WITHDRAWN: Modulatory effect of α-Bisabolol on induced apoptosis via mitochondrial and NF-κB/Akt/PI3K Signaling pathways in MCF-7 breast cancer cells

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Abstract

Breast cancer is a highly feared form of cancer that predominantly affects women. In pursuing effective treatments, herbal medicine has garnered attention as a viable resource. It holds promise as an alternative approach for managing and combating breast cancer. The primary objective of the research was to explore how α-Bisabolol hinders the growth of MCF-7 human breast cancer cells and decipher its molecular mechanisms of reducing cell proliferation and promoting apoptosis. In the experiment, cultured MCF-7 cells were divided into four distinct groups: The first group functioned as the control, whereas the second, third, and fourth groups received separate treatments of α-Bisabolol at varying concentrations. After allowing the cells to incubate for a 24-hour, we examined them to assess any alterations in their morphology after applying α-Bisabolol. This treatment led to the suppression of cell growth, an elevation in the generation of reactive oxygen species (ROS) and the initiation of apoptosis. Furthermore, examination through western blot and real-time PCR unveiled that cell treated with α-Bisabolol exhibited reduced levels of the cell survival gene Bcl-2, alongside elevated levels of the pro-apoptotic genes Bax, Bad, Caspase-3, Caspase-9, and cytochrome c. Meanwhile, NF-κB, p-PI3K, and p-Akt proteins were downregulated in α-Bisabolol treated cells. These results suggest that α-Bisabolol diminishes the cell viability of MCF-7 cells and triggers cellular apoptosis through both the mitochondrial pathway and the NF-κB/Akt/PI3K signaling pathways.

1. INTRODUCTION

Breast cancer (BC) is recognized as one of the most severe diseases affecting women, serving as a leading cause of cancer-related deaths on a global scale. As per the information provided by GLOBOCAN in 2020, it's worth noting that breast cancer (BC) is progressively emerging as the most prevalent form of cancer, with 2.3 million new cases recorded. This trend is anticipated to surpass the occurrence of lung cancer.[1] Even though advancements have been achieved in medical treatments, the ailment remains a substantial contributor to female mortality globally.[2,3] Like many other cancer types, breast cancer also exhibits a fundamental trait of heightened cell proliferation. MCF-7 cells, characterized by the presence of hormone receptors, were originally derived from breast adenocarcinoma patients. These cells are widely utilized as a prominent model for studying how natural compounds can effectively hinder the proliferation of human breast cell lines.[4]

Continuous activation of cancer-causing signaling pathways has consistently shown a connection with the development of fundamental cancer traits, including increased cell proliferation, evasion of programmed cell death, invasion, and the stimulation of blood vessel formation (angiogenesis).[5,6] Specifically, the PI3K/Akt and NF-κB signaling pathways exhibit the most significant degree of dysfunction across diverse malignant tumors.[7,8] Phosphatidylinositol-3-kinase (PI3K) is an initial lipid kinase that becomes active due to the influence of different growth factors through receptor tyrosine kinases. Phosphorylation plays a crucial role in activating PI3K and is closely overseen by the anti-cancer gene PTEN. Upon PI3K activation, protein kinase B/Akt is recruited to the cell membrane. Akt, a

serine/threonine kinase, undergoes phosphorylation at threonine 308 and serine 473 by phosphatidylinositol-dependent kinase (PDK) 1 and 2, respectively. Subsequently, the activated Akt proceeds to phosphorylate numerous downstream proteins, including inhibitory kappa B kinase (IKK).[9–12]

NF-κB, a transcription factor positioned in the signaling pathway downstream of Akt, oversees various cellular processes, including growth and developmental mechanisms. In unstimulated cells, NF-κB is a heteromeric complex comprising NF-κB1 and RelA subunits, kept in check within the cytoplasm by inhibitors of kappa B (IκB). When cells are stimulated, the inhibitory kappa B (IκB) undergoes a series of changes: it undergoes phosphorylation through the inhibitory kappa B kinase complex, followed by ubiquitin-mediated modification and subsequent breakdown within the proteasome. This process frees NF-κB, allowing it to move into the cell nucleus and activate effector genes crucial for driving neoplastic progression.[13–15]

Evasion of apoptosis is a widely recognized characteristic observed in various cancers, including breast carcinoma. This phenomenon plays a substantial part in the fundamental aspects of cancer, such as promoting cell survival and advancement, thereby contributing to the distinctive features of cancer development.[16] Cancer cells evade apoptosis primarily due to alterations in signaling pathways, including genetic mutations and imbalances in apoptosis-promoting and apoptosis-inhibiting proteins.[17] Utilizing natural agents to induce apoptosis could potentially trigger a targeted cell death process in cancer cells, while sparing adjacent normal cells from any harmful effects.[18–19]

Growing focus has been directed towards sesquiterpenes due to their potentially valuable biological properties, particularly their potential as agents against tumor development. [20–22] α-Bisabolol is an oily sesquiterpene alcohol obtained from plants. It serves various purposes, including gastroprotection,[23] antimicrobial action,[24] and alleviation of inflammation.[25] Furthermore, α-Bisabolol can trigger apoptosis in glioma cells,[26] acute leukemia cells,[27] and liver carcinoma cells.[28] Research offers proof regarding the apoptosis-stimulating and anti-tumor effects of α-Bisabolol. These studies revealed that α-Bisabolol can specifically initiate apoptosis in numerous cancerous tumor cells through the mitochondrial pathway. This effect is likely achieved by targeting lipid rafts on the cell membrane.[29,30] Although there have been reported instances of α-Bisabolol demonstrating anticancer properties, its specific impact on breast cancer remains an unexplored area. Hence, the current investigation was structured to assess the anticancer efficacy of α-Bisabolol against the MCF-7 mammary carcinoma cell line.

2. MATERIALS AND METHODS

2.1. Chemicals

The α-Bisabolol and Oligonucleotide primers were acquired from Sigma chemicals Co. located in St. Louis, USA. The RNA extraction kit and quantitative RT PCR kit were obtained from Qiagen, USA.

Antibodies such as NF-κB, pI3K, p-AKT and β-actin, along with corresponding secondary antibodies were procured from Santacruz, USA. Other supplementary cell culture substances, including DMEM medium, Phosphate-Buffered Saline, Fetal Bovine Serum, DCFH-DA, Rhodamine 123, Acridine Orange, Ethidium Bromide, and various other chemicals, were sourced from Thermo Fisher Scientific based in the USA.

