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Anti-Cancer Efficacy of Niosomal Encapsulated Withania somnifera on Breast Cancer Cells: An in Vitro Study

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Abstract

Molecular analysis of breast cancer cells by niosomal encapsulated *Withania somnifera* (WS) extract is the focus of this investigation. Characterization of ASH-loaded niosomes produced by a thin film technique showed that they have an appropriate zeta potential, small particle size, and low polydispersity index. The concentration-dependent suppression of cell proliferation was seen in MCF-7 breast cancer cells treated with ASH-NIO, with an IC50 of 24.13 µM, and olaparib at values of 10.45 µM. Molecular investigation showed that ASH-NIO treated cells had lower levels of B-cell lymphoma 2 (Bcl2) mRNA expression compared to untreated cells, and upregulated levels of Tumor protein 53 (P53), Bcl-2-associated X protein (Bax), caspase-9, and caspase-3. These results provide more evidence that ASH-NIO has potential anti-cancer effects and may be useful as an adjunctive treatment for breast cancer.

Keywords Niosomes, Withania somnifera, Breast cancer, Molecular analysis, Anti-cancer efficacy

Introduction

The incidence of breast cancer among Arab women has been steadily increasing every year for the previous 24 years. Among women aged 45-55 years, breast cancer ranks as the second most common cause of death. Age, hormonal fluctuations, and stress are risk factors linked to breast cancer (1). The typical treatment options for breast cancer often include surgery to remove the damaged tissue, as well as radiotherapy, hormone therapy, and chemotherapy. Chemotherapy is primarily characterized by the occurrence of significant adverse reactions. Radiation therapy generates reactive free radicals that induce DNA damage, resulting in cell death and genomic harm in the stem cells (2). According to an assessment by the World Health Organization, 80% of the people in developing nations heavily rely on traditional medicine as their primary form of healthcare. An herbal composition with strong anticancer properties might be considered as a viable substitute for chemotherapy, since it avoids toxic side effects. Additionally, it can be used as a beneficial supplement to breast cancer treatment by reducing the adverse effects of the drug (3,4).

Plants are well recognized as one of the foremost suppliers of therapeutic compounds. Out of the 250,000 higher plant species in the globe, about 80,000 are used for medicinal purposes. For many years, medicinal plants have served as a valuable source of diverse bioactive components, which are commonly utilized in the

form of entire extracts or isolated compounds to treat various disorders (5). Phytochemicals are metabolites found in plants that serve as a defense mechanism, shielding them against microbial illnesses and pest infestations. These bioactive substances have pharmacological activities and are utilized as medicinal agents or pharmaceuticals. Herbal pharmaceuticals and formulations have become increasingly popular due to their potential therapeutic advantages, lower cost, and fewer side effects compared to allopathic medications. Additionally, they can enhance the bioavailability of prescriptions (6).

Various parts of plants have traditionally been utilized to address a range of medical conditions. The root is commonly employed for its aphrodisiac, hepatology, antifungal, antidepressant, astringent, neurasthenic, and muscle toning properties. *Withania somnifera* (WS), commonly referred to as Ashwagandha (ASH) is a member of the Solanaceae family. ASH is a renowned medicinal herb recognized for its phyto-pharmacological attributes, including anti-inflammatory, antioxidant, anti-stress, and immunomodulatory effects. Furthermore, ASH extract possesses anti-cancer effects. The pharmacological actions of ASH are mostly attributed to its withanolides, a group of chemicals found in its leaves, stems, and roots. Withaferin A is the main compound in humans that exhibits anticancer and antioxidant effects, as it acts as an immunomodulator. Moreover, withaferin A possesses adaptogenic properties and acts as an angiogenesis inhibitor. It also exhibits a radiosensitization impact on cancer cells, hence aiding in the regeneration of neurons (7).

Nanomedicine shows great potential in treating various chronic diseases, such as hepatic and neurological disorders. Various drug delivery systems have the capacity to overcome the restrictions related to the physical and chemical properties, as well as the distribution and effectiveness of phytopharmaceuticals. These methods achieve this by improving the controlled release, biodistribution, stability, and efficacy of both isolated compounds and purified extracts (8). Niosomes are a specific category of nanoparticle drug delivery devices known as non-ionic surfactant vehicles. They are spherical structures formed by the self-assembly of non-ionic amphiphiles in water. Niosomes have the capacity to encapsulate both hydrophilic and hydrophobic medicines within their central region and between the layers of their structure, respectively. Thus, they are regarded as an effective drug delivery method for various active agents such as phytochemicals, extracts, medicines, and numerous anticancer therapies (e.g., methotrexate, doxorubicin, and cisplatin) (9). Niosomes are regarded as uncomplicated, cost-effective, and very durable nanocarriers in comparison to various other nanocarriers that could be employed in cancer therapy for treatment and diagnosis (10). These lipid drug delivery methods have significant advantages over liposomes, such as enhanced chemical stability, extended shelf life, high purity, consistent content, cheap cost, and easier storage (11). They possess the capacity to extend the duration of trapped pharmaceuticals in the bloodstream, reduce drug breakdown and deactivation following injection, hence reducing unwanted side effects and toxicity. Additionally, they enhance the availability of the drug and enable targeted delivery to the affected area (12).

This study aimed to develop a niosome carrier (ASH-NIO) loaded with ASH extract to assess the anti-cancer efficacy of niosomal encapsulated ASH on breast cancer cells.

