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Enhanced Anticancer Efficacy of Noscapine-Loaded Lipid Nanocapsules: *In-vitro* and *In-vivo* Evaluation.

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Abstract

Noscapine (NOS), an alkaloid with several interesting biological applications, has demonstrated antitumor action and cough suppressant potential. However, the strong hydrophobic nature of NOS makes it poorly water soluble. Therefore, it was necessary to figure out how to make NOS more soluble. In this study, we investigated the anticancer effect of free NOS as compared to that of NOS-loaded lipid nanocapsules (NOS-LNCs) on the 4T1 cell line, and in an orthotopic breast cancer (BC) mouse model. Spherical-shaped NOS-LNCs were prepared with a particle size (PS) of 213.4 ± 1.41 nm and a zeta potential (ZP) of -13.6 ± 1.17. The IC50 of free NOS, NOS-LNCs, and plain LNCs was measured on the 4T1 cell line by MTT assay, and the results revealed that the IC50 was 66.107 µg/ml, 13.209 µg/ml, and 121.132 µg/ml, respectively. On the other hand, NOS-LNCs demonstrated stronger anticancer effects in-vivo along with strong antiproliferative and antioxidant properties. Also, the immunohistochemistry analysis of BAX and BCL-2 in the tumor tissue confirmed the apoptotic activity of the NOS-LNCs, as it increased the expression of BAX and downregulated BCL-2. In conclusion, the current investigation showed that the developed NOS nanoform has a great deal of potential as a therapeutic agent for the treatment of BC.

Keywords: Noscapine; Breast cancer; Anticancer effect; Lipid nanocapsules.

1. Introduction

Natural anticancer medications are becoming more and more popular since they effectively inhibit the growth of cancer cells. An herb is a plant or a component produced from it that is utilized for flavor, smell, and/or medicinal qualities in addition to other uses **[1].** Traditional medicinal herbs are naturally occurring plant-derived drugs that have been utilized for hundreds of years in local or regional healing traditions to treat a variety of illnesses, including cancer, with little to no chemical modification to their formulation **[2].** However, poor solubility, limited bioavailability, instability, and poor permeability are among the biopharmaceutical problems that many natural compounds face **[3, 4].** In order to increase the bioavailability of various bioactive chemicals or herbs, decrease side effects, and prevent drug degradation, a number of innovative drug delivery systems have been developed over the past 20 years **[3, 5, 6].** These delivery methods also increase stability, target specific drug sites, boost therapeutic efficacy, and stop first-pass metabolism.

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An alkaloid known as noscapine was extracted from Papaver somniferum, a plant used to make opium. Noscapine (NOS) is an antitussive heterocyclic chemical that is based on isoquinoline. In addition, it is a well-liked suppressor for coughs and is said to be a prospective cancer-curing chemical. It can attach to tubulin and alter its conformation. This inhibits mitosis, interferes with microtubule mobility, and ultimately kills cancer cells. Additionally, it has been claimed to be a potential contender against the suppression of Chikungunya's non-structural protease, the major protease of SARS-CoV-2, and others **[7-9].** NOS is also a drug with antitumor qualities, as demonstrated by earlier research, which alters the course of the cell cycle and, as a result, causes apoptosis in cell lines of various malignancies **[10].** NOS was reported in a previous study to upregulate BAX expression and downregulate BCL-2 expression in breast cancer (BC) cell lines **[11].** Its qualities make it a desirable medication to research for the treatment of BC. The tumor models have also demonstrated NOS' antiangiogenic properties **[12].** There are two possible pathways for NOS's antiangiogenic effects. Initially, NOS downregulates the expression of hypoxia-inducible factor-1 (HIF-1) in hypoxic tumor cells, which in turn decreases the production of vascular endothelial growth factor (VEGF) **[13].** Second, NOS inhibits the formation of blood vessels by blocking endothelial cells' response to VEGF. Additionally, NOS may disrupt endothelial cell migration, which is essential for the development of arteries and veins **[14, 15].**

The application of nanotechnology in BC therapy is commonplace since it is one of the scientific and technological domains with the quickest rate of growth worldwide. As time goes on, nanotechnology helps uncover new cancer research approaches, even at the molecular level. Differentiating themselves in various ways, nanoparticles can be applied as nanomedicine in therapeutic settings. Regarding market share, licensed nanomedicines consist mostly of nanopharmaceuticals (drug delivery products), accounting for 75% of the total **[16].** The main benefits of nanocarriers comprise enhanced drug solubility, defences against drug deterioration, enhanced bioavailability, regulated release, target-specific medication delivery, and fewer dose intervals. This allows medications to be delivered safely while shielding non-target cells and tissues from the negative effects of the medication **[17].** Nanocarriers have been made from a variety of materials, such as metals, lipids, and polymers **[18].** Among them, LNCs come in first. It is believed that LNCs have a hybrid structural configuration that is a cross between polymeric nanoparticles and liposomes. They are composed of an emulsifier agent and a solid lipid-based outer shell enclosing an oily core **[19, 20].** The two main benefits of LNCs are their biological suitability and their biological degradation. Particles with sizes between 20 and 100 nm are anticipated to be created by a phase inversion technique. Physical stability in LNCs has been observed to last for up to 18 months. The convenience of production is derived from the straightforward preparatory procedure **[21].** Compared to traditional nanoparticles, LNCs have a higher drug encapsulation efficiency. LNCs formulation greatly increased the drug's water solubility and increased its bioavailability. Furthermore, LNCs preferred intra-tumour bio-distribution and potential functionalization for cancer targeting, nanocapsule-based drug delivery systems have been employed successfully in the treatment of cancer **[22].** Many previous studies used LNCs to enhance the delivery of treatment for breast tissue cancer. The loading of paclitaxel and salinomycin

into LNCs for the treatment of BC was carried out in a previous study **[23].** Also, the loading of some vitamin A derivatives, which have anticancer properties as Tretinoin, into LNCs to treat breast cancer, as well as synergistic with anti-cancer drugs as doxorubicin (DOXO) and 5-fluororacil (5-FU) **[24**].

BC is the most prevalent kind of cancer overall **[25].** About 41,000 women in the United States alone lose their lives due to BC each year, making up about 15% of all cancer-related fatalities **[26].** In the past twenty years, the prognosis of patients with breast cancer has improved due to early identification and extensive treatment procedures, which have lowered cancer mortality **[27].** The diagnosis, therapy, and screening for BC have advanced significantly **[28].** BC is caused by a variety of physiological and molecular circumstances in the breast, with risk factors that differ depending on the kind and hereditary predisposition of the disease. The BRCA1 or BRCA2 genes, in particular, continue to be important sources of mutations **[29].**

In the present study, the 4T1 cell line was utilized to evaluate the cytotoxic effect of both the free form and nanoformulated NOS. Besides, the anticancer potential of free NOS and NOS-LNCs was evaluated in an orthotopic BC animal model that is carrying 4T1 cells. A physiochemical analysis was performed on the prepared NOS-LNCs. In-vitro evaluation of NOS release was also conducted. To the best of our knowledge, NOS can play a key role in enhancing the antioxidant status and has a powerful anticancer effect due to its proapoptotic and antiproliferative characteristics. This work is the first to investigate this along with enhancing the anticancer efficacy of NOS through its nanoencapsulation into LNCs.