2.2. Culturing MCF-7 cells

For this study, the MCF-7 cells employed were obtained from NCCS in Pune, India. The cells were nurtured in DMEM (Dulbecco's Modified Eagle Medium), which was enhanced with 1% glutamine and penicillin-streptomycin, and additionally fortified with 10% FBS (Fetal Bovine Serum). The cells were kept within a controlled setting at a temperature of 37°C, housed in a humidified atmosphere comprising 5% CO_2. To transfer the attached cells, the process involved initially washing them with PBS (Phosphate Buffered Saline) followed by subjecting them to trypsin treatment at 37°C for a duration of 1 to 2 minutes. Cells from passages 3 to 5 were employed for each experimental trial.

2.3. MTT assay

MCF-7 breast carcinoma cells were cultivated in a 96-microplate at a measure of 1 million cells per milliliter. Different concentrations (1-500 μM) of α-Bisabolol were applied to these cells. Following a 72-hour incubation at 37°C in an environment consisting of 5% CO_2 and 95% O_2, a solution of MTT (0.5 mg/mL) from Sigma Chemicals Co. was introduced to the cells and left to incubate for an additional 4 hours. Afterward, the cells underwent centrifugation, and the liquid above the sediment was extracted. Subsequently, DMSO was introduced into each well to dissolve the formazan crystals. Ultimately, a microplate reader was employed to gauge cell viability by measuring absorbance at 540 nm.

2.4. Experimental framework

The study encompassed MCF-7 breast carcinoma cells cultivated within four separate groups. Group I functioned as the control cells, whereas Groups II to IV comprised breast carcinoma cells that were subjected to varying levels of α-Bisabolol. Following the administration of α-Bisabolol, the cells were left to incubate for 24 hours, during which meticulous observation and analysis of morphological alterations were conducted. After this incubation period, the cells were utilized for a range of additional experiments.

2.5. Cellular ROS detection

The level of intracellular ROS was evaluated through the utilization of the DCF assay. Isolated cells were readied in a 2 ml solution of PBS having a pH value of 7.4. Afterward, 1 mL of the cell suspension was mixed with 10 μL of DCFH-DA and subjected to an incubation period at a temperature of 37°C for 30 minutes. Following that, the cells were subjected to three rinses with PBS, and the magnitude of fluorescence was measured using a spectrofluorometer. In addition, fluorescence microscope images were acquired using an excitation wavelength of 460 nm.

2.6. Evaluation of antioxidants status

The activities of superoxide dismutase (SOD), Catalase (CAT), and Glutathione peroxidase (GPx) in cardiac tissue were assayed with the methods of Kakkar et al.,[31] Sinha[32] and Rotruck et al.[33] respectively. The level of reduced glutathione (GSH) was estimated by Ellman et al.[34] in MCF-7 cells.

2.7. Mitochondrial membrane potential (Δψm)

Rhodamine-123 staining was employed to assess alterations in Δψm. A droplet containing 1 µL of the dye was administered to both treated and control MCF-7 cells, allowing them to incubate for 30 minutes. Subsequent to a PBS wash, the cells were examined under a fluorescence microscope using a range of 450–490 nm wavelengths.

2.8. AO/EB apoptotic assay

To detect apoptosis, α-Bisabolol was subjected to staining using Ethidium bromide/acridine orange (EtBr/AO). MCF-7 cells ($1x10^6$/well) were cultured for 24 hours, treated with α-Bisabolol, and subsequently fixed using a solution of methanol and glacial acetic acid. The cells underwent AO/EtBr staining and were then observed using a fluorescence microscope. Apoptosis-like traits were ascertained by assessing the overall cell count within the field of view.

2.9. Apoptotic gene expression profiling by qPCR

The entirety of RNA extracted from cells was carried out using the RNeasy Mini kit (Qiagen, USA), adhering to the guidelines provided by the manufacturer. The evaluation of mRNA expression levels pertaining to Bax, Bad, Bcl-2, Caspase-3, -9 and cytochrome-c were conducted using real-time PCR on the Eppendorf Thermocycler from the USA. This analysis was normalized to GAPDH mRNA. The calculation of relative gene expression levels (fold change) was carried out through the utilization of the $2^{-\Delta\Delta Ct}$ formula.

2.10. Protein expression by western blotting

Proteins extracted from the cell lysate were segregated through gel electrophoresis with SDS and then transferred onto a nitrocellulose sheet. The nitrocellulose membranes were obstructed using a blocking buffer (Tris-buffered saline [TBS] supplemented with 5% BSA) for a duration of 2 hours at ambient temperature. The nitrocellulose membranes were subjected to an incubation with primary antibodies (Bax, Bcl-2, Caspase-3, -9, cytochrome-c, NF-κB, p-I3K, p-AKT, and β-actin) in the above-mentioned solution while placed on an orbit shaker at a temperature of 4°C for a duration of 12 hours. The nitrocellulose membranes underwent rinsing using a TBST (TBS containing 0.1% Tween 20) solution, after which they were exposed to horseradish peroxidase-conjugated secondary antibodies for a period of 2 hours at ambient temperature. Subsequently, the nitrocellulose membranes underwent an additional wash with a TBST solution, and the bands were visualized and identified using a chemiluminescence substrate.

2.11. Statistical analysis

The results for both control and experimental cells were expressed as mean values along with their corresponding standard deviations. Statistical assessment was carried out using one-way ANOVA in the SPSS software, followed by Duncan's Multiple Range Test (DMRT) to compare the averages among groups. Significance was observed in the outcomes with a P value below 0.05.

3. RESULTS

3.1. α-Bisabolol influence on MCF-7 cell cytotoxicity

The cytotoxicity of α-Bisabolol has been investigated in MCF-7 cells for 72 hours at various doses (ranging from 1 to 500 μM). We found that increasing concentration of α-Bisabolol progressively declined the cell viability and proliferation, indicating a dose-dependent response (Fig. 1). The IC_{50} values, denoting the concentration that inhibits 50% of cell viability, were identified as 80 μM. The concentrations of α-Bisabolol spanning from 100 to 500 μM displayed a cell viability range between 44% and 18%. To proceed with subsequent assays, the investigation concentrated on non-toxic α-Bisabolol concentrations (5, 10, and 20 μM) that exhibited minimal impact on cell viability and were considered suitable for further experimentations.