Materials and methods

Plant extract

The plant material and ASH extract were acquired from a herbal store in Cairo, Egypt, which preserved a voucher specimen. An Ultra-Turrax® T25 homogenizer (Janke & Kunkel IKA Lab., Staufen, Germany) was used to exhaustively extract 1 kilogram of powder with 3 × 2.5 L of distilled water for 20 minutes at around 60 °C. The biological assays and nanopreparations were made possible by freeze-drying the aqueous extract, which yielded 9% w/w compared to the dry plant.

Preparation of Niosomes Loaded with ASH

In order to prepare the niosome, the thin film approach was employed. A thin lipid film was formed by dissolving 100 mg of Span 60 and 20 mg of cholesterol in 10 ml of chloroform and then removing the solvent using a rotary evaporator set at 120 rpm, 60 °C, and 1 hour. An ASH solution was used to hydrate the produced thin film in order to produce ASH-NIO. To get the final concentration, 50 mg/ml of ash was dissolved in 10 ml of phosphate buffer saline solution which was heated to 60°C and maintained at pH 7.4. Then, the lipid layer was mixed with the water and subjected to sonication in an ultrasonic bath (Sonics and Materials Inc. USA) set at 60 Hz for 5 minutes in order to produce the niosomal formulation. The characterization of niosomes involved the utilization of dynamic light scattering and transmission electron microscopy to assess their size, polydispersity index, and morphology (It is preferable to have a reference).

Cells and *in vitro* treatment

A 2ml of DMEM was added to 96-well culture plates containing MCF-7 breast cancer cells at a density of $5x10^5$ cells/ml after 48 hours. The incubating medium was taken out and replaced with new medium that contained different concentrations of ASH-NIO (5-100 μM). One well was left untreated and incubated for 48 hours at 37 ° C in a humified atmosphere of 95% CO_2, which was maintained until further analysis (It is preferable to have a reference).

Effect of MTT assay on human cell lines for cytotoxicity

One colorimetric method for measuring cellular metabolism is the MTT test. The conversion of yellow MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) to purple formazan, as described by Mosmann (1983), was used to assess cell viability (Reference). In an *in vitro* study, breast cancer cells were cultivated and exposed to different concentrations of ASH-NIO and olaparib. Cell viability was evaluated using the MTT test.

Molecular biological determination

Determination of the levels of expression of Tumor protein 53 (P53), Bcl-2-associated X protein (Bax), B-cell lymphoma 2 (Bcl2), Caspase-9, and Caspase-3, were detected in MCF-7 cell line by PCR. RNA extraction was then performed, followed by cDNA synthesis. The expression of these genes was measured using a SYBR Green kit by real-time PCR. The internal control utilized in the experiment was GAPDH. The expression levels of the target genes were adjusted to match the expression levels of GAPDH. The fold change in gene expression compared to the control was determined using the $2^{-\Delta\Delta CT}$ technique (13).

Statistical analysis

The findings were presented as mean ± standard deviation (SD). We utilized analysis of variance (ANOVA) with the Duncan multiple range test as a post hoc test to investigate the influence of the treatment groups on the relevant parameters. Statistical significance was indicated by a P-value less than 0.05. All charts and statistical analyses were conducted using GraphPad Prism 8.0.2 (GraphPad Software, Inc.) and SPSS version 28.0 (IBM Corp., NY, USA).

Results

Examination of ASH-NIO Zeta potential, particle size, and polydispersity index

The ASH-NIO experiments demonstrated small particles with low PDI and dynamic light scattering. Figure 1 showed how the ASH-NIO small particle size values improve oral bioavailability and drug absorption. The average particle size, PDI, and zeta potential were assessed using the Malvern Zetasizer dynamic scattering light analysis. The PDI value fell within the permissible range at 0.276±0.12. Approximately -15±0.48 mV was the ASH-NIO ZP value.

