the three cell lines. The results of the second aim of study were that the MDA had a significant decrease in the highest level of concentration in the three cancer cell lines, however, the decrease was more substantial in the cancer cell lines of BT549 and PC3 in comparison to the DMSO control cells. The dose range of the crude extract was 2.52 µg/ml to 5.03 µg/ml. We determined that the crude extract had a significant effect on the MDA levels in all three cancer cell lines. The third aim of the study was the assessment of the activation of caspase-3 by the crude bitter melon extract on the three human cell lines, and it was assessed using the enzyme-linked immunosorbent assay (ELISA) using anti-caspase-3. The dose range of concentrations was 2.52 to 5.03 ug/ml, and the results reveal a significant increase in the apoptosis levels in the three highest concentrations 4.03, 4.53 and 5.03 µg/ml in the BT549, A549 and PC3 cancer cell lines in comparison to DMSO the control. However, there was no increase in the BT549 and A549 in the lowest concentrations of 2.52, 3.02, and 3.52 ug/ml, but there was an increase in the apoptosis levels in PC3 in the lowest concentrations of 2.52, 3.02 and 3.52 µg/ml.

The effect of the lower concentrations of 0.079, and 0.157 µg/ml of crude bitter melon extract was an increase in proliferation of the cancer cells, however, in the higher concentrations 0.315, 0.629, 1.26, 2.52 and 5.03 µg/ml there was a gradual decrease in the cancer cells proliferation. The observed increase in cell proliferation at lower doses (*i.e.*, 0.079 and 0.157 µg/ml) for the cell lines followed a classic biphasic, or hormetic, dose-response pattern (low-dose stimulation, high-dose inhibition), consistent with adaptive responses to mild, non-toxic stress. While initial metabolic assays (e.g., cell proliferation assay (WST-1)) suggested increased viability, it was crucial to distinguish this from increased metabolic activity alone. To validate that this effect represents true increased proliferation, we conducted BrdU incorporation assays, confirming that low-dose treatment significantly increased DNA synthesis compared to control. In the highest concentration 5.03 µg/ml all three cancer cell lines had over 94% decrease in proliferation, in comparison to DMSO the control. Similar in the effect of the bitter melon extract on lipid peroxidase in the three cell lines, only the highest concentration had a significant effect. Lastly, in the assessment of the Activation of Caspase-3 by bitter melon extract, in BT549 and A549 cancer cell lines only the three highest concentrations had a substantial effect. While in the PC3 cancer cell line all the concentrations had an effect with the three highest concentrations having a more significant effect.

5. Conclusions

The findings from this study show that bitter melon extract stimulates proliferation in BT549 and A549 cancer cell lines in the two lowest concentrations of 0.079 µg/ml and 0.157 µg/ml. The reproduction is also encouraged in the PC3 cancer cell line but only in the lowest level of 0.079 µg/ml. However, starting at level 0.315 µg/ml, there is a gradual increase in cancer cell deaths. The decrease was significant in BT549 and A549 in the two highest levels of 2.52 and 5.03 µg/ml; however, in PC3, the decrease was significant only in the highest concentration of 5.03 μg/ml. Due to the cytotoxic effect of the bitter melon extract, the highest concentration 5.03 μg/ml had the most significant decrease in cell viability in the BT549, A549 and PC3 cancer cell lines. These results were compared to those of DMSO control. The concentration of DMSO in all treatment groups was less than 0.5% [24].

Other phytochemicals have shown anticancer potential either in these cell lines or in viability assays. A study on balsamic, a 28 kDa Type I ribosome-inactivating protein, in the seeds of Momordica balsamina reported the following findings [28]. In those studies, the molecular mechanism, and the possible effects of balsamin on the two critical hallmarks of cancer were investigated [28]. The induction of apoptosis in human breast cancer MCF-7 and BT549 cells showed that balsam induced an increase in caspase-3 and caspase-8 activity. Furthermore, balsam inhibited proliferation of breast cancer cells in a dose-dependent manner in MCF-7 and BT549 breast cancer cell lines [28]. In a similar study, other authors [29] determined the effect of lutein on the proliferation of human PC3 as well as rat prostate carcinoma cells (AT3 cells). The anti-cancer activity of lutein was effective against both rat and human prostate cancer in induction of apoptosis [29].

Since BME efficiently inhibited cell proliferation, we further examined the effect BME on the lipid peroxidation levels in the three cell lines. The results indicate that BME lowers the MDA levels significantly in A549 at concentrations of 2.52 μg/ml and 5.03 μg/ml. However, the MDA levels were only significantly reduced in BT549 and PC3 in the highest concentration 5.03 μg/ml. Due to the cytotoxic effect of BME, the MDA levels are significantly lowered in BT549, A549 and PC3 cancer cell lines. These results were compared to those of the control (DMSO).

Furthermore, since BME efficiently reduced cell viability as well as reduced MDA levels in the three cancer cell lines, the effect of BME on the caspase-3 activation in the cell lines was examined. Our findings indicate that BME induces apoptosis in the three cell lines. In BT549 cells, the lower concentrations had trivial effect. However, for the highest concentrations of 4.03 μg/ml, 4.53 μg/ml and 5.03 μg/ml, there was a significant gradual increase in the apoptotic levels. With regards to A549 cells, the low concentrations did not have much apoptotic effect, compared to the highest concentrations of 4.53 μg/ml and 5.03 μg/ml. Conversely, in the PC3 cells, there was substantial increase in the apoptotic levels in a dose-dependent manner, with the highest dose 5.03 μg/ml having a more significant response. The results were compared to those of the control (DMSO). The above studies support the findings by other authors [30] where increase in dose of carvacrol treated A549 cells, caused a decrease in cell number, degeneration of cell morphology and a decrease in total protein content. To characterize carvacrol induced changes in cell morphology, cells were examined by light microscopy. Cells treated with the respective carvacrol doses were reported to have detached from the disk, with cell rounding, cytoplasmic blebbing and irregularity in shape. The authors concluded that carvacrol was a potent inhibitor of cell growth in A549 cell lines. Likewise, the antiproliferative and apoptotic effect of honey and chrysin, which is a natural product found in honey, on cultured human prostate cancer cells was investigated [31]. The research findings revealed that both compounds had antiproliferative effects on PC3 cells in a dose and time dependent manner. Furthermore, chrysin induced apoptosis in PC3 cells as determined by flow cytometry.

The present study provides evidence that all tested cell lines were sensitive to BME growth-inhibitory potential. The data showed a significant decrease in the number of viable cells in all three cell lines on treatment with BME. The maximum effect was observed in the 5.03 μg/ml concentration, where BME induced over 90% decrease in percentage of cell viability in all tested cancer cell lines. BME also significantly decreased the MDA level in all the established cancer cell lines. Furthermore, a significant increase in apoptosis was observed in all cancer cell lines when cells were treated with BME. The findings show the capacity of BME to inhibit cell proliferation, activate caspase-3 activity, and decrease MDA levels in all tested cell lines. Taking together these findings denotes that BME has the potential to be a useful tool in the battle against cancer. The results might be due to the phytochemicals present in BME.

The present study is the first to examine the effects of BME against human breast cancer cell line BT549, human lung cancer cell line A549 as well as the human prostate cancer cell line PC3. In conclusion, the current study demonstrates that BME has anti-cancer potential. The results herein provide essential information on the action of BME for justification as a potential candidate for anticancer drug.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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