3.2. Reactive oxygen species (ROS) generation and anti-oxidants in MCF-7 Cells upon α-Bisabolol treatment

The unbalanced redox environment in the cancer cells could protect them from oxidative stress and prolong their proliferation. Therefore, we studied the role of α-Bisabolol in inducing oxidative stress-mediated cell death in breast cancer cells. Initially, we noted that untreated MCF-7 cells have decreased ROS levels. Further treatment with α-Bisabolol (5, 10 & 20 μM) resulted in a significant increase in intracellular ROS, indicated by elevated DCF fluorescence intensity (Fig. 2a & b).

Conversely, we observed that α-Bisabolol treatment regulated the antioxidant enzyme system in MCF-7 cells. The antioxidants like GPx, SOD, catalase, and GSH were significantly decreased activities in α-Bisabolol treatment in a concentration-dependent manner (5, 10 & 20 μM; Fig. 3a & b).

3.3. Modulation of mitochondrial electrochemical potential in MCF-7 cells by α-Bisabolol

The influence of α-Bisabolol treatment across varying concentrations (5, 10, and 20 μM) on the mitochondrial membrane potential was studied. Our findings revealed that concentration-dependent treatment of α-Bisabolol corresponded to more pronounced declines in mitochondrial membrane potential in MCF-7 cells (Fig. 4). Particularly, treatments with 20 and 10 μM of α-Bisabolol exhibited notably more significant reductions in mitochondrial membrane potential compared to the 5 μM treatment and untreated control cells ($P < 0.05$). These observations robustly imply that α-Bisabolol induces oxidative instability, ultimately disrupting the mitochondrial membrane potential in MCF-7 cells.

3.4. Visualization of apoptotic morphological alterations induced by α-Bisabolol via EtBr/AO staining

The early apoptotic nuclear changes were studied to prove the apoptotic inducing property of α-Bisabolol in MCF-7 cells. We found that administration of α-Bisabolol led to noticeable alterations in the visual appearance of MCF-7 cells, characterized by chromatin assuming yellowish-orange or red, indicative of apoptosis initiation. Notably, the higher concentrations of α-Bisabolol (20 and 10 μM) exhibited dual staining of AO and EtBr, indicating late apoptosis stages. Conversely, the untreated control MCF-7 cells displayed no significant apoptotic morphological changes with round-shaped nuclei, devoid of any apoptotic features (Fig. 5a & b)

3.5. Exploring the influence of α-Bisabolol on the apoptotic proteins in MCF-7 Cells

In this study, we explored the regulatory role of α-Bisabolol in the intrinsic apoptosis pathway in MCF-7 breast cancer cells. We found that α-Bisabolol prompted the elevation of death-inducing molecules like Bax, Bad, Caspase-3, Caspase-9, and Cytochrome-c, alongside the reduction of the apoptosis-resistant factor Bcl-2 in MCF-7 cells. Among the three doses of α-Bisabolol, the most noticeable impact was observed at the 20 μM concentration (Fig. 6)

3.6. Investigating the impact of α-Bisabolol on the NF-κB/ p-PI3K/p-AKT pathway in MCF-7 Cells

The abnormal activation of the NF-κB/p-PI3K/p-Akt pathway is strongly linked to several forms of cancer, including breast cancer. We investigated the influence of α-Bisabolol on the NF-κB/p-PI3K/p-Akt signaling pathway in MCF-7 cells. We found that α-Bisabolol (20 μM) treatment led to a reduction in the levels of NF-κB/p-PI3K/p-Akt in comparison to the doses of 5 μM and 10 μM (Fig. 7).

4. DISCUSSION

In our investigation, we explored the anti-cancer effects of α-Bisabolol on the MCF-7 mammary cancer cell line. In the present study, we found that decreased cell viability and proliferation, in the concentration dependent manner. These findings are consistent with earlier research that has highlighted α-Bisabolol's ability to impede the proliferation of diverse cancer cell types including pancreatic,[20] glioma,[26] liver,[28] and NSCLC cells.[35]

Elevated quantities of reactive oxygen species (ROS) within the body can result in oxidative stress, harming essential cellular elements, notably DNA. This process plays a role in the progression of persistent illnesses, such as cancer.[36] Moreover, creating unbound radicals makes cells susceptible to harmful outcomes, such as chromosomal harm, protein degradation, and lipid oxidation. When the

generation of ROS surpasses the capabilities of the body's protective antioxidant mechanisms, oxidative stress is triggered.[37] Xie et al. revealed that oxidative harm is initiated by creating lipid peroxides and disturbing membrane potential.[38] Consequently, the stimulation of ROS is viewed as a practical approach to encourage apoptosis. For the first time in this investigation, we report that the presence of ROS in MCF-7 cells was identified by utilizing the DCFH-DA fluorescent marker. Following a 24-hour exposure to α-Bisabolol, the MCF-7 cells exhibited amplified fluorescence intensity compared to untreated cells, indicating heightened ROS generation. Applying α-Bisabolol led to a quantitatively measurable increase in intracellular ROS production.

A defense mechanism known as antioxidants is present in humans and animals to combat the detrimental effects of free radicals. Antioxidants prevent lipid peroxidation by interfering with the chain reaction initiated by free radicals. The findings from our present investigation unveiled a reduction in the functioning of SOD, GPX, catalase and GSH in MCF-7 breast carcinoma cells, attributed to heightened oxidative stress generation. Nonetheless, α-Bisabolol successfully counteracted this impact by scavenging free radicals and bolstering the antioxidant systems. These results are consistent with earlier research, which demonstrated that the administration of α-Bisabolol increased the levels of antioxidants in a rat model of Parkinson's disease induced by rotenone, compared to the control group of mice.[39]

Mitochondria perform a vital function in triggering apoptosis by increasing the outer mitochondrial membrane's permeability and diminishing the mitochondrial membrane's potential. This mechanism is essential for triggering apoptosis and eventual cell demise. Within these cellular components, a range of factors governing apoptosis are situated, including Bcl-2 family members and cytochrome c. Activating the apoptosis signal controls the transition in mitochondrial permeability, resulting in a reduction of mitochondrial membrane potential.[40] In our investigation, the administration of α-Bisabolol led to the disruption of mitochondrial membrane potential, causing the compromise of mitochondrial membrane integrity within MCF-7 cells. Correspondingly, Cavalieri et al. documented that α-Bisabolol distinctly provoked the rupture of mitochondrial electrochemical potential, resulting in the release of cytochrome C, ultimately initiating apoptosis in glioma cells.[26] Furthermore, α-Bisabolol exhibited noticeable chromatin condensation and fragmentation of the nucleus within MCF-7 cells, indicating the initiation of apoptosis. This observation is supported by the results of AO/EtBr staining. The staining of MCF-7 cells with ethidium bromide highlighted the disruption of membrane integrity, while the presence of bright green and orange dots allowed for the identification of both early and late apoptotic cells, respectively.