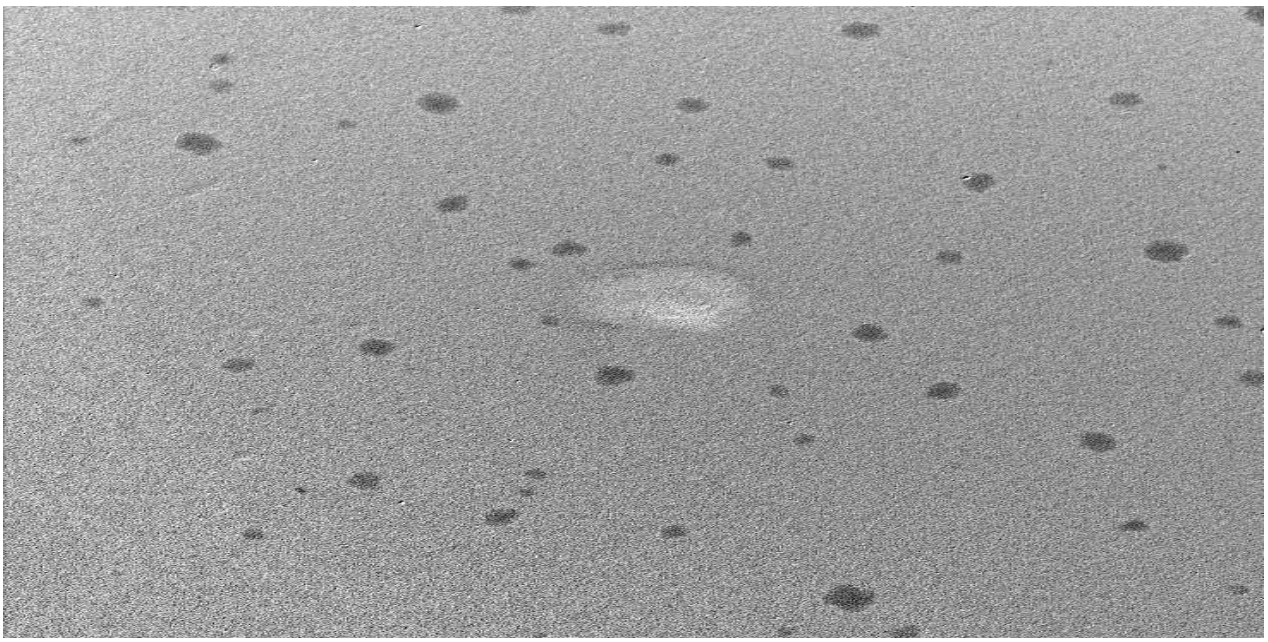

Figure 1*. Transmission electron microscope image of the ASH-NIO.*

The Impact of ASH-NIO on MCF-7 Cells

Cell proliferation rate in MCF-7 samples treated with different concentrations of ASH-NIO and olaparib was measured using the MTT test. By employing the MTT test, we investigated the *in vitro* impacts of these medications on the viability of MCF-7 cells after 48 hours. A concentration inhibitory impact of ASH-NIO and olaparib on MCF-7 cells was demonstrated by the results. The concentration-dependent inhibition of MCF-7 cell growth was seen with ASH-NIO and olaparib. The cytotoxic impact was observed in cell when exposed to ASH-NIO at concentrations of 24.13 µM, as well as in olaparib at values of 10.45 µM (Table 1).

Table 1. *IC_{50} of ASH-NIO, and olaparib on MCF-7 cells using MTT assay.*

Cells / **Drugs**	**ASH-NIO (µM)**	**Olaparib (µM)**
MCF-7 (IC_{50})	24.13	10.45

IC_{50}: Inhibitory concentration of the sample, which causes the death of 50% of cells in 48 h.

Evaluation of marker gene mRNA expression levels on MCF-7 cells

When comparing cancer cells treated with ASH-NIO and olaparib to untreated cancer cells, figures 2-6 illustrated that mRNA expression levels of P53, Bax, caspase-9, and caspase-3 are significantly upregulated compared to untreated cells. Additionally, Bcl2 mRNA expression levels were significantly downregulated in groups treated with ASH-NIO and olaparib in MCF-7 compared to untreated cells.

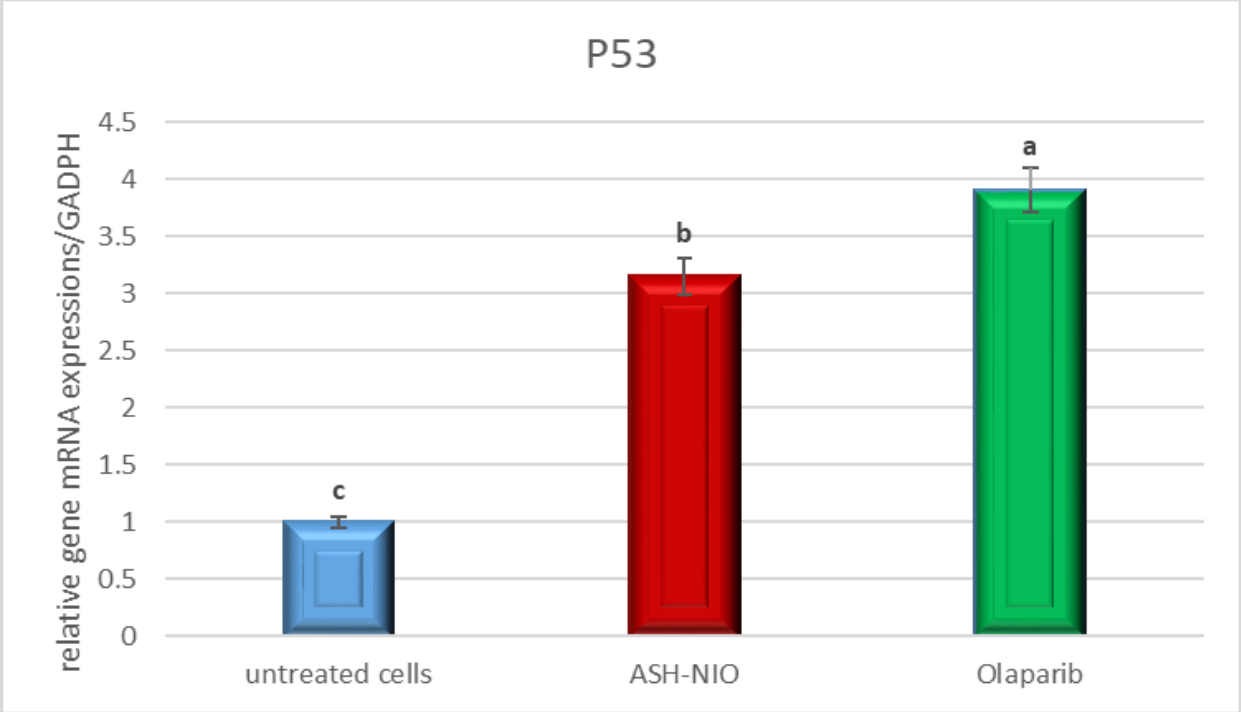

Figure 2. *ASH-NIO, and olaparib impacts on the gene expression of P53 (relative gene mRNA expressions/GADPH) in the MCF-7 cells. The analysis of quantitative variables was conducted using ANOVA, followed by a Tukey LSD post-hoc test. Means ± SD with different superscript are statistically different according to Duncan's multiple range test ($p < 0.05$). A significance level of $p < 0.05$ was considered statistically significant.*

