CHAPTER 6

RESULTS

6.1 Cytotoxicity activity of *Anzia ornatoides*

6.1.1 Evaluation of cytotoxicity potential

Lichen extracts were tested for their ability to inhibit the cancer cell lines PC-3, OVCAR-3, MCF-7, HeLa, Hep-G2, and h-1299. The potential of extracts for cytotoxicity was evaluated at dosages ranging from 5 µg/ml to 160 µg/ml. Methanol extract in the studies inhibited the maximum proliferation of cancer cells in comparison to other extracts. Effectiveness at inducing cell death was directly correlated with extract concentration. A strong increase of cancer cell death is seen in the cell line OVCAR-3 (Fig. 6.1), finally leading to apoptosis of OVCAR-3, therefore further research was performed with it. The cytotoxicity of the cell line was dependent on concentration and time as shown by the IC50 values (Fig. 6.3). The IC50 values of methanolic extract were 52, 38, 48, 47, 52, and 78 µg/ml for PC-3, OVCAR-3, MCF-7, HeLa, Hep-G2, and h-1299 (Fig. 6.2).

Hexane, diethyl ether, ethyl acetate, methanol, and water extracts were utilised to compare among the cell lines. Multiple cancer cell types PC-3, OVCAR-3, MCF-7, HeLa, Hep-G2, and h-1299 are shown in Fig. 6.1. Each of these abbreviations stands for a particular kind of cancer cell line that was employed in the study. The Y-axis of each picture illustrates the measure of suppression of proliferation, which is expressed as a percentage of growth rate. This indicates the effectiveness of extracts at inhibiting cancer cell growth. A distinct cancer cell line is represented by each data point or graph on the X-axis, which shows the rising concentration of extracts in a particular solvent. These graphs or data points demonstrate for each cell line, the inhibitory effect on cancer cell proliferation changes as extract concentration increases.

6.1.2 Immunofluorescence imaging to detect apoptosis

The effectiveness of the methanol extract in inducing apoptosis was tested using Ao and Etbr staining. Confocal image distinguishes, apoptotic cells were fragmented, while control cells showed a uniform nucleus (Fig. 6.3). Following a 48 h methanol extract treatment, nuclear condensation was seen in every treated cell.