Apoptosis is a complex procedure that encompasses numerous signaling pathways, encompassing activation through death receptors, mitochondrial malfunction, caspase initiation, DNA impairment, and the influence of reactive oxygen species (ROS).[41–43] Cytotoxic substances utilize these routes to trigger controlled cell death in susceptible target cells, rendering them valuable across various therapeutic strategies.[44] This study focused on investigating the influence of α-Bisabolol treatment on cell apoptosis. The expression of genes and proteins associated with cell apoptosis was evaluated using

qPCR with real-time monitor and immunoblotting to delve into the underlying mechanisms. The outcomes indicated that treating MCF-7 cells with α-Bisabolol prompted a substantial increase in the expression of Bax, Bad, Cas-3, and Cas-9, along with a reduction in Bcl-2 expression. This shift in the balance between proapoptotic and antiapoptotic B-cell lymphoma 2 family proteins showed that α-Bisabolol could foster apoptosis. Noteworthy changes were observed in MCF-7 cells upon α-Bisabolol treatment, including a decrease in mitochondrial electrochemical potential, significant cytochrome c efflux, and caspase-9 induction. These mitochondrial events were closely tied to α-Bisabolol's impact on Bcl-2 family proteins. Furthermore, numerous reports have concurred that α-Bisabolol triggers apoptosis in various cancer cell lines. For instance, Cavalieri et al. found that α-Bisabolol rapidly initiates apoptosis through the mitochondrial pathway in glioma cells without adversely affecting non-cancerous glial cells.[26] α-Bisabolol has also been found to induce apoptosis in the primary human acute leukemia cell line CML-T1.[27] Likewise, α-Bisabolol prompts apoptosis in HepG2 cells via extrinsic and intrinsic pathways, demonstrating a response linked to dosage and duration. Murata et al. and Seki et al. reported α-Bisabolol-induced apoptosis in pancreatic carcinoma cells KLM1, Panc1, and KP4.[20,45] In line with our findings, Wu et al. demonstrated that α-Bisabolol triggers apoptosis in A459 cells in a concentration-responsive pattern accompanied by alterations in the Bax/Bcl-2 expression ratio. Collectively, these results underscore α-Bisabolol potential as a potent anticancer agent.[35]

The abnormal initiation of signaling pathways is acknowledged to enhance the persistence of tumor cells and the development of resistance to drugs, consequently intensifying the aggressiveness of cancer.[46] Numerous pieces of evidence highlight that PI3K and AKT molecules function as a central nexus, interfacing with multiple signaling pathways both upstream and downstream.[47,48,49] In our current research, we discovered that α-Bisabolol effectively hindered the phosphorylated state of PI3K and diminished the expression of its downstream effector, p-Akt. Western blot analysis of protein expression demonstrated α-Bisabolol's capacity to curtail the levels of p-Akt and p-PI3K. PI3K and Akt, being among the most frequently activated kinases in breast cancer, have been linked to an unfavorable prognosis in individuals with mammary cancer. The activation of PI3K and Akt contributes to apoptosis resistance, potentially rendering tumor cells unresponsive to treatment.[50,51] Similarly, investigations by Seki et al. indicated that α-Bisabolol impeded PI3K and Akt in pancreatic cancer cells.[45] Again, another study demonstrated that α-Bisabolol reduced the activation of p-PI3K and p-Akt in human non-small cell lung carcinoma cells.[35] As such, we hypothesize that the antitumor impact of α-Bisabolol is, to some extent, triggered through the PI3K/Akt signaling pathway cascade.

It is evident that the activation of the PI3K/Akt signaling pathway ultimately leads to the activation of various transcription factors, including NF-κB. NF-kB serves as a downstream element of the PI3K/Akt pathway, and its activation stems from the phosphorylation of IkB kinase (IKK), subsequently triggering the degradation of IkB. Elevated levels of active NF-kB have also been linked with chemoresistance, as it governs target genes, including apoptosis inhibitors. Our results indicated that α-Bisabolol deterred the activation and translocation of NF-κB into the nucleus. Our findings align with Ramazani et al. and Arunachalam et al. (2022)[52,53] who demonstrated α-Bisabolol's ability to suppress NF-kB in renal toxicity

in rats. Kim et al. (2011) reported that α-Bisabolol exhibited anti-inflammatory effects through the suppression of iNOS and COX-2 genes, achieved by inhibiting NF-κB signaling in LPS-induced RAW264.7 macrophages.[54]

5. CONCLUSION

Considering the outcomes of our study, we can assume that α-Bisabolol triggers apoptosis by suppressing the p-PI3K/p-Akt/NF-κB signaling pathway in MCF-7 cells. Furthermore, α-Bisabolol prompts the generation of ROS, triggers chromatin condensation, and diminishes mitochondrial membrane potential. These outcomes imply that α-Bisabolol holds promise as a significant compound for addressing mammary carcinoma and merits further exploration through research initiatives.

Declarations

ETHICAL APPROVAL

Not applicable

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None

DATA AVAILABILITY STATEMENT

Data is available on request from the authors.

AUTHOR CONTRIBUTIONS

Manuscript concept and design: A. Sundaresan and B. Deivasigamani. Analysis and Data interpretation: B. Deivasigamani and A. Sundaresan. Manuscript Preparation: A. Sundaresan and Mohammed Ibrahim Alquraishi. All the authors reviewed the manuscript.