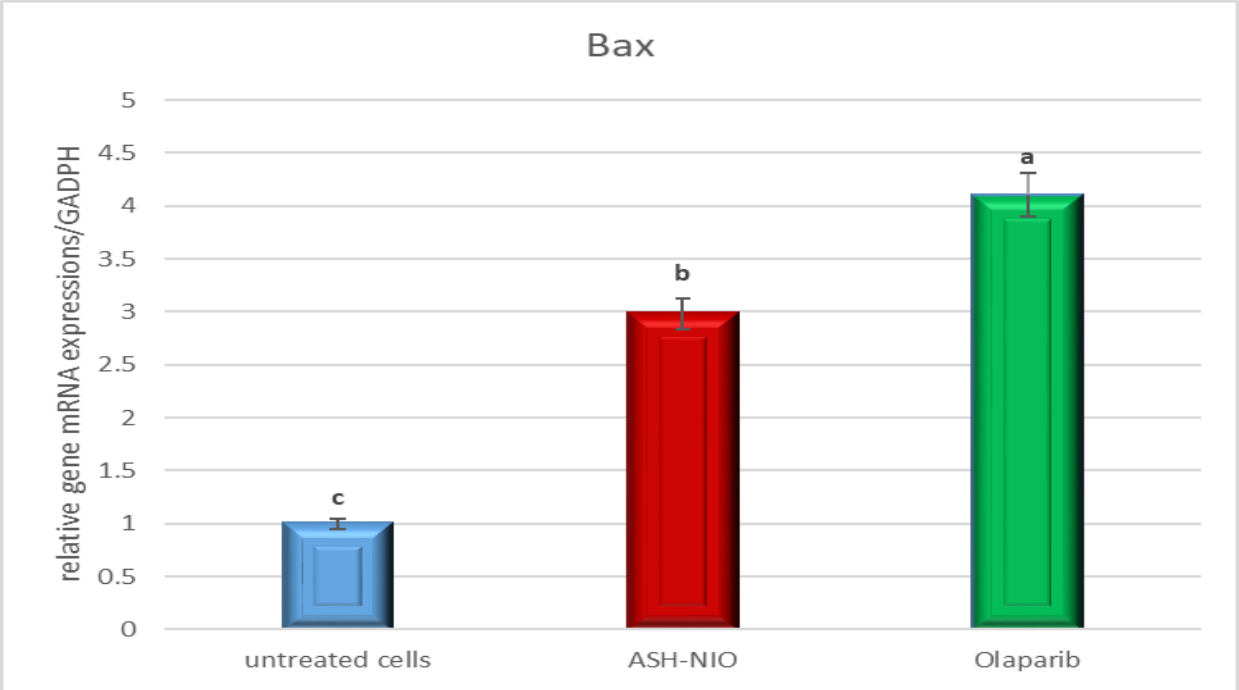

Figure 3. *ASH-NIO, and olaparib impacts on the gene expression of Bax (relative gene mRNA expressions/GADPH) in the MCF-7 cells. The analysis of quantitative variables was conducted using ANOVA, followed by a Tukey LSD post-hoc test. Means ± SD with different superscript are statistically different according to Duncan's multiple range test ($p < 0.05$). A significance level of $p < 0.05$ was considered statistically significant.*