2. Materials and methods.

2.1. Reagents and cell lines

The American Type Culture Collection (ATCC) provided the mouse 4T1 triple-negative breast cancer cell line. The DMEM-cultured cells were supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin (Pen/Strip). Sigma-Aldrich (Germany) was the source of noscapine (NOS). The source of Lipoid® S75-3 (soybean lecithin, unsaturated phosphatidylcholine) was GmbH in Ludwigshafen, Germany, while BASF (Germany) supplied Cremophor® RH 40. GMBH (Ludwigshafen, Germany) provided Labrafac Lipophile oil, and BASF (Ludwigshafen, Germany) provided Solutol HS15

2.2. Lipid nanocapsules (LNCs) preparation

Heurtault et al.'s protocol **[30-32]** was followed in the preparation of noscapine-loaded lipid nanocapsules (NOS-LNCs). In summary, water (3 mL), Lipoid® S75 (200 mg), Labrafac Lipophile oil (1 gm), NOS (50 mg), and Solutol HS15 (1 gm) were combined and stirred until a homogeneous solution (W/O) was formed. Three heating-cooling cycles between 70 and 90°C were then applied to the combination. Dropping five milliliters of cooled water into the heated emulsion shocks the system and ultimately turns the created emulsion's exterior phase into (O/W).

2.3. Evaluation of lipid nanoparticles (LNCs)

Particle size, polydispersity index, and zeta potential determination Using the Zeta Sizer Nano series (Malvern Instruments SA, Worcestershire, UK), the developed plain and NOS-loaded LNCs were diluted using deionized water to maintain a particle count of approximately 250 kcps before being measured for particle size (PS), polydispersity index (PDI), and zeta potential (ZP) as previously described **[33].**

2.4. Investigation of *in-vitro* release

Following the previously mentioned procedures for dialysis, the cumulative release percentage of

NOS from LNCs was tracked and assessed **[31, 34].** The release media, phosphate buffer saline with a pH of 7.4 (10 ml), was placed into bottle chambers and incubated in a shaking water bath with 10% methanol present at 37°C and 150 rpm to create sink conditions. After that, at various intervals, aliquots were taken out of the bottles and replaced with brand-new release medium. NOS quantification was carried out using a UV-Vis spectrophotometer at a wavelength of 210 nm.

2.5. Evaluation of the stability of LNCs

After three months at room temperature, any changes in the PS and PDI of the prepared NOS-LNCs were monitored in order to evaluate their stability **[35]**.

2.6. Evaluation of the *in-vitro* cytotoxicity

A density of 2.5×10^3 cells/well was used to seed the murine 4T1 cells in 96-well plates. The cells were placed in an incubator set at 37ºC and 5% CO_2 throughout the whole night. A serial dilution of free NOS was then added, at concentrations of (200µM, 100, 50, 25, 12.50, 6.25) to each 2 wells after cell adhesion as control. NOS-LNCs was serially diluted to the following concentrations (25 µg/ml, 12.5, 6.25, 3.125, 1.5625, 0.78125) and added to the cells in duplicate, 200 µl for each concentration. The medium was disposed of after 48 hours, and 100µl of prepared MTT (3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide) was added to each well. The plate was then incubated for 4 hours until crystals formed, after which 100µl of SDS was added. The plate was then left in the dark for an additional night until the crystals were dissolved and the color was released (SERVA Electrophorese GmbH, Germany, cat. No. 002039501). The IC50 of the NOS and NOS-LNCs on the 4T1 breast cancer cell line was calculated using the Prism 8 programme on the plate using the Eliza reader (BioTek, Elx800, US) at the wavelength (570 nm) **[36].**

2.7. Animals:

The Faculty of Medicine and Health Sciences at Mansoura University in Egypt provided 36 female BALB/c mice, aged 6 to 8 weeks, for the purpose of this study. For seven days prior to the start of the trial, mice were housed in a 24°C laboratory with a 12-hour light/dark cycle.

2.7.1. Treatment with either free or nano-formulated NOS in BALB/c mice with tumor xenograft

In the lower fourth inguinal mammary fat pads S/C, $2x10^6$ 4T1 cells suspended in 100 µl of PBS were injected, as previously reported **[37].** A 100 µl PBS injection was given intraperitoneally (IP) to nine mice as a control. A Vernier caliper was used to calculate the tumor volume every two days. Utilizing the formula $V=1/2$ ($width^2$ × length), the tumor volumes were computed. The mice were split into three groups of nine animals each, two weeks following the inoculation of 4T1 cells. The groups were as follows: carboxymethyl cellulose (CMC) was administered orally to the first group (the cancer control group) every other day for 45 days; 2nd group were subjected to an oral gavage of NOS suspended in CMC for 45 days at a dosage of 150 mg/kg on alternate days [38]; and 3rd group received an oral gavage of NOS-LNCs prepared in a stock solution at a concentration of 7.5 mg/ml and suspended in PBS for 45 days at a dosage of 150 mg/kg on alternate days **[38].** In addition to, Carboxymethyl cellulose (CMC) was given orally to the control group every other day for 45 days.

2.7.2. Evaluation of oxidative stress biomarkers

Using commercial kits from Biodiagnostic®, Cairo, Egypt, the levels of nitric oxide (NO) **[39],** malondialdehyde (MDA) **[40]**, and the activities of catalase (CAT) **[41]** and superoxide dismutase (SOD) **[42]** were tested in accordance with the manufacturer's instructions. After the mammary glands were weighed and collected, they were homogenized at a 1:9 ratio using cold PBS. We ground the tissue with a mortar and gun using sterilized sand. The samples were centrifuged for 10 minutes at 4000 rpm, the sediment was removed, and the supernatant was collected for the oxidative stress parameters to be measured. The samples were kept at -80 for further examination.

2.7.3. Flowcytometry assay

For flow cytometry analysis, tumor tissues were removed and processed. To rinse the tissues, 2ml of PBS was utilized. Cut into small (12 mm3) pieces, the tissues were first arranged on a plate using ophthalmic scissors. Tissues were homogenized with a syringe plunger in order to get a suspension of individual cells. By centrifuging at 300 g for five minutes at room temperature, tissue lumps were removed using a strainer with a 200–300 screen size. After that, the supernatant was disposed of. The cells were divided into two batches and centrifuged twice in PBS containing 0.1% BSA at room temperature for five minutes. The supernatant was disposed of. Following an adjustment of the cell number to 1×107/mL, the cells were resuspended in PBS containing 0.1% BSA.

