Antiproliferative Effects of *Nerium oleander* Leaves and Its Cardiac Glycosides Odoroside A and Oleandrin on MCF-7 Cancer Cells

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**Abstract:** *Nerium oleander* leaves extract preparations have been used for centuries against sores, abscesses and solid tumors. The aim of this study was to identify active compound(s) responsible for the antiproliferative activity of *N. oleander* leaves against MCF-7 breast cancer cell line and to explore their mechanisms of action. The methanolic extract and fractions were screened against four human cancer cell lines: HT-144, MCF-7, NCI-H460 and SF-268 using sulforhodamine B assay. The most active ethyl acetate soluble fraction led to the isolation of eleven pure compounds viz adynerigenin, adynerin, odoroside A, odoroside B, 5-O-kaempferobinoside, β-neriurtate, oleanderocioic acid, oleandignoside, oleandrin, 5-O-queretebinoside and ursolic acid. The methanolic extract and the ethyl acetate soluble fraction exhibited significant growth inhibition (TGI: 0.14 to 3.3 µg/ml) against HT-144, MCF-7, NCI-H460 and SF-268 cell lines. The effects of the methanolic extract, ethyl acetate soluble fraction, odoroside A and oleandrin on the cytoskeleton and nuclei of MCF-7 cells were evaluated using immunofluorescence microscopy. Significant reduction in the mitotic index of MCF-7 cells were exhibited by the methanolic extract, ethyl acetate soluble fraction, odoroside A and oleandrin accompanied by morphological changes such as band-like arrangement of cells, reduction in F-actin processes, disruption of interphase microtubule network and condensation of nuclei. These results support that the *N. oleander* leaves possess antiproliferative activity against the aforementioned cell lines particularly estrogen receptor positive MCF-7 cells, thereby justifying its traditional use against tumors. Moreover, the antiproliferative effects induced by the methanolic extract, ethyl acetate soluble fraction, odoroside A and oleandrin were possibly related to skeletal and nuclear morphological changes.

**Keywords:** Antiproliferative, cytoskeleton, human cancer cell lines, *Nerium oleander*, odoroside A, oleandrin.

1. INTRODUCTION

Globally, cancer causes ~8 million deaths annually, 64.9% of which occur in less developed regions of the world [1]. In females, breast cancer is the most frequently diagnosed cancer worldwide, including Pakistan, accounting for about one fourth of the total cancer cases [1, 2]. Conventionally, anthracycline-, alkylating agent-, taxane-, or antimetabolite- based regimens are the mainstay for the treatment of breast cancer [3]. However,
due to the risk of cumulative cardiac toxicity of anthracycline-based regimens and the unfavorable toxicity profile of taxanes, there is a need to develop alternative novel and safer anticancer drugs for the treatment of breast cancer. Many of the anticancer drugs are derived from plants; the most popular being taxol from the bark of Taxus brevifolia, [4] and vinca-alkaloids from Catharanthus roseus [5]. Oral vinorelbine (a semi-synthetic third generation vinca-alkaloid with a modified catharanthine ring) has been added to this list as a highly effective and well tolerated agent [6]. Furthermore, combination therapies such as that of vinorelbine and oral fluoropyrimidine capecitabine with bevacizumab, a humanised monoclonal antibody directed specifically against vascular endothelial growth factor (VEGF)-A, are also undergoing phase II clinical trials in recurrent breast cancers [7].

Nerium oleander (common names: Adelfa, Dafla, Olean, Kanair and Rose-bay) belongs to the Apocynaceae family and is distributed abundantly in Indo-Pakistan subcontinent as well as the Mediterranean, tropical and subtropical regions. Apart from various diseases like sores and warts, its extract is used in folk remedies for the treatment of tumors [8-11]. For many years, oleandrinogenin and oleandrin (cardiac glycosides) isolated from N. oleander leaves have been used for treating cardiac abnormalities in China and Russia, their beneficial effects were also reflected against various types of cancers including breast cancer [12]. Recently, oleandrin has been shown to induce DNA damage responses in cancer cells by suppressing the expression of Rad51 [13]. Two aqueous (anvirzel and breastin), and one supercritical carbon dioxide (PBI-05204) extracts have been prepared from oleander leaves. Anvirzel has under gone phase I clinical trial for cancer treatment in USA [14], while the extract breastin exhibited high activity in a panel of human tumor cell lines [15] and has also been clinically evaluated on more than 380 cancer patients [16]. PBI-05204, the oleandrin-containing N. oleander leaves-derived extract, with potent anticancer activity, has demonstrated its safety in phase I clinical trial in patients with advanced solid tumors [17]. Additionally, it has been reported that PBI-05204 induces a remarkable antitumor effect in mice transplanted with human pancreatic cancer cells [18]. Moreover, inhibition of glycolysis and selective killing of lung cancer cells have also been demonstrated by another hydroalcoholic extract from the leaves of Nerium oleander [19].