CONFLICT OF INTEREST

All the authors stated that there are no conflicts of interest

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Figures

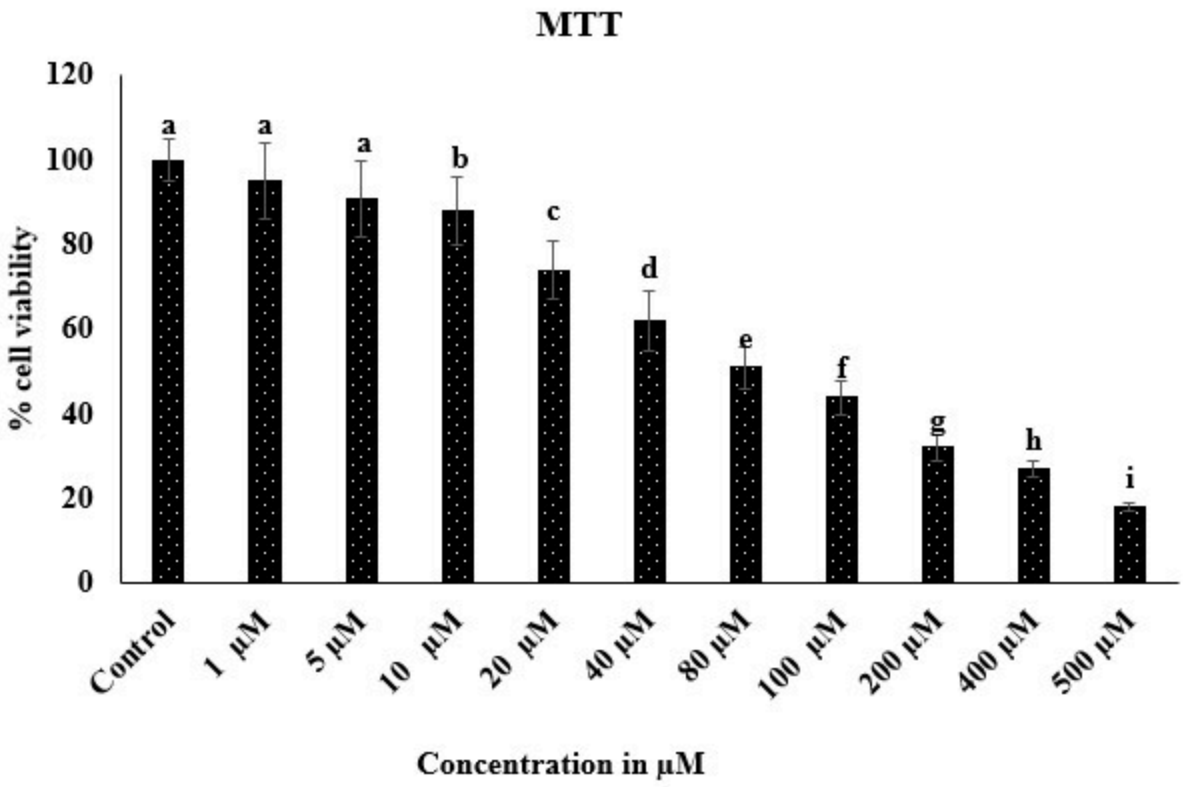

Figure 1

Effect of α-Bisabolol on cell viability in MCF-7 cells by MTT assay. The cells were treated with different concentrations of α-Bisabolol (1-500 µM) for a duration of 72 h, and the measurements were obtained using the MTT assay. The data presented as the mean ± SD, represent the results of six independent experiments in each group. values not sharing a common superscript differ significantly at $p \leq 0.05$ (DMRT).

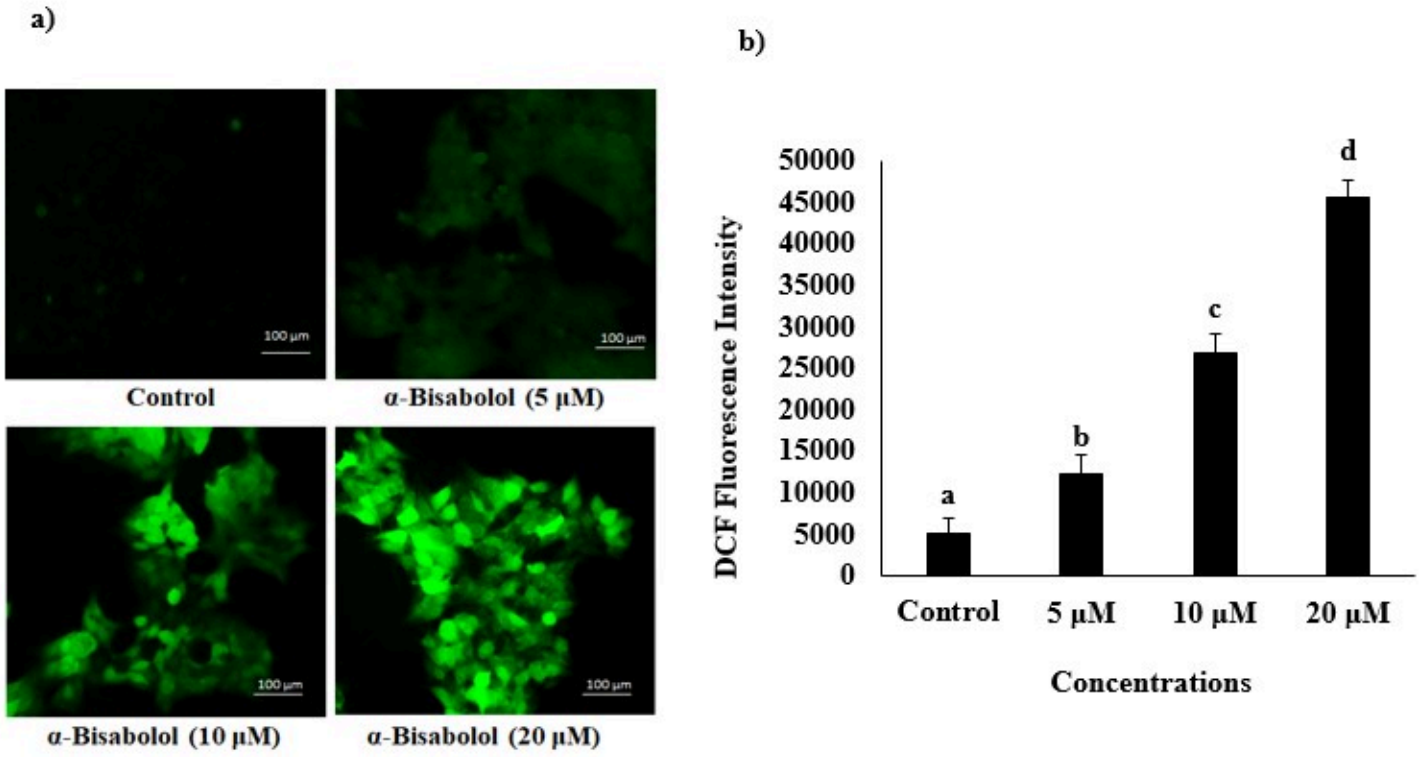

Figure 2

Effect of α-Bisabolol on intracellular ROS generation in MCF-7 cells. Fig. 2a. The Fluorescence microscopic images showed the increased DCF fluorescence due to the increase in intracellular ROS generation by different concentration of α-Bisabolol. Fig. 2b. The bar diagram depicts the fluorescence intensity of DCF. The points with error bar represented as Mean ± SD of three independent experiments and values not sharing a common superscript differ significantly at p≤0.05 (DMRT).