BD Bioscience (United States) supplied 10 µl of diluted antibody 1:100 distilled water, which was added to each fresh tube containing 100 µl of the cell suspension for Ki67 labelling. The tubes were then gently swirled. After 20 to 30 minutes of RT in the dark, the tubes were cleaned for five minutes at 1000 rpm using 2 ml of PBS, and the supernatants were aspirated. Each tube was filled with 0.5 cc of PBS. Following the addition of 20 µl of BD Via-ProbeTM Cell Viability Solution (Cat. No. 555816) for PE (Phycoerythrin) labelled antibody, the sample was examined using a flow cytometer (BD FACSCalibur) **[43].**

2.7.4. Histopathological examination

10% neutral-buffered formalin was used to fix tumor and lung tissues for the whole night before they were embedded in paraffin. Hematoxylin and eosin (H&E) Stain was applied to tissue slices, and they were then visually inspected under a microscope **[44].**

2.7.5. Immunohistochemical analysis

Tissue sections were separated into layers that were 4 m thick. The next procedures were deparaffinization with xylene, rehydration in ethanol at a graded concentration of 100-95-75%, and epitope retrieval. Using a pressure cooker and Cell Marque triology, the heat-induced epitope retrieval (HIER) approach was the suggested course of action. Rinse five times with distilled water and PBS after finished. Tissue sections were boiled in Quartett's ProTaqs® Antigen EnhancerV (Berlin, Germany) for 20 minutes to retrieve antigens, and then they were cooled at room temperature for an additional 20 minutes. Before immunohistochemistry, tissue slices were treated with 3% H2O2 for ten minutes to stop the activity of endogenous peroxidase. 5% goat serum was used to block tissue slices for one hour at room temperature. BAX polyclonal antibody (AB clonal technology, Cat. No. 12009) and Bcl-2 mouse monoclonal antibody (Quartett, Berlin, Germany) were diluted 1:100 and incubated for 1 hour at room

temperature on tissue slides. a 15-minute UltraVision One HRP Polymer incubation time came next. Finally, chromogen and substrate Make a ready-to-use DAB substrate solution using the supplies from Quartett (Berlin, Germany). DAB Chromogen Solution (Reagent B1) and DAB Buffer Solution (Reagent B2) should be combined in a 1:1 ratio once the volume has been adjusted for the number of slides to be stained. Typically, 200 µl of mixed substrate solution is required to completely cover one tissue slide in order for the color to turn brown. Tissue slices were then cleaned with distilled water and counterstained with hematoxylin. The following step is dehydration with increasingly potent xylene and alcohol. The final step in the process was to mount the slides using mounting material and cover them with a cover slide.

2.7.6. Statistical analysis

In triplicate (n = 3), all results were shown as means and standard error. GraphPad Prism version 8 was utilized to evaluate the statistical significance of the collected data using one-way ANOVA with LSD post-hoc testing. Pair-wise comparison between the mean of each parameter was determined using LSD post-hoc test was displayed on the top of each bar, where ns= nonsignificant, * $p<0.05$ (significant), ** $p<0.01$ (high significant) and *** $p<0.0001$(very high significant).

3. Results

3.1. NOS-LNCs characterization

Table 1 displays the findings from the prepared NOS-LNCs' dynamic light scattering (DLS) investigation. The prepared NOS-LNCs had a mean hydrodynamic particle size (PS) of 213.4 ± 1.41 nm, as the table indicates. Furthermore, it was discovered that the particle aggregation tendency (PDI) was less than 0.3. As a result, the produced nanoformulations distributed uniformly and exhibited no agglomeration tendency. It was discovered that the inherent surface charge was negative, measuring -13.6 ± 1.17 mV. Additionally, the PS of the prepared NOS-LNCs and the PDI of the fresh LNCs and after a period of 3 months showed a slight change ($p > 0.05$), and this was less than the crucial threshold of aggregation, indicating high stability of the prepared NOS-LNCs.

Table 1: DLS analysis of the prepared NOS-LNCs at 0 and after 3 months (n = 3).

	Freshly prepared formulations			**Formulations after 3-months**	
	PS ± SD	**PDI ± SD**	**ZP ± SD**	**PS ± SD**	**PDI ± SD**
NOS-LNCs	213.4 ± 1.41	0.25 ± 0.02	- 13.6 ± 1.17	221.7 ± 2.21	0.21 ± 0.016

Particle size (PS), polydispersity index (PDI) and Zeta potential (ZP).

3.2. NOS release from LNCs *in-vitro*

Using the sink condition approach, the release profile of the NOS-LNCs and free NOS suspension was assessed during a 48-hour period. In the instance of free NOS suspension with diffusing slightly over 80%, a burst drug release was seen during the first 12 hours. Regarding NOS-LNCs, the medication was continuously released from the LNCs during a 48-hour period at a rate of 72.33% ± 1.72 (Figure 1). As a result, loading the NOS into LNC may allow for the administration of a smaller dosage of the medication while also reducing the likelihood of negative effects and increasing its effectiveness.

Figure 1

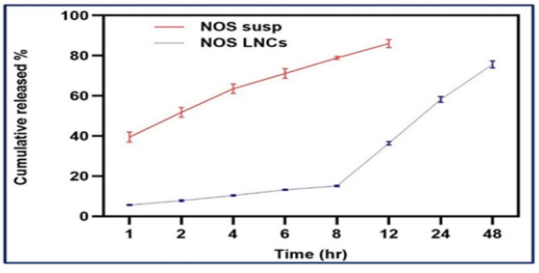

Figure 1: The 48-hour release performance of NOS from LNCs in comparison to its suspension form. The data points are shown as mean ± SD, with n = 3.

3.3. The cytotoxicity of free NOS and NOS-LNCs

The drug's in vitro cytotoxicity was evaluated using 4T1 cells. The computed IC50 value for free NOS exceeded (being less potent) that of the NOS-LNCs, as they attained 66.107 µg/ml and 13.209 µg/ml, respectively (Figure 2). This effect can be attributed to the role of nanoformulation in enhancing the solubility and consequently the cytotoxic effect of the drug, taking into consideration that the LNCs have demonstrated almost a none cytotoxic effect on cells with an attained IC50 value of 122.132 µg/ml.

Figure 2:

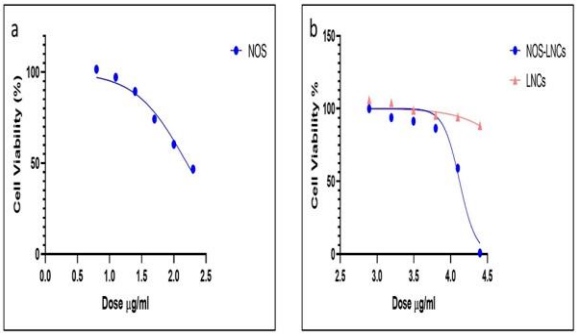

Figure 2: Curves showing the cell viability of the 4T1 cell line with various concentration of free NOS and NOS-LNCs. The cell viability was evaluated using the MTT test with different concentration of free NOS (a) NOS-LNCs and blank LNCs (b).

3.4. In tumor-bearing mice, loading NOS into LNCs enhances its antioxidant activity

The cancer control group had the greatest amounts of NO and MDA. NO and MDA levels in tumor-bearing mice returned to normal after receiving NOS treatment. But the treatment with NOS-LNCs had the most significant effect (Figure 3 a& b). In contrast, the cancer control group's CAT and SOD activity were the lowest and were subsequently recovered by NOS treatment. Nevertheless, the restoration of CAT and SOD activity was more markedly affected by the NOS-LNCs treatment (Figure 3c &d).