It has been previously reported by our group that odoroside A (GI50: 0.001; LC50: 0.14 μg/ml) and oleandrin (GI50: 0.11; LC50: 0.5 μg/ml; drug concentrations causing 50% growth inhibition (GI50) and killing 50% (LC50) of the cells) were the most cytotoxic compounds present in N. oleander leaves against MCF-7 cells [20]. In continuation, the present study for the first time describes bioassay-directed fractionation of N. oleander leaves methanolic extract leading to pure compounds; and their antiproliferative evaluation against four human cancer cell lines: HT-144 (melanoma, skin), MCF-7 (adenocarcinoma, breast), NCI-H460 (non-small cell lung cancer) and SF-268 (astrocytoma, CNS). The mechanism of their antiproliferative action was evaluated by their effects on the cytoskeleton and nuclei of estrogen receptor positive MCF-7 cells using immunofluorescence microscopy.

2. MATERIAL AND METHODS

2.1. Materials and Preparation of Stock Solutions

Alexa 488 phalloidin, fetal bovine serum (heat inactivated) and zymed clearmount mounting medium were obtained from Invitrogen (Grand Island, NY 14072, USA). Cy3-labeled β tubulin clone TUB 2.1 monoclonal antibody, 4, 6-diamidino-2-phenylindole (DAPI), dimethyl sulfoxide (DMSO), gentamycin sulphate, L-glutamine penicillin-streptomycin solution, poly-L-lysine, RPMI-1640, sulfonhodamine B (SRB), trichloroacetic acid, trypsin blue and trypsin-EDTA were purchased from Sigma (St. Louis, Mo, USA). Other chemicals were supplied by the companies listed within parentheses: Amphotericin B, doxorubicin, paclitaxel and vinblastine (MP Biochemicals, Solon, Ohio 44139, USA); tris base (Aldrich, Stenheim, Germany); acetic acid (Riedel-de-Haen, Sigma-Aldrich GmbH D-30926, Germany); formaldehyde 4% (Carl Roth, Gmbh Co., Schoemperlenstraße 76185, Karlsruhe, Germany); and triton-X-100 (Wako Pure Chemical Industries Ltd., Japan). The stock solutions of N. oleander leaves extract (40 mg/ml), fractions (20 mg/ml), and pure compounds (20 mM) were prepared in DMSO (100%) with final concentration of DMSO not exceeding 0.5% (v/v), while sterile
water was used for doxorubicin (20 mM). All the stock solutions were kept at -20°C and working dilutions were freshly prepared in RPMI-1640.

2.2. Extraction of N. oleander Leaves and Isolation of Compounds

Fresh and uncrushed N. oleander leaves (40 kg), obtained from the University of Karachi garden, were extracted 3x with methanol (MeOH) at room temperature. The concentrated syrupy residue (methanolic extract, ME) obtained on removal of the solvent from the combined extract under reduced pressure was shaken with ethyl acetate (EtOAc) and water (1:1). Both EtOAc and aqueous phases were dried under reduced pressure to yield respective fractions which were evaluated for their anticancer activity against aforementioned human cancer cell lines. Considering that ethyl acetate soluble fraction (EA) demonstrated better activity, it was further extracted with aqueous Na2CO3 solution (4%) to separate the acidic and neutral fractions. The EtOAc phase containing the neutral fraction was washed, dried over anhydrous Na2SO4, charcoaled and concentrated. It was subjected to various chromatographic techniques and led to the isolation of eleven pure compounds (adynigerinin, adynerin, odoroside A, odoroside B, 5-O-kaempferobinoside, β-neriurstate, oleanderocioic acid, oleandiginoside, oleandrin, 5-O-quercetinobioside and ursolic acid) as described previously [1].