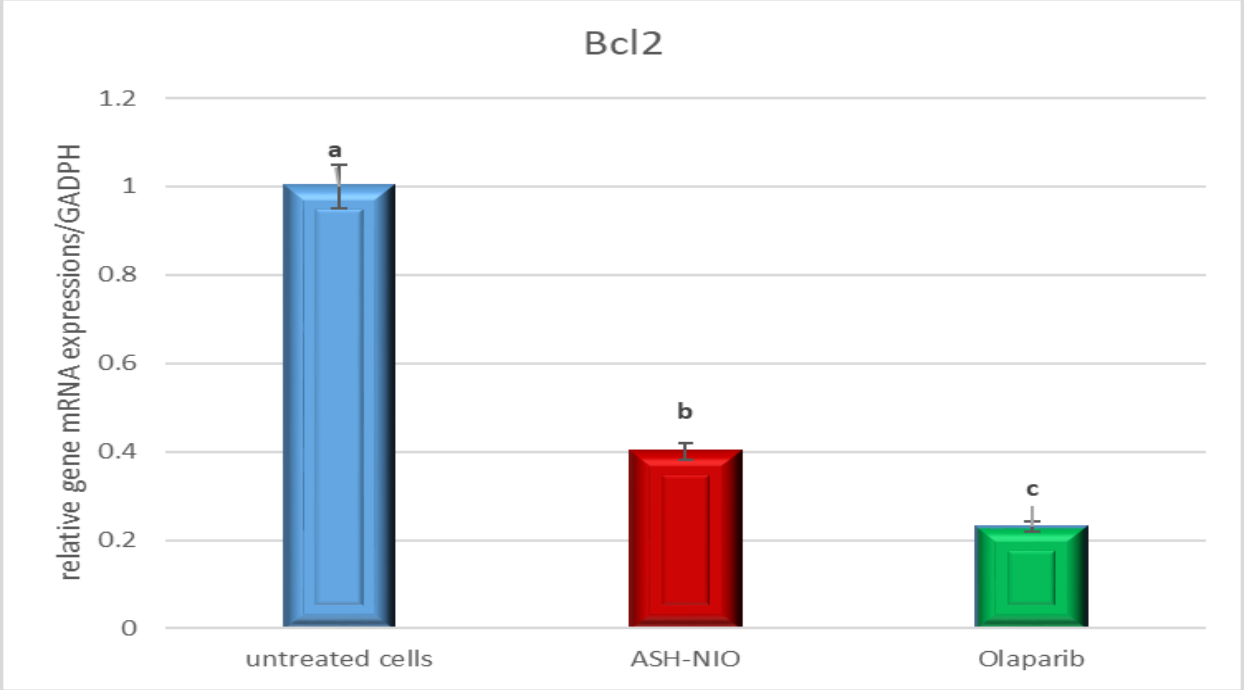

Figure 4. *ASH-NIO, and olaparib impacts on the gene expression of Bcl2 (relative gene mRNA expressions/GADPH) in the MCF-7 cells. The analysis of quantitative variables was conducted using ANOVA, followed by a Tukey LSD post-hoc test. Means ± SD with different superscript are statistically different according to Duncan's multiple range test ($p < 0.05$). A significance level of $p < 0.05$ was considered statistically significant.*

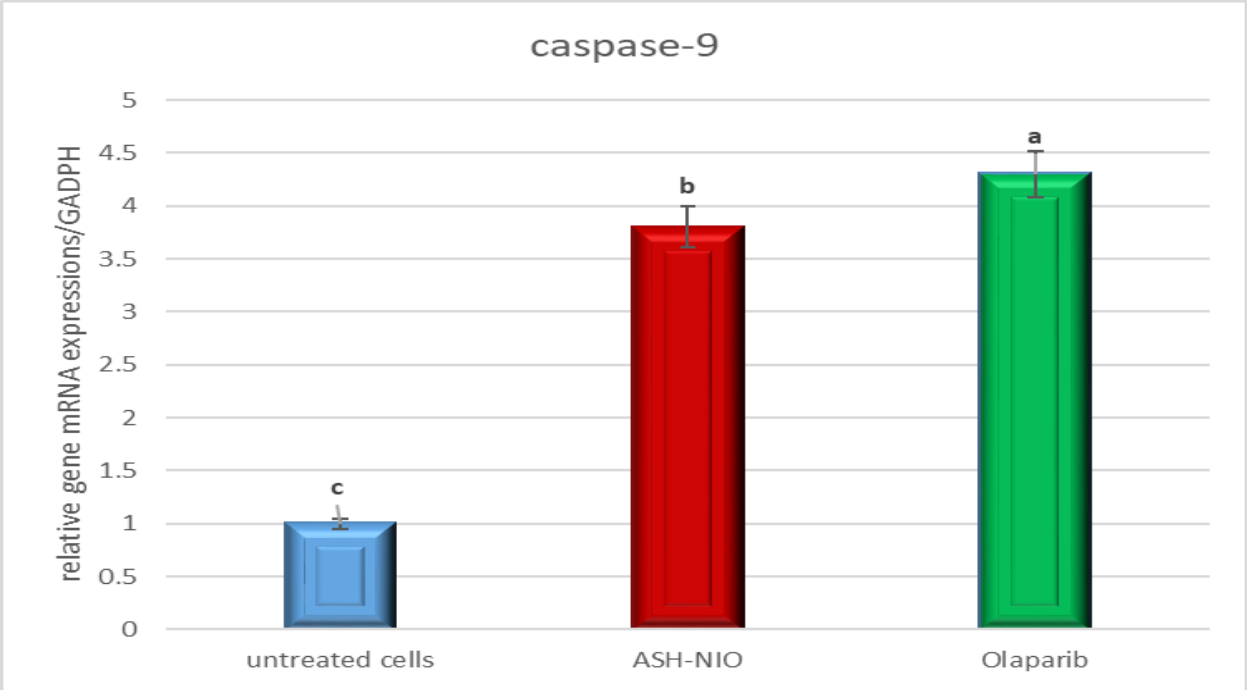

Figure 5. *ASH-NIO, and olaparib impacts on the gene expression of caspase-9 (relative gene mRNA expressions/GADPH) in the MCF-7 cells. The analysis of quantitative variables was conducted using ANOVA, followed by a Tukey LSD post-hoc test. Means ± SD with different superscript are statistically different according to Duncan's multiple range test ($p < 0.05$). A significance level of $p < 0.05$ was considered statistically significant.*