Figure 3:

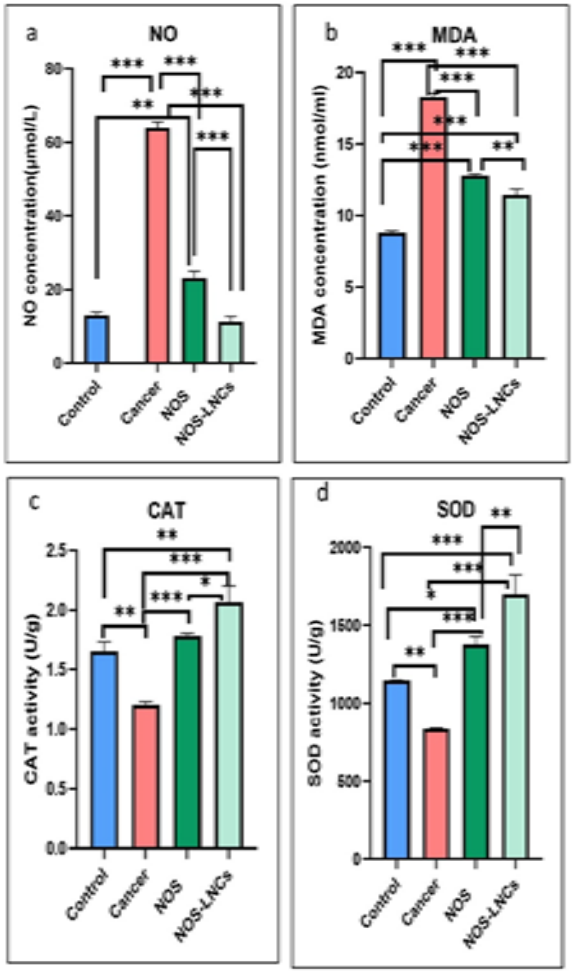

Figure 3: The antioxidant and oxidative stress status in mammary gland tissue desiccated from different groups. a) NO concentration, b) MDA concentration, c) Catalase activity, d) SOD activity. Data presented as Mean ±SEM, ns= non-significant, * P<0.05 (significant), **P<0.01 (high significant) and ***P<0.001 (very high significant).

3.5. The antiproliferative action of NOS in mice bearing tumours is increased when it is loaded into LNCs.

By using Ki67 staining, the tumor tissues removed from mice were evaluated for cell proliferation. The cancer control group exhibited the greatest expression of Ki67, as shown in (Figure 4). Ki67 expression was reduced by NOS therapy; however, NOS-LNC treatment had the greatest effect. The anti-proliferative effect of NOS on neoplastic cells is demonstrated by these findings, and this effect was amplified upon loading into LNCs.

Figure 4

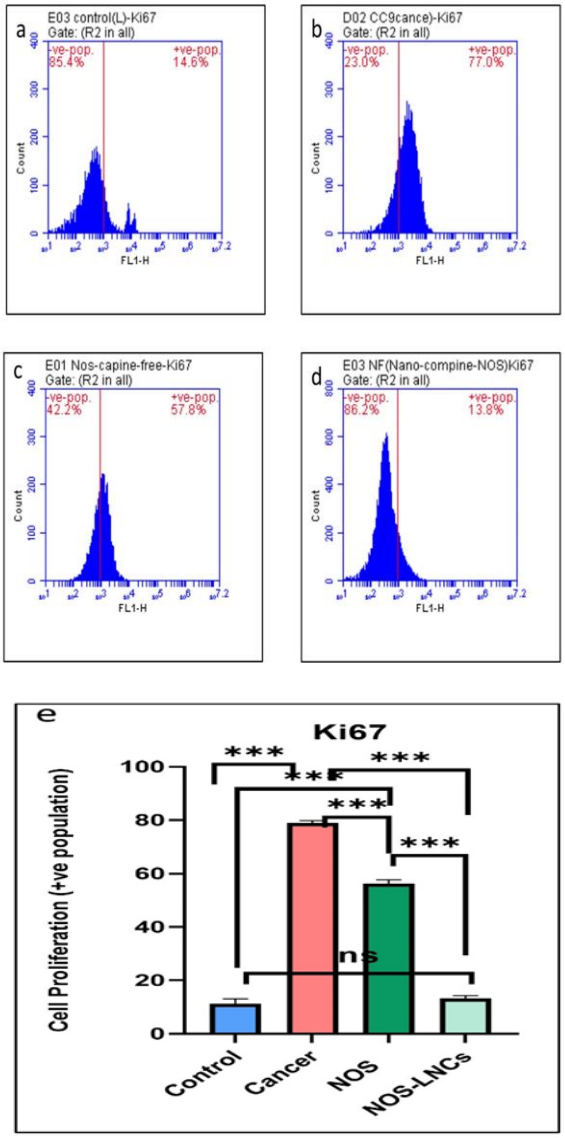

Figure 4: Evaluation of Ki67 in tumor tissue from different groups by flow cytometry assay. Ki67 expression was evaluated in control group (a), cancer group (b), free NOS (c) and NOS-LNCs (d). e) Data presented as mean ± SEM, ns= non-significant, * P<0.05 (significant), **P<0.01 (high significant) and ***P<0.001 (very high significant).

3.6. The loading of NOS into LNCs improves its necrotic effect on tumor cells.

A marked infiltration of hyperchromatic neoplastic cells was visible on histopathological analysis of mammary gland tissues from the cancer control group. When compared to the NOS-free treated group, the NOS-LNC-treated groups had moderate necrosis of malignant cells (Figure 5).

Figure 5

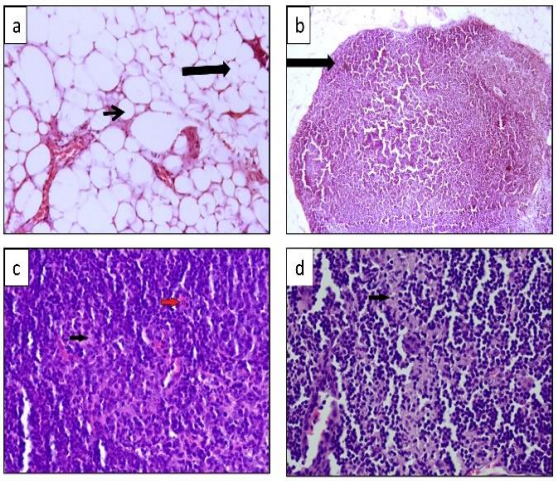

Figure 5 H&E-stained breast tissue sections from different experimental groups were examined histopathologically. a) Control group showed normal architecture of breast with Lactiferous duct (thick arrow) embedded in adipose tissue (thin arrow). b) Cancer group showed Well circumscribed solid papillary adenocarcinoma (thick arrow) with projection of proliferated cells. c) Free noscapine treated group showed Neutrophil infiltration (red arrow) and localized neoplastic cell necrosis (black arrow). d) NOS-LNCs treated group showed a mass of hyperchromatic small cells with moderate necrosis of the neoplastic cells (black arrow). All images are at magnification = 400, except image (a), which is at magnification power = 100×.

3.7. NOS-induced overexpression of BAX and downregulation of BCL2 is enhanced when loaded into LNCs

(Figure 6B) illustrates that the cancer control group had less BAX immunostaining than the groups that received free NOS (Figure 6C) or NOS-LNCs treatment (Figure 6D). Notably, the significant increase in BAX expression was seen in NOS-LNCs. In contrast, the cancer control group had the greatest level of BCL-2 expression, and BCL-2 expression was found to be downregulated during NOS medication (Figure 7). Furthermore, loading into LNCs appears to enhance NOS's ability to downregulate BCL-2.