2.3. Cell Lines

HT-144 (melanoma, skin), MCF-7 (adenocarcinoma, breast), NCI-H460 (non-small cell carcinoma, lung) and SF-268 (anaplastic astrocytoma, CNS) human cancer cell lines were kept at 37°C in 5% CO2 incubator. They were maintained in RPMI-1640 cell culture medium supplemented with heat inactivated fetal bovine serum (10%), glutamine (2 mM), penicillin (100 units/ml), streptomycin (100 µg/ml) and amphotericin-B (2.5 µg/ml). The adherent monolayer was trypsinised for 3 to 6 minutes depending on the cell type using trypsin-EDTA (0.05%). The cell concentration and viability were determined by trypan blue exclusion assay and cells having > 90% viability were used for the experiments.

2.4. Growth Inhibitory and Cytotoxicity Assay

Measurement of in vitro growth inhibition and cytotoxicity was carried out by employing sulforhodamine B (SRB) assay [21, 22]. Briefly, cell suspension (100 µl) of MCF-7 and NCI-H460 (1×10^5), SF-268 (1.5×10^5), and HT-144 (2×10^4) cells were added in each well of 96-well plates and incubated. After 24 hours, various doses of N. oleander leaves’ methanolic extract (0.01 to 250 µg/ml), fractions (0.001 to 100 µg/ml), pure compounds (0.0001 to 100 µM) and doxorubicin (0.001 to 10 µM) were added followed by further incubation for 48 hours. The cells were fixed with trichloroacetic acid (50 µl, 50%), washed with distilled water, and left for overnight air drying. The cells were stained with SRB dye (100 µl/well) and air-dried for 24 hours. The bound dye was solubilized in Tris-base (100 µl/well, 10 mM) and absorbance was measured at 545 nm. The mean absorbance reading of control cells of the aforementioned cell lines was 2.7±0.2. Drug concentrations causing 50% and total growth inhibition (GI50, TGI) and killing (LC50) were calculated graphically by plotting the percentage of cell growth or cell killing versus drug concentrations [22].

2.5. Immunofluorescence Microscopy

Fluorescent phalloidin derivative Alexa Flour 488 phalloidin, DAPI and Cy3-labeled β tubulin antibody were used to visualize F-actin, nuclei and the microtubules, respectively [23-25]. Lab-Tek II 8-chambered slides (Nalgene Nunc, USA) were used to sub-culture MCF-7 cells (1x 10^4/well). After 24 hours incubation, culture medium (400 µl) containing methanolic extract (5 and 10 µg/ml), ethyl acetate soluble fraction (1 and 5 µg/ml), odoroside A (0.001, 0.01 and 0.1 µM), oleandrin (0.01, 0.05 and 0.1 µM), or vehicle (0.1% DMSO) was added in each well. The cells were incubated at 37°C for further 24 hours. The slides were fixed with buffered formalin (400 µl/well) and washed with 1x phosphate buffered saline (PBS). The cells were permeabilized in Triton X-100 (1%, 400 µl/well) and incubated with Alexa Fluor 488 phalloidin, Cy3-labeled tubulin antibody and finally with DAPI. The slides were mounted and viewed using Nikon’s 90i epi-fluorescence microscope (NA of objective being 1.4). Nikon DXM1200C cooled CCD camera was used to capture images with DAPI, FITC and TxRed filter cubes. Real-time deconvolution was performed on the images using NIS-elements 3.0 software (AR version) and the images were processed with Adobe Photoshop (for details please see [26].
Table 1. Antiproliferative effects of *N. oleander* leaves methanolic extract, fractions and doxorubicin against HT-144, MCF-7, NCI-H460 and SF-268 human cancer cell lines.

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>HT-144</th>
<th>MCF-7</th>
<th>NCI-H460</th>
<th>SF-268</th>
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<tbody>
<tr>
<td>Methanolic extract</td>
<td>3.3 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.3 ± 0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.9 ± 0.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.85 ± 0.06&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aqueous fraction</td>
<td>8.1 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.0 ± 1.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.6 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.7 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethyl acetate soluble fraction</td>
<td>0.4 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.14 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.52 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.44 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>0.09 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.09 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.15 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.28 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are mean ± SEM of three independent experiments analyzed by ANOVA. The mean absorbance reading (at 545nm) of control cells of the aforementioned cell lines was 2.7 ± 0.2. For comparison, least significant difference and Duncan’s multiple range tests were used. In columns statistically different values are represented by dissimilar alphabetical superscripts. In rows statistically different values are represented by dissimilar numerical superscripts. TGI = Concentration of the extract/fraction/doxorubicin causing total growth inhibition of cells.