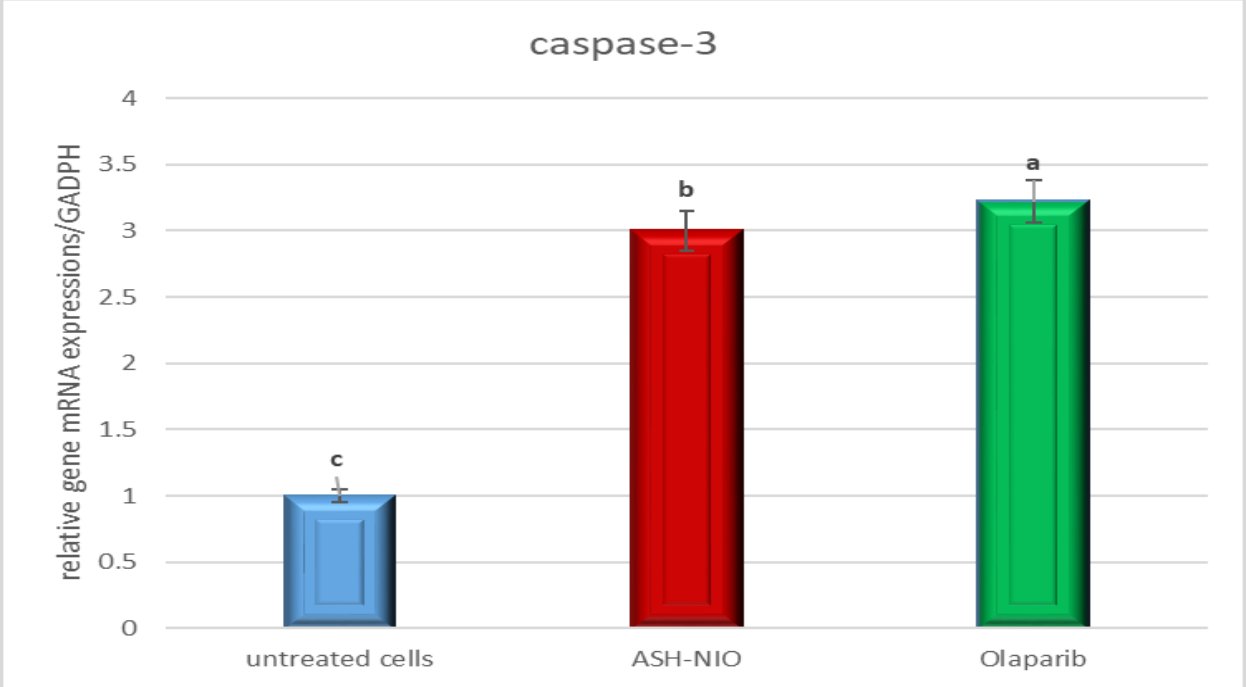

Figure 6. *ASH-NIO, and olaparib impacts on the gene expression of caspase-3 (relative gene mRNA expressions/GADPH) in the MCF-7 cells. The analysis of quantitative variables was conducted using ANOVA, followed by a Tukey LSD post-hoc test. Means ± SD with different superscript are statistically different according to Duncan's multiple range test ($p < 0.05$). A significance level of $p < 0.05$ was considered statistically significant.*

Discussion

Pharmacologically, ASH is significant as an antioxidant, immunomodulatory, hypolipidemic, chemopreventive, anti-inflammatory, anxiolytic, and antidepressant agent due to its biologically active

chemical constituents. However, due to the properties of its main bioactive compounds, ASH has poor bioavailability. Therefore, the goal of developing ASH-NIO was to increase both the therapeutic efficacy and physical stability of ASH in the gastrointestinal tract. It should be noted that no prior testing has been conducted on the ASH-NIO on MCF-7. Our results revealed that ASH-NIO had anti-cancer efficacy against MCF-7 breast cells. There has been no prior discussion in the literature regarding the use of niosomal encapsulation to improve the delivery and efficacy of WS, despite its potential therapeutic capabilities in cancer treatment. This is especially true when it comes to breast cancer models. Our research provides the first evidence that ASH-NIO inhibits cell proliferation in MCF-7 breast cancer cells and alters important molecular markers linked to cell death. This innovative strategy offers hope for better patient outcomes in this challenging disease setting, paving the way for further study into the use of ASH-NIO as an adjunctive treatment in breast cancer.

Although WS has no impact on healthy human cells, it is cytotoxic to several types of tumor cells, suggesting that it targets cancer cells specifically (14). Multiple *in vitro* and *in vivo* studies have shown that WS has a variety of useful qualities, including those that alleviate anxiety, reduce angiogenesis, alleviate depression, fight tumors, cytotoxicity, genotoxicity, antibacterial, and antifungal activities. WS affects cancer cell cytotoxicity through increasing intracellular ROS levels (15,16). WS blocks various abnormal pathways that are involved in inflammation and proliferation (e.g., IL-6, TNF-α, and cycloxygenase-2 (COX-2)), angiogenesis and metastasis (e.g., VEFG, MMP9, TWIST, NF-κB, and STAT), cell survival (e.g., Bcl-2, Bcl-xL, survivin, and cIAP1/2), and cell cycle regulation (e.g., cyclin A, cyclin D1, Cdks, p21, and p53) (17). In addition, WS is an adaptogenic Ayurvedic herb that is well-known for its stress-relieving and general health-enhancing properties; multiple research have demonstrated WS's efficacy in this regard (18). Individuals report higher levels of happiness and well-being after taking a full-spectrum WS root extract at a high concentration, which increases their resilience to stress (19).

Several challenges, including chemotherapy's limited therapeutic index, damage to normal cells, and multi-drug resistance, make it difficult to effectively treat breast cancer. The latter is particularly problematic because multidrug-resistant infections are the cause of treatment failure and, ultimately, mortality. Nowadays, a common strategy for overcoming MDR is to combine chemotherapy with natural products. A current study investigated the efficacy of water extracts from WS with cisplatin in two different laboratory settings: one using EMT6/P and EMT6/CPR breast cancer cell lines in a petri dish, and the other using female Balb/C mice that had been injected with the same cell lines. The results from both in vitro and in vivo experiments were encouraging and significant in the development of breast cancer treatment, particularly given that no prior testing had been conducted on WS extract in this context (20).