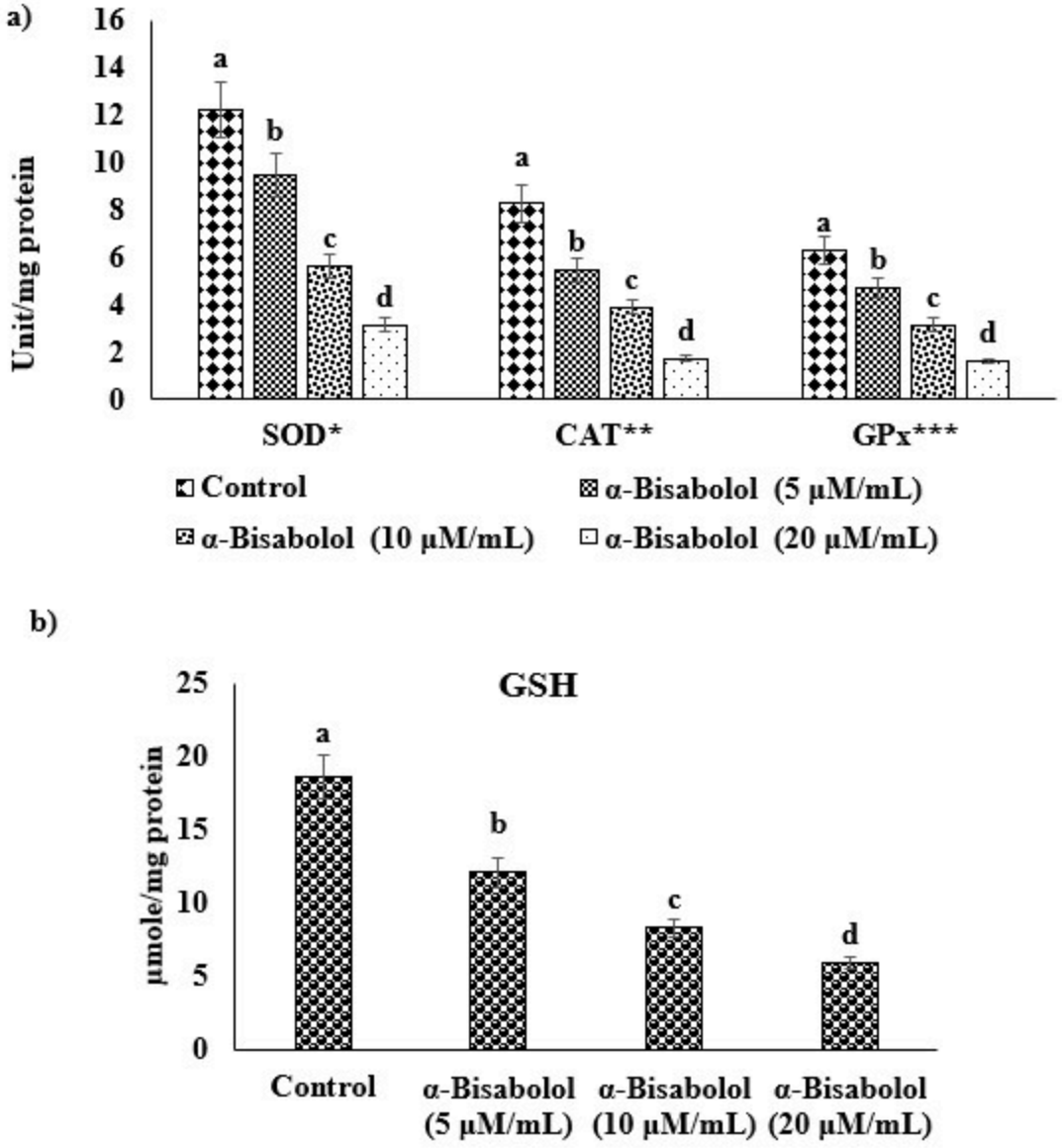

Figure 3

Effect of α-Bisabolol on antioxidant status in MCF-7 cells. Fig. 3a. Effect of α-Bisabolol on SOD, CAT and GPx activities in MCF-7 cells. Fig. 3b. Effect of α-Bisabolol on GSH level in MCF-7 cells. Values are given as means ± SD of six experiments in each group. Values not sharing a common marking (a, b, and c) differ significantly at p≤0.05. *-Enzyme concentration required for 50% inhibition of nitroblue tetrazolium reduction in 1 min. **-µmol of hydrogen peroxide consumed per minute. ***-µg of glutathione consumed per minute.