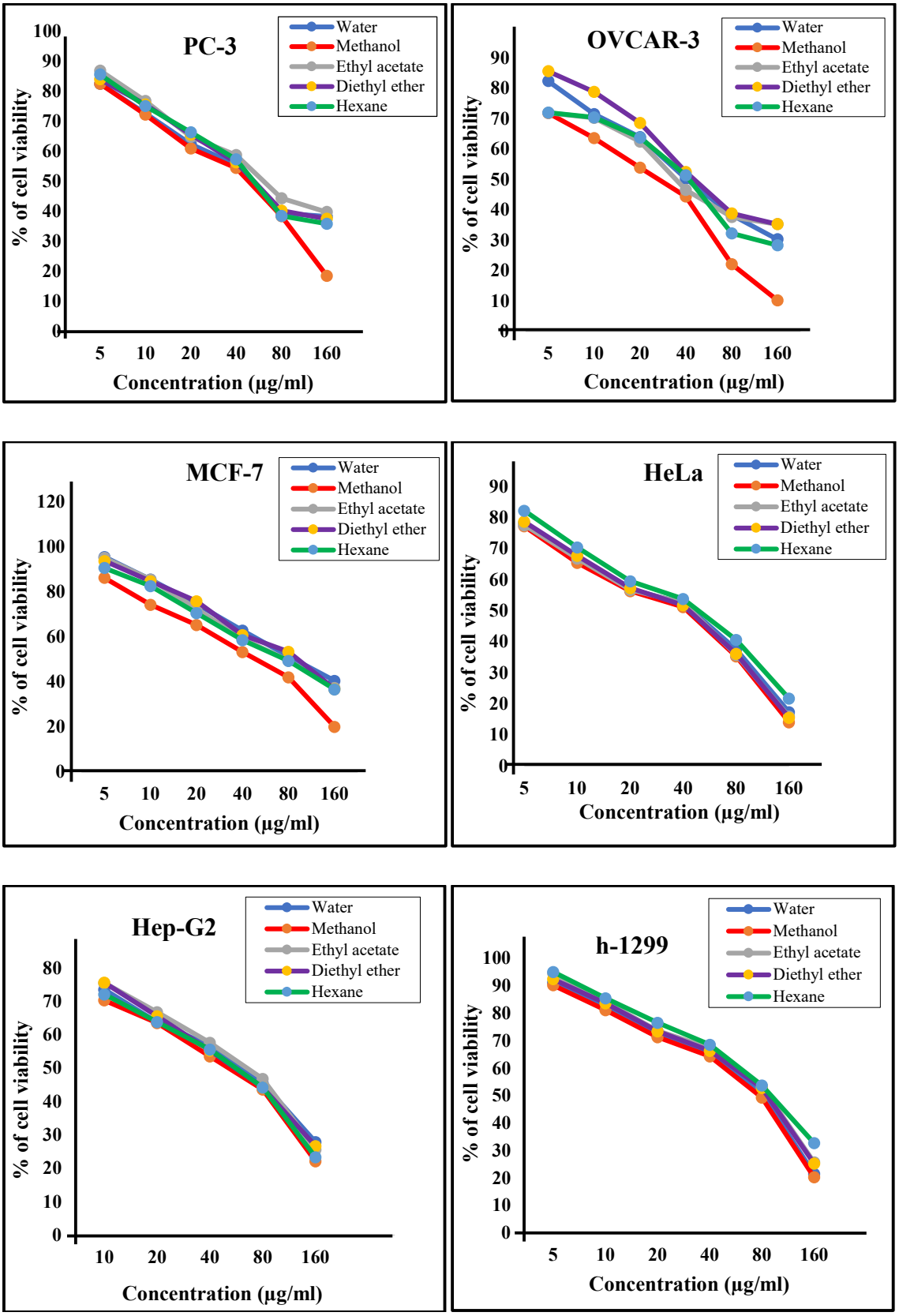

Fig. 6.1. Inhibition of proliferation of cancer cells against all the extracts

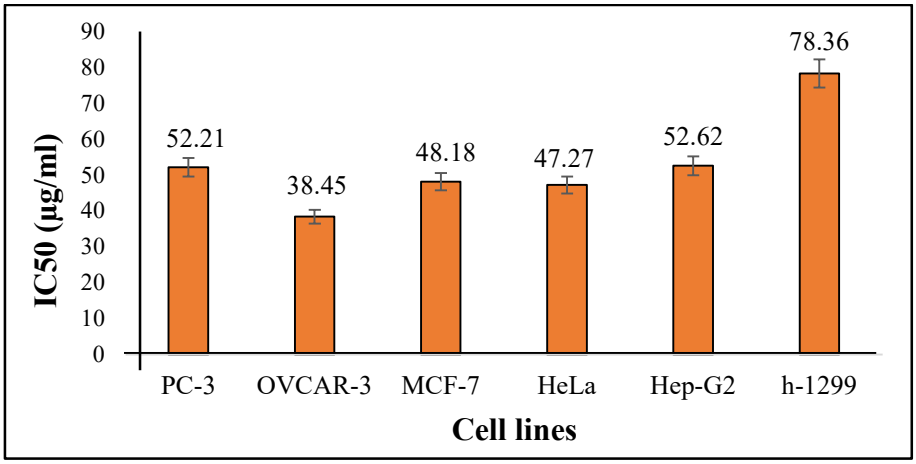

Fig. 6.2. IC50, Cancer cell lines of methanol extract

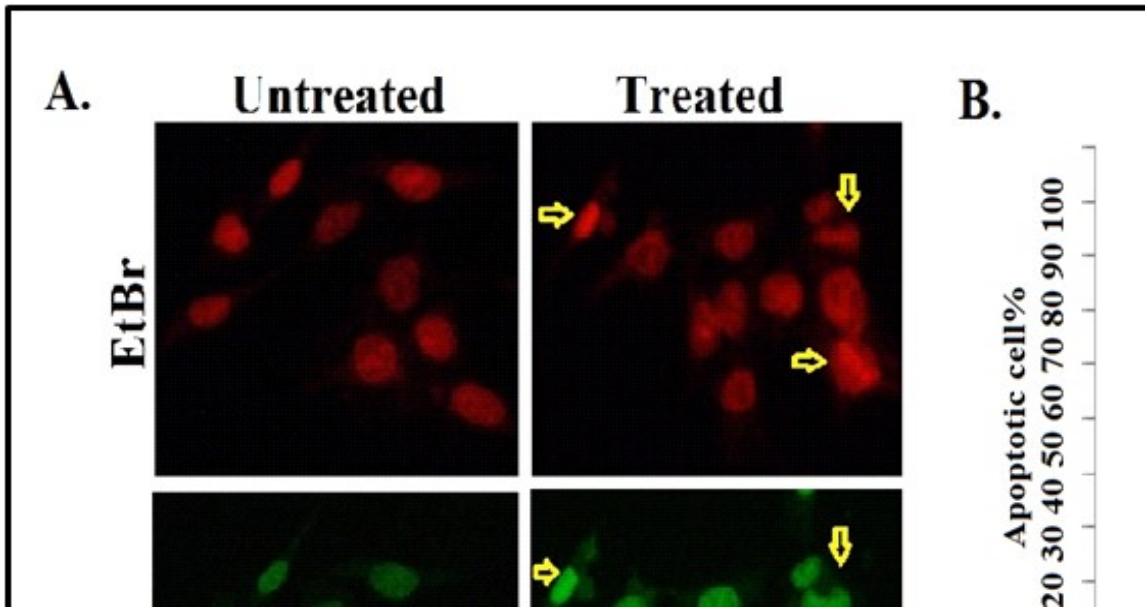

Fig. 6.3. A - Treatment of OVCAR-3 cancer cells with methanolic extract of *A. ornatoides* at a specific IC50 concentration, B - Viability and apoptotic percentage of the OVCAR-3 cancer cell

The Fig. 6.3 focuses on the treatment of OVCAR-3 cancer cells with the methanolic extract of *A. ornatoides* at a specific IC50 concentration, which is 38 µg/ml. The image in Part A of the figure was captured with a microscope. The OVCAR-3 cancer cells in this image have been stained with two different dyes: Ethidium Bromide (EtBr) and Acridine Orange (AO). To differentiate between living and apoptotic cells, these stains were frequently employed. The apoptotic cells have been highlighted in the image. It indicates the size represented in the image by providing the scale bar (20 µm) and the

magnification level (200X). The graph in Part B of the image displays the viability and apoptotic percentage of OVCAR-3 cancer cell. Data measuring the impact of treating OVCAR-3 cells with methanolic extract at the IC50 concentration are shown in this graph. It displays the quantity of living cells and cells that have died (undergone apoptosis) as a result of the treatment. Error bars or standard deviation are also included in the graph to show the viability in the data.

6.1.3 Flow cytometry and confocal analysis for apoptosis

To understand more about how cells react to the methanol extract by dying, apoptosis was created. A cell-impermeant DNA-binding fluorescent dye (Hoechst) can readily penetrate the cells only after apoptosis, when membrane permeability is reduced. The number of apoptotic cells was measured using FACS analysis and confocal imaging. Following treatment with the IC50 concentration of methanol extract, confocal microscopy and flow cytometry were used to evaluate apoptotic cells against OVCAR-3. The illustration includes a flow cytometry representative (Fig. 6.4). There were only a few (5%) apoptotic cells in the untreated cell, which was probably background cell death due to normal cell culture damage. In contrast, the fraction of apoptotic cells in OVCAR-3 treated with the extract was 55%, higher than that the untreated.