Our viability assay results showed that WS extract inhibited the viability of EMT6/P and EMT6/CPR cell lines in a concentration-dependent way when tested *in vitro*. Previous investigations have found similar outcomes. The anti-breast cancer effect of the crude water extract of WS on MCF-7 cell lines was shown to be dose-dependent, according to Prasad et al. (21). The anticancer components found in the ASH-NIO are responsible for its antiproliferative action. According to the previous observation, succinic acid component in ASH (68.52% concentration) boosted caspase-3 activity in human leukemic lymphoblasts (CCRF-CEM cell line) and had an apoptotic effect on acute lymphoblastic leukemia *in vitro* (22). The antiproliferative action and widespread usage of anthranilic acid (16.87%) and its derivatives in cancer treatment are further noteworthy aspects (23). Also, gallic acid (7.52%), a phenolic substance, can upregulate Fas and FasL and induce p53 and caspase-3, which in turn reduces viability and promotes apoptosis (24). These results were consistent with an earlier investigation that found that WS extract increased caspase-3 activity in MDA-MB231 cells (25). Another study declared that in comparison to the control, cells treated with WS extract, and cells treated with cisplatin, this combination's antiapoptotic effects successfully increased caspase-3 levels in both cell lines. The resistant cell line was made more sensitive to cisplatin at lower dosages by WS extracts, which indicate that the extracts significantly enhanced the cisplatin response (26). These results also are in line with earlier research showing that WS water extract shrank tumors in mice with colorectal (HT-29) and cervical (HeLa) cell-derived malignancies. Withaferin A, an ingredient in WS, was found in another study to reduce tumor size in mice after they were injected with HeLa cells (27).

According to the published mechanistic studies, WS can make cancer cells more sensitive to chemotherapy by activating the apoptotic pathway, the most common mechanism for cell death caused by cisplatin. In order to overcome resistance and trigger cisplatin-induced apoptosis, WS activates tumor suppressor p53 (28). One potential strategy for reversing chemoresistance is the ability of WS to inhibit the autophagy flux in the MCF7 and MDA-MB-231 breast cancer cell lines (29). *In vivo* xenograft animal models using triple-negative breast cancer cells, Jian et al. (30) investigated the effects of PARP1 inhibitors on proliferation inhibition and apoptosis induction. They proposed that reducing human apurinic endonuclease 1 could enhance the sensitivity of olaparib in treating triple-negative breast cancer cells. Researchers Zhao et al. (31) discovered that MDA-

MB-231 cells were cytotoxic to PARP inhibitors, PI3K inhibitors, and carboplatin when used together. The MDA-MB-231 cell lines were also significantly affected by its synergistic effects. All medications affected cell cycle progression; immunofluorescence and western blotting showed that the combination of drugs damaged DNA, which led to an increase in non-homologous end joining repair and an inhibition of homologous recombination repair, ultimately resulting in cytotoxicity. Based on these results, it is highly recommended to study the therapeutic effects of olaparib with carboplatin and BKM120 (a PI3K inhibitor) in animal studies before moving on to the MDA-MB-231 clinical trials in humans. According to Vysyaraju et al. (32), olaparib NPs significantly reduced tumor growth in 4T1-Luc tumor-bearing mice by enhancing apoptotic ROS generation and lowering Ki-67 expression, two antiproliferative markers. When compared to free olaparib, olaparib NPs considerably inhibited the growth of lung metastases and halted the cell population in the G2/M phase in both cell lines that were studied. Olaparib NPs show promise as a nanomedicine for breast cancer treatment. In their study on olaparib resistance in Ewing sarcoma, Heisey et al. (33) showed that Bcl2 and BCL-XL work together. Potentially trialed in clinical trials was their innovative and rational combo therapy. While they did find that EWS-FLI1 increases Bcl2 expression, they also found that venetoclax is insufficient on its own to render Ewing sarcoma cells responsive to olaparib. Accordingly, Ewing sarcoma survival depends on Bcl2 and BCL-XL. According to Paraghamian et al. (34), the anti-apoptotic protein Bcl2 expression and the phosphorylation of S6, a downstream target of the mTOR system, were both improved by combining olaparib and ONC206, as compared to using either drug alone. Combined administration of ONC206 and olaparib had a stronger impact on reducing Bcl2 and Ki-67 expression in both obese and lean animals.

This work examines the anti-cancer potential of niosomal encapsulated WS extract on breast cancer cells. It has been demonstrated that ASH-NIO has concentration-dependent effects on apoptotic molecular markers and cell proliferation inhibition. Furthermore, these findings suggested that ASH-NIO might be helpful as a breast cancer adjunctive treatment.

Conclusion

The results demonstrated that the MCF-7 cells exhibited enhanced therapeutic efficacy when loaded with ASH extract, in comparison to cells that were not treated. As a conventional drug approved by the FDA, our findings showed that ASH-NIO had anti-cancer effects against *in vitro* cancer trials compared to the untreated group and the group treated with olaparib. Additional effects of upregulated Bcl-2 expression and enhanced P53, Bax, caspase-9, and caspase-3 were shown by our results. The study highlights the positive impact of ASH-NIO as a possible supplementary treatment that could help various cancer models and as an additional medication to existing standard medications, which is now the norm for cancer management and treatment. When given alongside other conventional medicines, ASH-NIO should only be the subject of rigorous research into its effects in various cancer models.