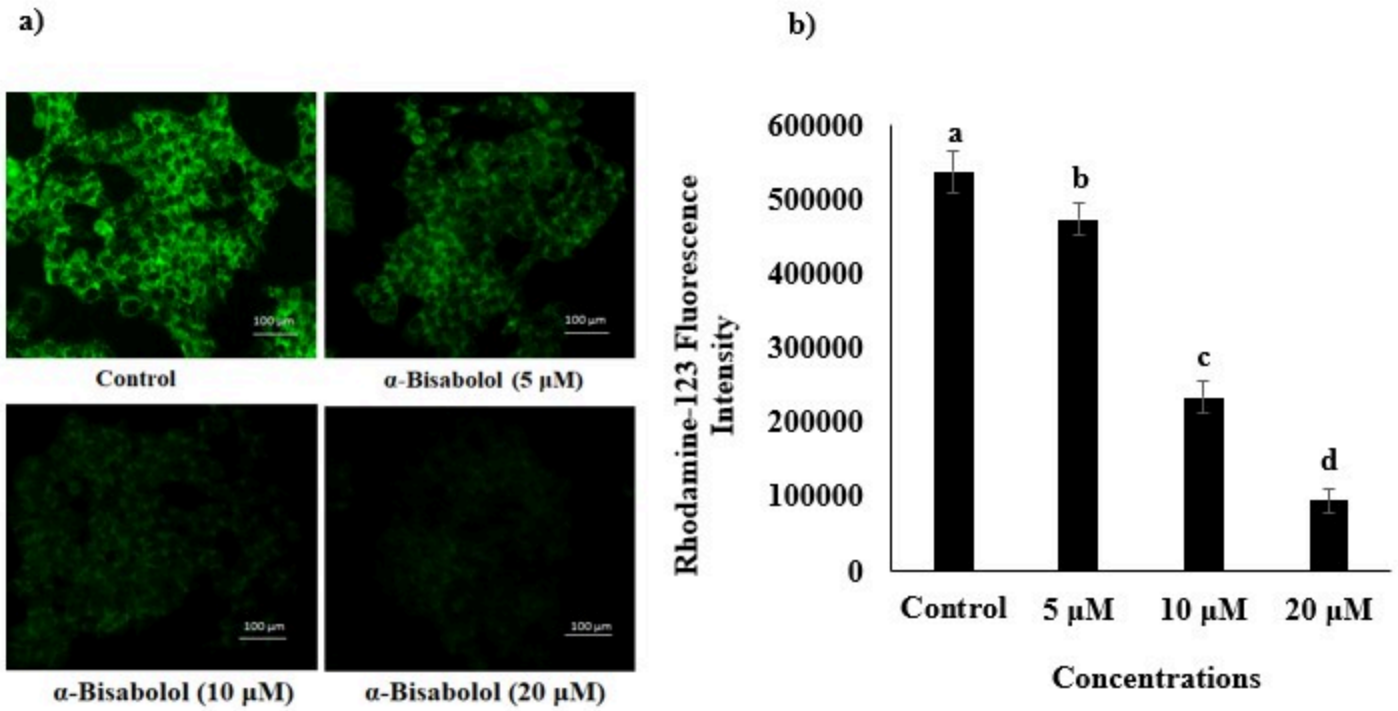

Figure 4

Effect of α-Bisabolol on alterations of mitochondrial membrane potential (MMP) in MCF-7 cells. Fig. 4a. The Fluorescence microscopic images showed the decrease in Rh-123 fluorescence due to the loss of MMP by different concentration of α-Bisabolol. Fig. 4b. The bar diagram depicts the fluorescence intensity of Rh-123. The points with error bar represented as Mean ± SD of three independent experiments and values not sharing a common superscript differ significantly at p≤0.05 (DMRT).

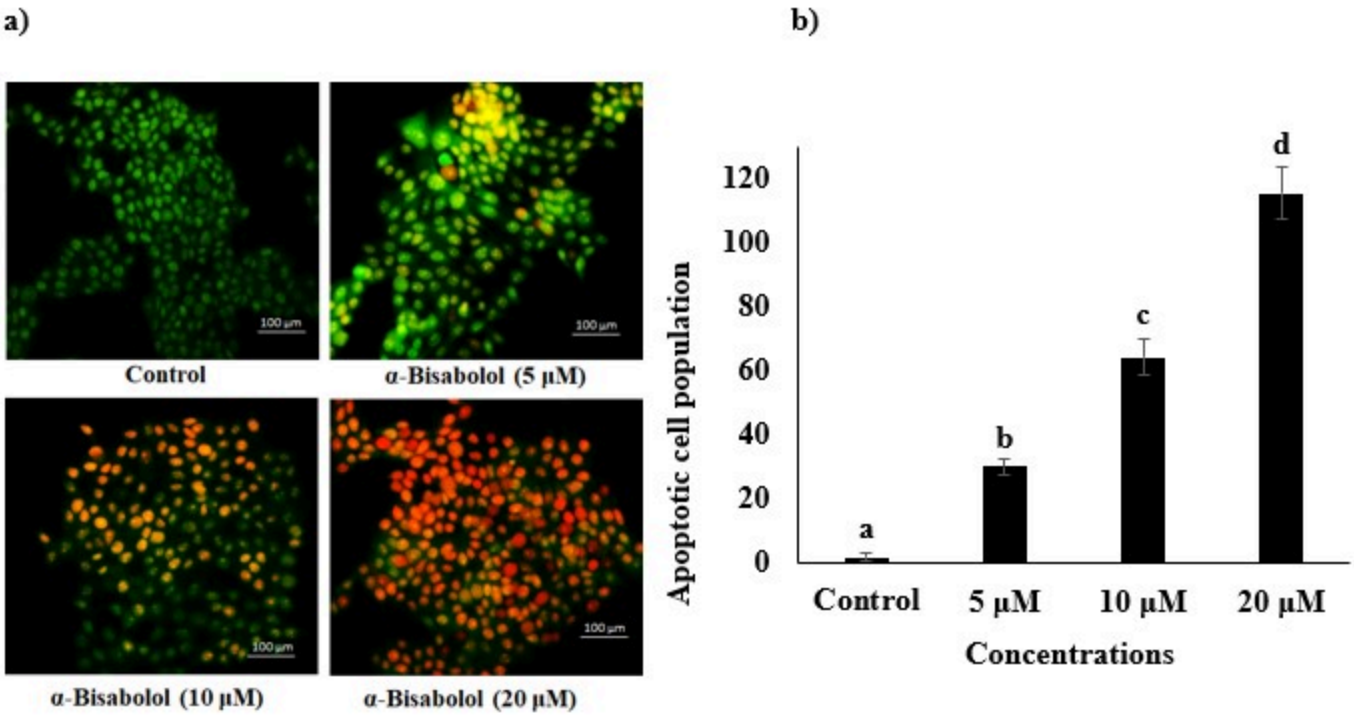

Figure 5

Effect of α-Bisabolol on apoptotic morphological changes in MCF-7 cells. Fig. 5a. The Fluorescence microscopic images showed the increased dual staining of AO/EtBr due to the nuclear damage caused by different concentration of α-Bisabolol. Fig. 5b. The bar diagram depicts the apoptotic cell population (EtBr-stained cells). The points with error bar represented as Mean ± SD of three independent experiments and values not sharing a common superscript differ significantly at p≤0.05 (DMRT).