Figure 6

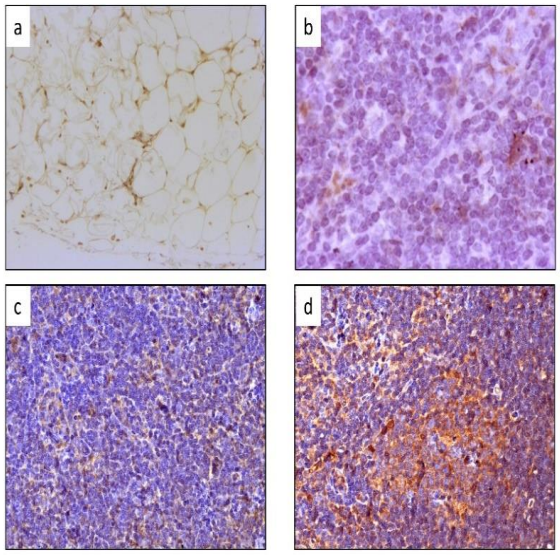

Figure 6: Immunohistochemical analysis of breast gland tissue sections from different groups stained with BAX immunostaining. a) Control group showed negative immunostaining against BAX. b) Cancer group showed negative immunostaining against BAX. c) Free noscapine treated group showed mild positive immunostaining against BAX. d) NOS-LNCs treated group showed strong positive immunostaining against BAX. All sections stained with DAB immunostain, hematoxylin as counter stain. All images are at magnification = 400, except image (a), which is at magnification power = 100×.

Figure 7

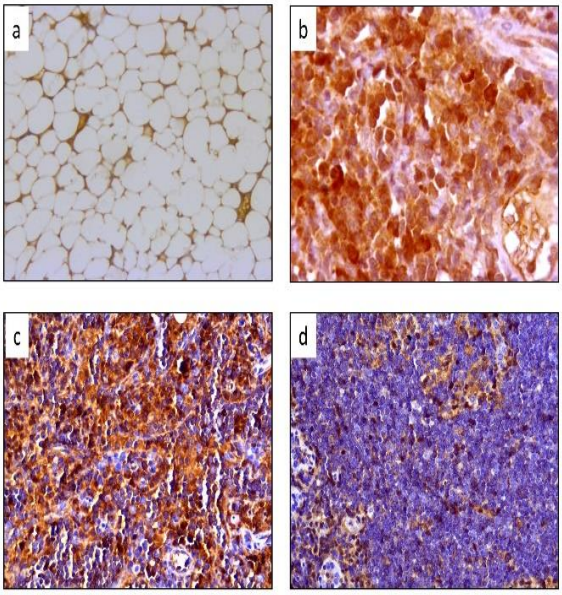

Figure 7: Immunohistochemical analysis of breast gland tissue sections from different groups stained with BCL-2 immunostaining. a) Control group showed negative immunostaining against BCL-2. b) Cancer group showed strong positive immunostaining against BCL-2. c) Free noscapine treated group showed strong positive immunostaining against BCL-2. d) NOS-LNCs treated group showed mild positive immunostaining against BCL-2. All sections stained with DAB immunostain, hematoxylin as counter stain. All images are at magnification= 400, except image (a), which is at magnification power = 100×.

4. Discussion

The MTT assay confirmed the cytotoxic effect of NOS on cancer cell and the computed IC50 of NOS-LNCs is much lesser than free NOS. Compared to free NOS, NOS-LNCs had a higher cytotoxic effect. Thus, as nanoparticles may effectively carry drugs and provide an enhanced permeability and retention (EPR) effect, they are used to target breast cancer cells with noscapine. Additionally, the plain LNCs proved to have no cytotoxic effect on the cell line in our study. LNCs have the following unique qualities; a high surface-to-mass ratio, a regulated release, long-term stability, minimal toxicity, and a high entrapment effectiveness for hydrophobic medicines **[17, 45, 46].** LNCs are also preferred because of their biological degradation and the fact that they do not stimulate the immune system **[17, 45].**

Also, we assess anticancer properties of NOS on orthotopic BC mouse model induced by mouse breast cancer cell line 4T1 that would mimic human triple-negative breast cancer.

Nitric oxide causes tumors through a variety of mechanisms, such as: (a) genotoxic effect by producing toxic and mutagenic species, directly causing DNA strand breaks, nucleic acid oxidation and deamination, and poor DNA repair; (b) antiapoptotic effects by suppressing p53 activity, inhibiting caspase activity, over-expressing BCL-2,

stimulating cyclo-oxygenase (COX), preventing cytochrome C release, and ceramide production; (c) The angiogenic impact of nitric oxide is mediated by vascular endothelial growth factor (VEGF) release, dilated endothelial nitric oxide synthase (eNOS)-mediated arteriolar arteries, increased vascular endothelial hyperpermeability, increased tumor vasculature permeability, and the synthesis of proangiogenic substances; (d) elevated matrix metalloproteinase 2 (MMP2), MMP-9, and VEGF production lead to a metastatic effect; (e) decreased leukocyte infiltration and proliferation, which diminishes the immune response to the tumor **[47]**. So, the cancer group had the greatest amount of nitric oxide in our results. nitric oxide levels are dramatically reduced with NOS therapy. As reported in previous studies, NOS downregulation BCL-2 expression, **[11]** as well as downregulates the expression of hypoxia-inducible factor-1 (HIF-1) in hypoxic tumor cells, which in turn decreases the production of vascular endothelial growth factor (VEGF) **[13]**. Second, NOS inhibits the formation of blood vessels by blocking endothelial cells' response to VEGF. Additionally, NOS may disrupt endothelial cell migration, which is essential for the development of arteries and veins **[14, 15].** Since noscapine has antioxidant qualities and prevents ROS-induced apoptosis, which lowers the growth of tumors by removing oxygen free radicals (ROS) **[48]**, Loading of noscapine into lipid nanocarriers would provide increased protection of tissues against dangerous reactive oxygen species **[49]**. This is consistent with our finding that NOS-LNCs are more effective in lowering NO levels.

The final product of polyunsaturated fatty acid peroxidation in cells is malondialdehyde (MDA), a marker of oxidative stress. While CAT (catalase) activity and SOD (superoxide dismutase) activity are used as antioxidant markers, the catalase enzyme is responsible for the conversion of hydrogen peroxide into oxygen and water. The SOD enzyme, which catalyzes the conversion of superoxide radicals into regular molecular oxygen and hydrogen peroxide, was also measured. A byproduct of oxygen metabolism, superoxide damages cells if it is not controlled. In our findings, the cancer control group had the highest level of MDA and the least activity of both CAT and SOD. While the group with NOS medication showed a lower level of MDA and improved activity of both CAT and SOD, loading NOS into LNCs showed a more significant decrease in MDA level and a more significant increase in both CAT and SOD activity. The reason behind this is that NOS, the second-most abundant alkaloid found in opium and found in poppy blossoms, is thought to be responsible for the antioxidant action of poppy flowers, which has been reported to decrease MDA and increase both CAT and SOD activity in many previous studies in agreement with our results **[50, 51]**.