List of Abbreviations

ANOVA	Analysis of Variance
SD	Standard deviation
LSD	least significant difference
FDA	Food and Drug Administration
MDR	Multidrug Resistance
WS	Withania Somnifera
ASH	Ashwagandha
ASH-NIO	Ashwagandha + Niosome
TNF-α	Tumor necrosis factor-alpha
NPs	Nanoparticles
ROS	Reactive oxygen species
MTT	Mean transit time
DNA	Deoxyribonucleic acid
cDNA	Complementary DNA
RNA	Ribonucleic acid
mRNA	messenger RNA
PCR	polymerase chain reaction

IL-6	Interleukin 6
COX-2	cycloxygenase-2
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma-extra large
VEFG	Vascular endothelial growth facto
MMP-9	Matrix metalloproteinase-9
TWIST1	Twist-related protein 1
Cdks	Cyclin-dependent kinases
DMEM	Dulbecco's Modified Eagle Medium
mTOR	mechanistic target of rapamycin
IC50	Half-maximal inhibitory concentration
cIAP1/2	cellular Inhibitor of Apoptosis Protein 1 and 2
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
STAT	Signal Transducer and Activator of Transcription
NF-κB	Nuclear Factor kappa-light-chain-enhancer of activated B cells

Conflict of interest

According to the authors, there is not any conflict of interest.

Acknowledgments

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Associated Content

SYBR Green I (SG): is an asymmetrical cyanine dye used as a nucleic acid stain
GAPDH: is a Protein Coding gene.
Polydispersity index (PDI): is used as a measure of broadness of molecular weight distribution.
MCF-7: is a breast cancer cell line
Olaparib (PARP Inhibitor): is a small molecule inhibitor of poly(ADP-ribose) polymerase (PARP), an enzyme involved in DNA repair processes.
VEGF: A protein that promotes the growth of new blood vessels (angiogenesis)
MMP-9: An enzyme that breaks down extracellular matrix components, allowing cancer cells to invade surrounding tissues and enter the bloodstream.
TWIST1: A transcription factor that plays a critical role in the epithelial-mesenchymal transition (EMT).
Survivin: A member of the inhibitor of apoptosis (IAP)
p53: is a tumor suppressor protein.
p21 (Cip1/Waf1): is a cyclin-dependent kinase inhibitor (CKI)
Cyclin A: Cyclin A is essential for the control of the cell cycle at the S (synthesis) and G2/M (gap 2/mitosis) phases.
Cyclin D1: Cyclin D1 is involved in regulating the transition from the G1 phase to the S phase of the cell cycle.
EMT6: is a murine (mouse) mammary carcinoma cell line frequently used in cancer research, particularly in studies involving drug resistance and cancer treatment efficacy.
EMT6/P: This denotes the parent or wild-type EMT6 cell line.
EMT6/CPR: This denotes a subline of the EMT6 cell line that has been specifically selected or engineered to be resistant to certain chemotherapeutic drugs, often including cisplatin (CP).
BALB/c: is a strain of laboratory mice widely used in biomedical research.
CCRF-CEM cell line: is a widely used human cell line in biomedical research, especially in the field of leukemia and cancer pharmacology.
Fas (also known as CD95 or APO-1) and **FasL** (Fas Ligand): are key components of the extrinsic pathway of apoptosis, a type of programmed cell death.
MDA-MB-231 cells: are a widely used human breast cancer cell line, specifically a model for triple-negative breast cancer (TNBC).
Caspase-9 and caspase-3: are key enzymes involved in the execution phase of apoptosis, or programmed cell death.
Both HT-29 and HeLa cells: are utilized extensively in laboratories worldwide for studying cancer biology, drug screening, and understanding the mechanisms of cell proliferation, apoptosis, and response to various treatments.
A xenograft: is a model specifically involves implanting human tumor cells or tissues into immunocompromised mice or other animal models to study tumor growth, progression, and response to therapies.
PARP1 inhibitors: are a class of drugs that target poly(ADP-ribose) polymerase 1 (PARP1), an enzyme involved in DNA repair processes.
Human apurinic/apyrimidinic endonuclease 1 (APE1), also known as REF-1 (redox effector factor 1), is a multifunctional enzyme involved in DNA repair and redox signaling.
Carboplatin: is a platinum-containing chemotherapy drug that forms DNA cross-links within cancer cells, disrupting DNA replication and causing cell death.
BKM120 (PI3K Inhibitor): is type of drug targets phosphoinositide 3-kinase and lead to reduced cancer cell proliferation and enhanced sensitivity to other anticancer therapies.

4T1-Luc cells: are a murine breast cancer cell line, and is widely used to study breast cancer metastasis and evaluate novel therapeutic strategies in preclinical settings.
G2/M phase: refers to a specific stage in the cell cycle where cells undergo preparation for and division into two daughter cells.
Ki-67: is a nuclear protein that is expressed during all active phases of the cell cycle, but not in the resting phase (G0) of the cell cycle.
EWS-FLI1: is a fusion oncogene that plays a pivotal role in the pathogenesis of Ewing sarcoma, a rare and aggressive type of bone and soft tissue cancer that primarily affects children and young adults.
Venetoclax: is a medication used in the treatment of certain types of blood cancers, particularly chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL).
Phosphorylation of S6: refers to a biochemical process where the ribosomal protein S6 undergoes phosphorylation, a modification involving the addition of a phosphate group to the protein molecule.
ONC206: is an investigational small molecule compound with potential therapeutic applications in oncology.
Bax: is a pro-apoptotic protein belonging to the Bcl-2 family, which plays a critical role in regulating apoptosis, or programmed cell death.