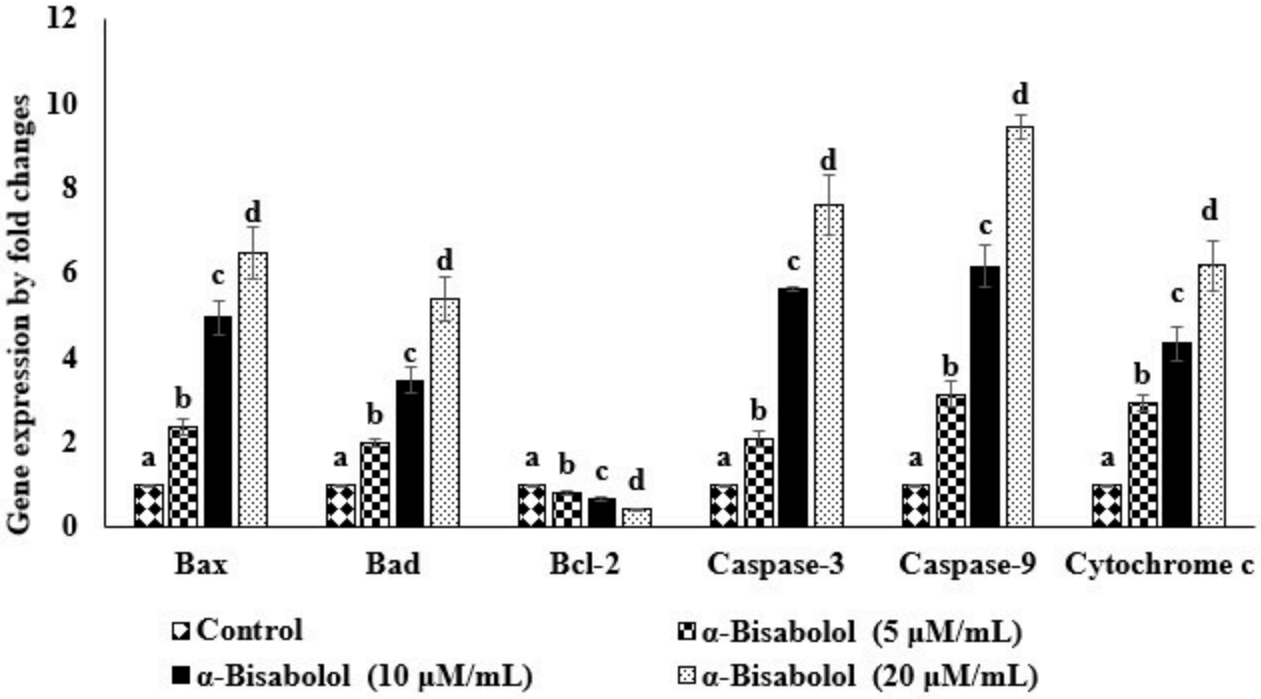

Figure 6

Effect of α-Bisabolol on apoptotic signaling in MCF-7 cells. The histogram depicts the quantitation of three independent experiments (means ± S.D). The Bax, Bad, Bcl-2, Caspase-3, -9 and cytochrome-c genes expression levels were normalized with the expression level of the GAPDH mRNA in each sample. Values not sharing a common superscript (a, b, c, d) differ significantly at $P < 0.05$ (DMRT).

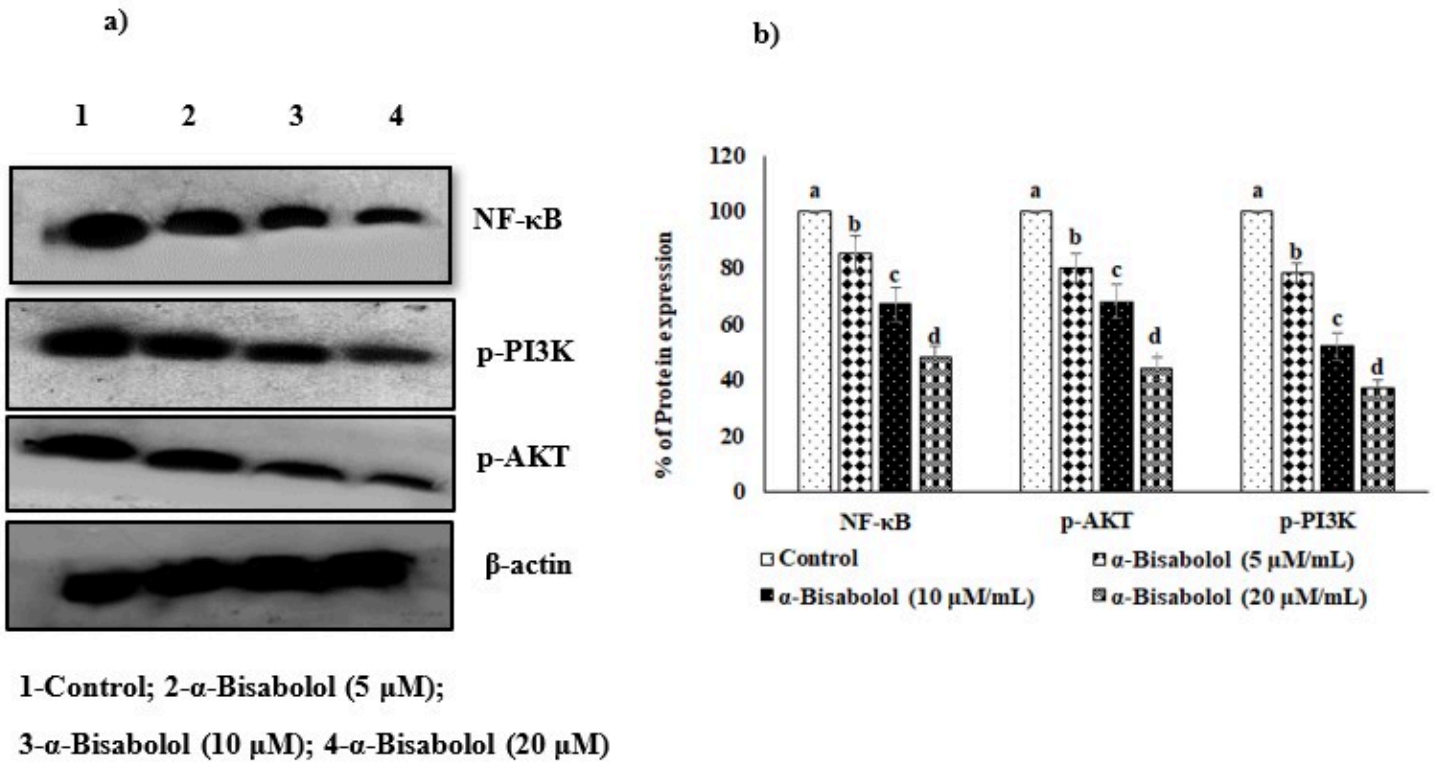

Figure 7

Effect of α-Bisabolol on NF-κB, p-PI3K and p-AKT protein expression in MCF-7 cells. Fig. 7a. Western blot analysis of NF-κB, p-PI3K and p-AKT protein expressions was acquired by chemiluminescence substrate. Fig. 7b. The quantification of protein was performed by densitometric analysis using Image J software. The densitometry data represent means ± SD. Values not sharing a common superscript (a, b, c, d) differ significantly at $P < 0.05$ (DMRT). The relative density of protein bands normalized to β-actin (n = 3).