2.6. Statistical Analysis

Statistical software SPSS 16 was used to analyze the data which was expressed as mean ± SEM. ANOVA (Analysis of variance) followed by LSD (least significance difference) and Duncan’s multiple comparison tests were used. <i>p</i> values of less than 0.05 were considered statistically significant (*<i>p</i> < 0.05; **<i>p</i> < 0.01; ***<i>p</i> < 0.001, versus control).

3. RESULTS

3.1. Total Growth Inhibition (TGI) of *N. oleander* Leaves Methanolic Extract and Fractions

The methanolic extract displayed significantly 2 to 4-fold better growth inhibition (TGI: 0.85 µg/ml) against SF-268 in comparison to other cell lines (Table 1). The aqueous fraction exhibited similar activity against all four cell lines (TGI: 5.7 to 8.1 µg/ml). The ethyl acetate soluble fraction was most potent (~3-fold) against MCF-7 (TGI: 0.14 µg/ml) as compared to other cell lines, and hence this cell line was selected for further investigation. It also exhibited comparable activity to the standard drug doxorubicin against MCF-7, NCI-H460 and SF-268. This fraction was also ~10 to 50-fold more potent than aqueous fraction against all cell lines tested. The potency order against MCF-7, NCI-H460 and SF-268 cell lines was: doxorubicin = EA > ME > Aq, while in case of HT-144, the potency order was: doxorubicin > EA > ME > Aq.

3.2. Growth Inhibition (GI<sub>50</sub>) and Cytotoxicity (LC<sub>50</sub>) Induced by the Extract, Fraction and Pure Compounds from *N. oleander* in MCF-7 Cell Line

The methanolic extract exhibited significantly minimum growth inhibition and cytotoxicity (Table 2). On the other hand, the ethyl acetate soluble fraction exhibited comparable GI<sub>50</sub> to doxorubicin. Odoroside A demonstrated maximum growth inhibitory activity (GI<sub>50</sub>: 0.001 µg/ml) which was 10-fold greater than that of doxorubicin. Regarding GI<sub>50</sub>, the potency order was odoroside A > doxorubicin = EA > oleandrin > odoroside B > ME. Odoroside A also exerted maximum cytotoxic effects (LC<sub>50</sub>: 0.14 µg/ml) that were ~4-fold greater than that of doxorubicin. The LC<sub>50</sub> potency order appeared to be odoroside A > doxorubicin = oleandrin = EA > odoroside B > ME.

3.3. Immunofluorescence Microscopy

The mechanism of antiproliferative effects induced by the methanolic extract, ethyl acetate soluble fraction, odoroside A and oleandrin against MCF-7 cell line was explored by employing immunocytochemistry. F-actin, nuclei and microtubules were visualized using Alexa 488 phalloidin, DAPI and Cy3-labeled tubulin antibody, respectively.
Table 2. Antiproliferative effects of *N. oleander* leaves methanolic extract, ethyl acetate soluble fraction, odoroside A, odoroside B, oleandrin and doxorubicin against MCF-7 human breast cancer cell line.

<table>
<thead>
<tr>
<th></th>
<th>$\text{GI}_{50}$</th>
<th>$\text{LC}_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic extract</td>
<td>$2.10 \pm 0.3^a$</td>
<td>$7.40 \pm 0.60^a$</td>
</tr>
<tr>
<td>Ethyl acetate soluble fraction</td>
<td>$0.04 \pm 0.01^b$</td>
<td>$0.75 \pm 0.03^b$</td>
</tr>
<tr>
<td>Odoroside A</td>
<td>$0.001 \pm 0.005^c$</td>
<td>$0.14 \pm 0.005^c$</td>
</tr>
<tr>
<td>Odoroside B</td>
<td>$0.40 \pm 0.05^d$</td>
<td>$4.40 \pm 0.05^d$</td>
</tr>
<tr>
<td>Oleandrin</td>
<td>$0.11 \pm 0.02^e$</td>
<td>$0.50 \pm 0.01^b$</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>$0.01 \pm 0^f$</td>
<td>$0.65 \pm 0.15^g$</td>
</tr>
</tbody>
</table>

Data are mean $\pm$ SEM of three independent experiments analyzed by ANOVA. The mean absorbance reading (at 545nm) of control MCF-7 cells was 2.683 $\pm$ 0.1. For comparison, least significant difference and Duncan’s multiple range tests were used. In columns statistically different values are represented by dissimilar alphabetical superscripts. The values within the parenthesis are expressed in $\mu$M. $\text{GI}_{50}$ = Concentration of the extract/fraction/compound causing 50% growth inhibition of cells. $\text{LC}_{50}$ = Lethal concentration of the extract/fraction/compound that killed 50% cells.