The proliferation biomarker Ki-67 is widely studied since it is found in all proliferating cells. Every active phase of the cell cycle, with the exception of the G0 phase, contains the nuclear nonhistone protein Ki-67 **[52].** Furthermore, thought to be a breast cancer prognostic factor, and it has been studied in several research **[53].** One of the characteristics of malignant tumors is increased proliferation. Accordingly, the cancer control group in our study had the largest percentage of $Ki67^+$ population. NOS-LNCs medication group demonstrated a statistically significant reduction in the $Ki67^+$ population level compared to the NOS medication group. Ki-67 expression indicates that NOS has potent anti-proliferative inhibitory effects **[15]**. In agreement with our study, numerous

investigations have reported on the antiproliferative activity of NOS on a variety of cancer types, including many drug-resistant variations **[54-59]**. The proliferation of cancer cells is inhibited due to NOS's antimitotic action. Noscapine is a cell cycle arresting anti-mitotic drug **[60].** As noscapine, a medication that interferes with microtubules, can inhibit mitosis. This is largely because, in addition to their function in chromosomal movement during mitosis, microtubules also perform a number of other functions, such as cytoplasmic organization and axonal transport. Furthermore, the therapeutic use of alkaloids has been limited due to drug resistance caused by overexpression of P-glycoprotein **[61].** In addition, LNCs enhanced the antiproliferative effect of noscapine.

The protein tubulin, which is heterodimeric and binds guanine nucleotides to create the dynamic cytoskeletal filament known as microtubules, is the target of noscapine. Microtubules play a multitude of roles in cells, one of which is the regulation of cell cycle progression, which makes them a promising target for cancer treatment **[62**]. It has been demonstrated that noscapine arrests cell cycle in its tracks during mitosis by stoichiometrically binding to tubulin, changing its structure, and influencing microtubule production **[63].** The anti-tubulin and anti-mitotic effects of noscapine are considered the main causes of cell death **[64].**

The expression of BAX and BCL-2 was investigated to evaluate the pro-apoptotic effect of NOS. In our study, there was a marked increase in the expression of BAX and a marked decrease in the expression of BCL-2 in the group that received NOS-LNCs medication as compared to the group that received NOS medication. In contrast, the cancer control group showed a marked decrease in BAX expression and a marked increase in BCL-2 expression. The primary pro-apoptotic protein in eukaryotic cells, BAX, is inactivated by a protein that is encoded by the BCL-2 gene. Furthermore, BCL-2 is linked to the suppression of BAX/BAK oligomerization, which in turn suppresses the activation of many apoptogenic molecules **[65]**. In line with our data from this study, the anticancer effect of NOS and its efficacy on stimulation of apoptosis were reported in many previous studies **[11, 59, 66-68].** Consequently, blocking Bcl2 expression could be a useful strategy in the battle against breast cancer. Noscapine causes apoptosis via upregulating the expression of genes that promote apoptosis and downregulating that of genes that oppose it. Consequently, it causes an increase of the BAX expression and a decrease of the BCL-2 expression **[11].**

5. Conclusion

In conclusion, NOS, a plant-derived compound with antitussive characteristics, has exhibited various pharmacological effects in recent cellular studies. These effects encompass its cytotoxic impact on cancer cells, antioxidant attributes through the reduction of ROS levels and inhibition of NO, inhibition of cancer cell proliferation, promotion of apoptosis via modulation of BAX and BCL-2 expression, and regulation of microtubule dynamics. Additionally, the encapsulation of NOS into LNCs enhanced its therapeutic potential by improving its solubility, facilitating the drug delivery to tumor cells, shielding the NOS from degradation, and prolonging its circulation half-life.

6. Conflict of Interest

The authors declare that they are not aware of any financial or interpersonal issues that may have affected the findings of the study they have published.

7. Funding sources

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8. Declarations and ethics approval

The medical research ethics committee at Mansoura University gave the current study preapproval (code number Ph.D./82).

9. References

[1] S.M. El-Sayed, A.M.J.H. Youssef, Potential application of herbs and spices and their effects in functional dairy products, (2019);5.

[2] Q. Li, H.-J. Li, T. Xu, H. Du, C.-L. Huan Gang, G. Fan, Y.J.F.i.P. Zhang, Natural medicines used in the traditional Tibetan medical system for the treatment of liver diseases, (2018);9:29.

[3] J.K. Patra, G. Das, L.F. Fraceto, E.V.R. Campos, M.d.P. Rodriguez-Torres, L.S. Acosta-Torres, L.A. Diaz-Torres, R. Grillo, M.K. Swamy, S.J.J.o.n. Sharma, Nano based drug delivery systems: recent developments and future prospects, (2018);16:1-33.

[4] S.S. Kesharwani, G.J.J.M. Bhat, Formulation and nanotechnology-based approaches for solubility and bioavailability enhancement of zerumbone, (2020);56:557.

[5] K.M. Yap, M. Sekar, S. Fuloria, Y.S. Wu, S.H. Gan, N.N.I. Mat Rani, V. Subramaniyan, C. Kokare, P.T. Lum, M.Y.J.I.J.o.N. Begum, Drug delivery of natural products through nanocarriers for effective breast cancer therapy: A comprehensive review of literature, (2021)7891-7941.

[6] V.P. Chavda, A.B. Patel, K.J. Mistry, S.F. Suthar, Z.-X. Wu, Z.-S. Chen, K.J.F.i.o. Hou, Nano-drug delivery systems entrapping natural bioactive compounds for cancer: recent progress and future challenges, (2022);12:867655.

[7] R. Chandra, J. Madan, P. Singh, A. Chandra, P. Kumar, V. Tomar, S.J.C.D.M. K Dass, Implications of nanoscale based drug delivery systems in delivery and targeting tubulin binding agent, noscapine in cancer cells, (2012);13:1476-1483.

[8] D. Kumar, P. Singh, A. Jayaraj, V. Kumar, K. Kumari, R.J.C. Patel, A theoretical model to study the interaction of erythro-noscapines with nsP3 protease of Chikungunya virus, (2019);4:4892-4900.

[9] V. Vishvakarma, K. Kumari, P.J.J.P.R.B. Singh, A model to study the inhibition of arginase II with noscapine & its derivatives, (2020);2:1-14.

[10] S. Li, J. He, S. Li, G. Cao, S. Tang, Q. Tong, H.C.J.P.o. Joshi, Noscapine induced apoptosis via downregulation of survivin in human neuroblastoma cells having wild type or null p53, (2012);7:e40076.

[11] E.O. Quisbert-Valenzuela, G.M. Calaf, Apoptotic effect of noscapine in breast cancer cell lines, Int J Oncol.(2016);48:2666-2674.

[12] J. Ferlay, M. Colombet, I. Soerjomataram, C. Mathers, D.M. Parkin, M. Piñeros, A. Znaor, F.J.I.j.o.c. Bray, Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods, (2019);144:1941-1953.

[13] W. Su, L. Huang, Q. Ao, Q. Zhang, X. Tian, Y. Fang, Y.J.C.l. Lu, Noscapine sensitizes chemoresistant ovarian cancer cells to cisplatin through inhibition of HIF-1α, (2011);305:94-99.