3.3.1. Cytoskeletal and Nuclear Morphology of Control MCF-7 Cells

In control, the polygonal-shaped MCF-7 cells formed large clumps after 24 hours of incubation (Fig. 1a). The oval interphase nuclei contained 5-6 nucleoli, while condensed chromatin was visible in the prophase, metaphase, anaphase and telophase stages of mitosis (Fig. 1b). Thread-like fine microtubules forming the interphase network and mitotic spindles were also visible (Fig. 1c).

3.3.2. Effects of Methanolic Extract and Ethyl Acetate Soluble Fraction

The methanolic extract (10 $\mu$g/ml) induced band-like arrangement of cells, whereas, no effect was evident on F-actin, nuclei, interphase microtubule network or mitotic spindles (Fig. 1d, 1e and 1f). Ethyl acetate soluble fraction (5 $\mu$g/ml) demonstrated multiple changes such as arrangement of cells in bands, reduction in F-actin processes (Fig. 1g), and condensed nuclei (Fig. 1h). Moreover, numerous intercellular connections were visible along with disrupted interphase microtubule network (Fig. 1i).

3.3.3. Effects of Odoroside A and Oleandrin

Odoroside A (0.1 $\mu$M) induced band-like arrangement of cells while many nuclei were condensed (Fig. 2a, 2b). Although thread-like microtubule fibers were visible but regular interphase network could not be observed (2c). In the presence of oleandrin (0.1 $\mu$M), cells were arranged in band- and whorl-like patterns (Fig. 2d). Additionally, most of the nuclei were condensed (e) and numerous intercellular connections were visible (Fig. 2f).

3.3.4. Effects of Methanolic Extract, Ethyl Acetate Soluble Fraction, Odoroside A and Oleandrin on the Mitotic Index of MCF-7 Cells

In control, the mean percent mitotic index was 3.35 $\pm$ 0.3 which was significantly reduced to 0.7 $\pm$ 0.08 after 24 hours of treatment with methanolic extract (10 $\mu$g/ml). Similar drastic decline in the mitotic index was seen after incubation with ethyl acetate soluble fraction (5 $\mu$g/ml; % MI: 0.5 $\pm$ 0.06) odoroside A (0.1 $\mu$M; % MI: 0.15 $\pm$ 0.04), and oleandrin (0.1 $\mu$M; % MI: 0.2 $\pm$ 0.05).
**Fig. (1).** Effects of *N. oleander* leaves methanolic extract and ethyl acetate soluble fraction on F-actin, nuclei and microtubules of MCF-7 cells after 24 hours of incubation. (a-c) Control MCF-7 cells: (a) F-actin, (b) Nuclei with condensed chromatin in various stages of mitosis: prophase (p), metaphase (m), anaphase (a) and telophase (t), and (c) Microtubules with mitotic spindles in mitotic stages (40x magnification). (d-f) *N. oleander* methanolic extract (10 µg/ml): (d) F-actin, (e) Nuclei with condensed chromatin in the metaphase stage of mitosis, and (f) Microtubules forming interphase network and control-like bipolar mitotic spindle (60x magnification). (g-i) *N. oleander* ethyl acetate soluble fraction (5 µg/ml): (g) F-actin, (h) Condensed nuclei, and (i) Microtubules with intercellular connections (60x magnification). Scale bar: 20 µm.

**4. DISCUSSION**

In the present study, we investigated the anti-proliferative effects of *N. oleander* leaves methanolic extract and fractions against HT-144 (melanoma, skin), MCF-7 (adenocarcinoma, breast), NCI-H460 (non-small cell carcinoma, lung) and SF-268 (anaplastic astrocytoma, CNS) human cancer cell lines. Odoroside A (5β) (LC_{50}: 0.14 µg/ml), isolated from the *N. oleander* leaves extract was the most potent compound exhibiting ~3- to 4-fold significantly better cytotoxicity than either oleandrin or doxorubicin against MCF-7 breast cancer cell line. Notably, it was 30-fold more cytotoxic than its stereoisomer odoroside B
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(5α). It is likely that an inverted configuration at C5 significantly affects its binding and hence is a critical factor in accounting for the difference in biological activity advocating the importance of sterospecificity as has been reported earlier in another study where the inverted configuration at C-1 of 4'-epi-daunorubicin, 4'-epi-adriamycin, and adriamycin led to their diminished cytotoxicity in mouse embryo fibroblasts cultures due to a significant decrease in the binding to DNA [27].

Plants containing well-known cardiac glycosides, digoxin and digitoxin, have been used for the treatment of cardiac failure since centuries. Dr. Stenkvist's observation that breast cancer patients who were on digoxin for treatment of heart failure exhibited a more benign phenotype with less proliferation of malignant disease than those patients not receiving cardiac glycoside therapy [29] led to an increased interest in their effects on cancer growth. Cancer can also be regarded as a disease resulting from impaired differentiation process [30] as the malignant cells have a potentially unlimited lifespan, uncontrolled proliferation and an inability to undergo normal differentiation [31]. Differentiating agents like all-trans retinoic acid (ATRA) have been used in the treatment of acute promyelocytic leukemia which transforms abnormal promyelocytes into mature granulocytes [32] and in combination with anthracycline and arsenic trioxide, ATRA led to marked improvement in acute promyelocytic leukemia patients [33]. Moreover, induction of differentiation in cancer

Fig. (2). Effects of odoroside A and oleandrin on F-actin, nuclei and microtubules of MCF-7 cells after 24 hours of incubation MCF-7 cells (at 60x magnification) after treatment with 0.1 µM of: (a-c) Odoroside A: (a) F-actin, (b) Condensed nuclei, and (c) Microtubules (d-f) Oleandrin: (d) F-actin, (e) Condensed nuclei, and (f) Microtubules with intercellular connections. Scale bar: 20 µm.

In the current study, N. oleander leaves ethyl acetate soluble fraction exhibited 2- and 10-fold better growth inhibition than pure compounds oleandrin (GI_{50}: 0.11 µg/ml) and odoroside B (GI_{50}: 0.4 µg/ml), respectively, against MCF-7 cell line, most probably due to the additive or synergistic effects of compounds residing in the fraction. Similar trend has also been noted for Chinese herb Coptidis rhizoma (Huanglian), where the plant extract was more effective than pure compound berberine favoring synergism [28].
cells is also reflected as a loss of their proliferative capacity as observed in acetaminophen-induced differentiation of human breast cancer stem cells and inhibition of tumor xenograft growth in mice [34]. In breast cancer cells, induction of differentiation leads to growth arrest, alterations in cytoplasmic and nuclear morphology, expression of milk components (lipids and casein), and regulation of Her2/neu protein [35-38]. In the current study, a virtual loss of the proliferative capacity of MCF-7 breast cancer cells was observed in the presence of 0.1 µM of cardiac glycosides odoroside A and oleandrin with mitotic index: ~0.2% vs 3.35% in control; and ~0.6% after treatment with the N. oleander methanolic extract (10 µg/ml) and ethyl acetate soluble fraction (5 µg/ml). Additionally, the aforementioned treatments induced band-like arrangement of MCF-7 cells in comparison to large clumps observed in untreated cells. Condensed nuclei and numerous intercellular connections were also visible (Fig. 2). It is thereby proposed that the process of differentiation could be responsible for the substantial decline in the proliferative activity along with the cellular and nuclear morphological changes in MCF-7 cells induced by N. oleander methanolic extract, ethyl acetate soluble fraction, odoroside A and oleandrin. One of the limitations of our study was that only morphological changes were noted, therefore, additionally other tests like expression of milk components (lipids and casein), and Her2/neu protein expression should be carried out [35-38].

CONCLUSION

N. oleander leaves methanolic extract and fractions possesses considerable antiproliferative activity against HT-144, MCF-7, NCI-H460 and SF-268 human cancer cell lines justifying the traditional use of this plant against tumors. Regarding MCF-7 cell line, odoroside A appeared to be the most potent compound isolated from the leaves exhibiting ~3 to 4-fold significantly better cytotoxicity than oleandrin and doxorubicin. In the present paper, it is reported for the first time that the antiproliferative activity of N. oleander leaves methanolic extract, ethyl acetate soluble fraction, and the pure compounds odoroside A and oleandrin against MCF-7 cells could be linked to changes in the cytoskeletal and nuclear morphology. Therefore, these data provide further support for N. oleander as a potential therapeutic agent for breast cancer that warrants further investigation.

CONFLICT OF INTEREST

There is no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations that could have inappropriately influenced, or be perceived to influence our work.

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