[14] N. Jhaveri, H. Cho, S. Torres, W. Wang, A.H. Schönthal, N.A. Petasis, S.G. Louie, F.M. Hofman, T.C.J.C.l. Chen, Noscapine inhibits tumor growth in TMZ-resistant gliomas, (2011);312:245-252.

[15] [S.S. Esnaashari, S. Muhammadnejad, S. Amanpour, A.J.A.P. Amani, A combinational approach towards treatment of breast cancer: an analysis of noscapine-loaded polymeric nanoparticles and doxorubicin, (2020);21:1-12.M.F. Kabil, M.Y. Mahmoud, A.F. Bakr, D. Zaafar, I.M.J.L.S. El-Sherbiny, Switching indication of PEGylated lipid nanocapsules-loaded with rolapitant and deferasirox against breast cancer: Enhanced in-vitro and in-vivo cytotoxicity, Life Sci., (2022);305:120731.

[16] D.K. Mishra, R. Shandilya, P.K. Mishra, Lipid based nanocarriers: a translational perspective, Nanomedicine: Nanotechnology, Biology and Medicine.(2018);14:2023-2050.

[17] M. Ghaffari, J.E.N. Dolatabadi, Chapter 17 - Nanotechnology for pharmaceuticals, in: S. Thomas, Y. Grohens, Y.B. Pottathara (Eds.) Industrial Applications of Nanomaterials, Elsevier2019, pp. 475-502.

[18] D. Sharma, C.M. Hussain, Smart nanomaterials in pharmaceutical analysis, Arabian Journal of Chemistry.(2020);13:3319-3343.

[19] L. Battaglia, E. Ugazio, Lipid Nano- and Microparticles: An Overview of Patent-Related Research, Journal of Nanomaterials.(2019);2019:2834941.

[20] N. Anton, J.-P. Benoit, P. Saulnier, Design and production of nanoparticles formulated from nano-emulsion templates—A review, Journal of Controlled Release.(2008);128:185-199.

[21] C.R.A. Mouzouvi, A. Umerska, A.K. Bigot, P. Saulnier, Surface active properties of lipid nanocapsules, PLOS ONE.(2017);12:e0179211.

[22] R. Klippstein, J.T. Wang, R.I. El-Gogary, J. Bai, F. Mustafa, N. Rubio, S. Bansal, W.T. Al-Jamal, K.T. Al-Jamal, Passively Targeted Curcumin-Loaded PEGylated PLGA Nanocapsules for Colon Cancer Therapy In Vivo, Small (Weinheim an der Bergstrasse, Germany).(2015);11:4704-4722.

[23] S.M. Basu, S.K. Yadava, R. Singh, J. Giri, Lipid nanocapsules co-encapsulating paclitaxel and salinomycin for eradicating breast cancer and cancer stem cells, Colloids and surfaces. B, Biointerfaces.(2021);204:111775.

[24] E. Schultze, J. Buss, K. Coradini, K.R. Begnini, S.S. Guterres, T. Collares, R.C.R. Beck, A.R. Pohlmann, F.K. Seixas, Tretinoin-loaded lipid-core nanocapsules overcome the triple-negative breast cancer cell resistance to tretinoin and show synergistic effect on cytotoxicity induced by doxorubicin and 5-fluororacil, Biomedicine & Pharmacotherapy.(2017);96:404-409.

[25] J.D. Figueroa, E. Gray, N. Pashayan, S. Deandrea, A. Karch, D.B. Vale, K. Elder, P. Procopio, N.T. van Ravesteyn, M.J.P.m. Mutabi, The impact of the Covid-19 pandemic on breast cancer early detection and screening, (2021);151:106585.

[26] Y. Liang, H. Zhang, X. Song, Q. Yang, Metastatic heterogeneity of breast cancer: Molecular mechanism and potential therapeutic targets, Seminars in cancer biology, Elsevier, 2020, pp. 14-27.

[27] P.A. Ganz, P.J.J.I.o.f.b.c.s.p.o.r.c. Goodwin, opportunities, Breast cancer survivorship: where are we today?, (2015)1-8.

[28] M.J.B.C.M. Fahad Ullah, D.R. Challenges, Progress, Breast cancer: current perspectives on the disease status, (2019)51-64.

[29] Y. Feng, M. Spezia, S. Huang, C. Yuan, Z. Zeng, L. Zhang, X. Ji, W. Liu, B. Huang, W.J.G. Luo, diseases, Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis, (2018);5:77-106.

[30] B. Heurtault, P. Saulnier, B. Pech, J.-E. Proust, J.-P.J.P.r. Benoit, A novel phase inversion-based process for the preparation of lipid nanocarriers, (2002);19:875-880.

[31] M.F. Kabil, M.Y. Mahmoud, A.F. Bakr, D. Zaafar, I.M.J.L.S. El-Sherbiny, Switching indication of PEGylated lipid nanocapsules-loaded with rolapitant and deferasirox against breast cancer: Enhanced in-vitro and in-vivo cytotoxicity, (2022);305:120731.

[32] M. Nasr, S.J.C.p.b. Abdel-Hamid, Lipid based nanocapsules: a multitude of biomedical applications, (2015);16:322-332.

[33] K. Mohsen, H.M. Azzazy, N.K. Allam, E.B.J.M.S. Basalious, E. C, Intranasal lipid nanocapsules for systemic delivery of nimodipine into the brain: in vitro optimization and in vivo pharmacokinetic study, (2020);116:111236.

[34] L. Ramzy, A.A. Metwally, M. Nasr, G.A.J.S.r. Awad, Novel thymoquinone lipidic core nanocapsules with anisamide-polymethacrylate shell for colon cancer cells overexpressing sigma receptors, (2020);10:1-15.S.S. Esnaashari, S. Muhammadnejad, S. Amanpour, A.J.A.P. Amani, A combinational approach towards treatment of breast cancer: an analysis of noscapine-loaded polymeric nanoparticles and doxorubicin, AAPS PharmSciTech., (2020);21 (5),1-12.

[35] S. Safwat, R.M. Hathout, R.A. Ishak, N.D.J.J.o.l.r. Mortada, Augmented simvastatin cytotoxicity using optimized lipid nanocapsules: a potential for breast cancer treatment, (2017);27:1-10.

[36] D.A. Volpe, S.S. Hamed, L.K.J.T.A.j. Zhang, Use of different parameters and equations for calculation of IC 50 values in efflux assays: potential sources of variability in IC 50 determination, (2014);16:172-180.

[37] S.E. Nigjeh, S.K. Yeap, N. Nordin, H. Rahman, R.J.M. Rosli, In vivo anti-tumor effects of citral on 4T1 breast cancer cells via induction of apoptosis and downregulation of aldehyde dehydrogenase activity, (2019);24:3241.

[38] S.G. Dash, S. Kantevari, S.K. Guru, P.K. Naik, Combination of docetaxel and newly synthesized 9-Br-trimethoxybenzyl-noscapine improve tubulin binding and enhances antitumor activity in breast cancer cells, Computers in biology and medicine.(2021);139:104996..

[39] O. Bassir, E.N.J.J.o.A. Maduagwu, F. Chemistry, Occurrence of nitrate, nitrite, dimethylamine, and dimethylnitrosamine in some fermented Nigerian beverages, (1978);26:200-203.

[40] S.J.C.c.a. Kei, Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method, (1978);90:37-43.

[41] P. Fossati, L. Prencipe, G.J.C.c. Berti, Use of 3, 5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine, (1980);26:227-231.

[42] M. Nishikimi, N. Appaji, K. Yagi, The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys Res Common 46: 849–854, 1972.

[43] M.J. Benson, R. Elgueta, W. Schpero, M. Molloy, W. Zhang, E. Usherwood, R.J.J.J.o.E.M. Noelle,

Distinction of the memory B cell response to cognate antigen versus bystander inflammatory signals, (2009);206:2013-2025.

[44] J.D. Bancroft, M. Gamble, Theory and practice of histological techniques, Elsevier health sciences2008.

[45] P. Ganesan, D. Narayanasamy, Lipid nanoparticles: Different preparation techniques, characterization, hurdles, and strategies for the production of solid lipid nanoparticles and nanostructured lipid carriers for oral drug delivery, Sustainable Chemistry and Pharmacy.(2017);6:37-56.

[46] Z. Niu, I. Conejos-Sánchez, B.T. Griffin, C.M. O'Driscoll, M.J. Alonso, Lipid-based nanocarriers for oral peptide delivery, Advanced Drug Delivery Reviews.(2016);106:337-354.

[47] [S. Korde Choudhari, M. Chaudhary, S. Bagde, A.R. Gadbail, V.J.W.j.o.s.o. Joshi, Nitric oxide and cancer: a review, (2013);11:1-11.

[48] M.R. Khazaei, Z. Rashidi, F. Chobsaz, M.J.I.J.o.B.M.S. Khazaei, Apoptosis induction of human endometriotic epithelial and stromal cells by noscapine, (2016);19:940.

[49] J.J. Otarola, M. Alejandra Luna, N. Mariano Correa, P.G. Molina, Noscapine-Loaded Nanostructured Lipid Carriers as a Potential Topical Delivery to Bovine Mastitis Treatment, (2020);5:5922-5927.

[50] V. Hill, T. Cairns, C.-C. Cheng, M.J.J.o.A.T. Schaffer, Multiple aspects of hair analysis for opiates: methodology, clinical and workplace populations, codeine, and poppy seed ingestion, (2005);29:696-703.

[51] R.L. Jayaraj, R. Beiram, S. Azimullah, N.M. M. F., S.K. Ojha, A. Adem, F.Y. Jalal, Noscapine Prevents Rotenone-Induced Neurotoxicity: Involvement of Oxidative Stress, Neuroinflammation and Autophagy Pathways, (2021);26:4627.

[52] R. Nishimura, T. Osako, Y. Okumura, M. Hayashi, Y. Toyozumi, N.J.E. Arima, t. medicine, Ki-67 as a prognostic marker according to breast cancer subtype and a predictor of recurrence time in primary breast cancer, (2010);1:747-754.

[53] R. Yerushalmi, R. Woods, P.M. Ravdin, M.M. Hayes, K.A.J.T.l.o. Gelmon, Ki67 in breast cancer: prognostic and predictive potential, (2010);11:174-183.

[54] Y. Ke, K. Ye, H.E. Grossniklaus, D.R. Archer, H.C. Joshi, J.A. Kapp, Noscapine inhibits tumor growth with little toxicity to normal tissues or inhibition of immune responses, Cancer immunology, immunotherapy : CII.(2000);49:217-225.

[55] M. Chougule, A.R. Patel, P. Sachdeva, T. Jackson, M. Singh, Anticancer activity of Noscapine, an opioid alkaloid in combination with Cisplatin in human non-small cell lung cancer, Lung cancer (Amsterdam,Netherlands).(2011);71:271-282.

[56] T. Jackson, M.B. Chougule, N. Ichite, R.R. Patlolla, M. Singh, Antitumor activity of noscapine in human non-small cell lung cancer xenograft model, Cancer chemotherapy and pharmacology.(2008);63:117-126. 117-126.

[57] J.W. Landen, R. Lang, S.J. McMahon, N.M. Rusan, A.M. Yvon, A.W. Adams, M.D. Sorcinelli, R. Campbell, P. Bonaccorsi, J.C. Ansel, D.R. Archer, P. Wadsworth, C.A. Armstrong, H.C. Joshi, Noscapine alters microtubule dynamics in living cells and inhibits the progression of melanoma, Cancer research.(2002);62:4109-4114.

[58] R. Aneja, A.M. Ghaleb, J. Zhou, V.W. Yang, H.C. Joshi, p53 and p21 determine the sensitivity of noscapine-induced apoptosis in colon cancer cells, Cancer research.(2007);67:3862-3870.

[59] M.B. Chougule, A.R. Patel, T. Jackson, M. Singh, Antitumor Activity of Noscapine in Combination with Doxorubicin in Triple Negative Breast Cancer, PLOS ONE.(2011);6:e17733.

[60] N. Dhiman, A. Sood, A.J.W.J.P.P.S. Sharma, Noscapine: an anti-mitotic agent, (2013);3:324-338.

[61] J. Zhou, K. Gupta, S. Aggarwal, R. Aneja, R. Chandra, D. Panda, H.C. Joshi, Brominated Derivatives of Noscapine Are Potent Microtubule-interfering Agents That Perturb Mitosis and Inhibit Cell Proliferation, (2003);63:799-807.

[62] N. Nambiar, P.K.R. Nagireddy, R. Pedapati, S. Kantevari, M. Lopus, Tubulin- and ROS-dependent antiproliferative mechanism of a potent analogue of noscapine, N-propargyl noscapine, Life Sciences.(2020);258:118238.

[63] M. Lopus, P.K.J.P.R. Naik, Taking aim at a dynamic target: Noscapinoids as microtubule-targeted cancer therapeutics, (2015);67:56-62.

[64] H. Gali-Muhtasib, R. Hmadi, M. Kareh, R. Tohme, N. Darwiche, Cell death mechanisms of plant-derived anticancer drugs: beyond apoptosis, Apoptosis.(2015);20:1531-1562.

[65] N. Bajwa, C. Liao, Z.J.E.o.o.t.p. Nikolovska-Coleska, Inhibitors of the anti-apoptotic Bcl-2 proteins: a patent review, (2012);22:37-55.

[66] R. Aneja, J. Zhou, B. Zhou, R. Chandra, H.C. Joshi, Treatment of hormone-refractory breast cancer: apoptosis and regression of human tumors implanted in mice, Molecular cancer therapeutics.(2006);5:2366-2377.

[67] K. Ye, Y. Ke, N. Keshava, J. Shanks, J.A. Kapp, R.R. Tekmal, J. Petros, H.C. Joshi, Opium alkaloid noscapine is an antitumor agent that arrests metaphase and induces apoptosis in dividing cells, (1998);95:1601-1606.

[68] S. Sajadian, M. Vatankhah, M. Majdzadeh, S.M. Kouhsari, M.H. Ghahremani, S.N. Ostad, Cell cycle arrest and apoptogenic properties of opium alkaloids noscapine and papaverine on breast cancer stem cells, Toxicology mechanisms and methods.(2015);25:388-395.