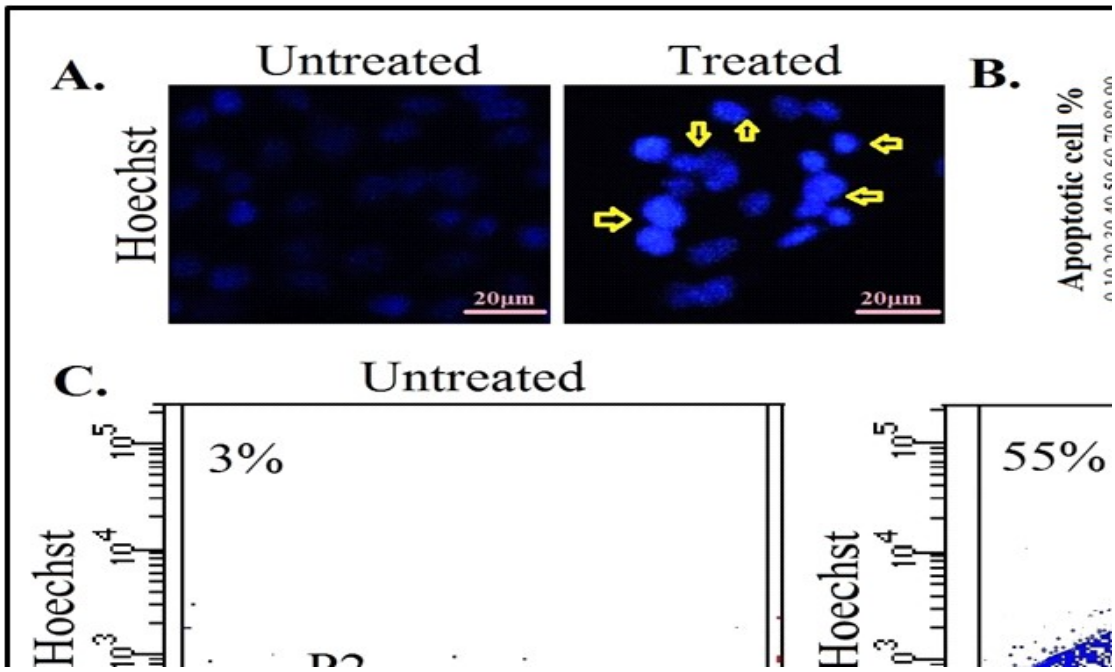

Fig. 6.4. A comprehensive visual representation of the impact of methanolic extract on OVCAR-3 cancer cells at the IC50 concentration

Part A of Fig. 6.4 illustrates the cell morphology, highlighting apoptotic cells, Part B quantifies the percentage of apoptotic and viable cells, and part C provides additional evidence of apoptotic cell death through flow cytometry analysis. This information is crucial for assessing the possible anticancer qualities of extracts as well as its effects on OVCAR-3 cell line.

6.1.4 In-vitro toxicity analysis

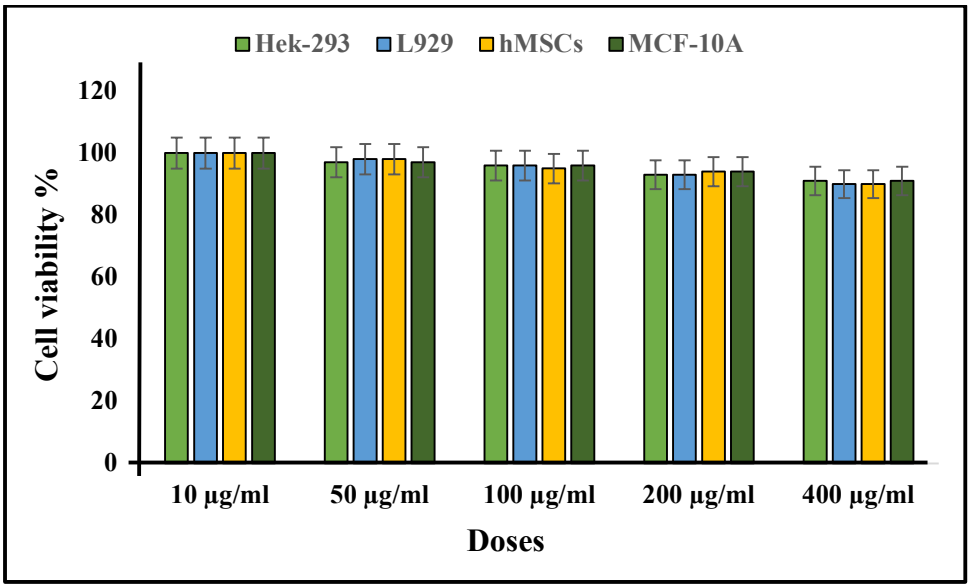

Fig. 6.5. Demonstrating the extract influences viability and apoptotic cell death of healthy cells across a range of concentrations

Of the Fig. 6.5, the concentration of the methanolic extract is represented by the X-axis on the graph, which ranges from 10 µg/ml to 400 µg/ml. This range shows the various concentrations at which the extract was given to the healthy cells. The Y-axis of the graph specifies different measures. The percentage of apoptotic cell death and viability are the two measurements stated here. The graph shows the concentration at which the extract most significantly effects the preservation of cell viability or the promotion of cell death (apoptosis). It demonstrates the extract's safety and possible therapeutic benefits on normal cells. It can assist in determining the extract's cytotoxicity, or capacity to kill cells, as well as any possible advantages in terms of inducing apoptosis, which may be advantageous in specific medical situations.

6.2 Discussion

To assess its potential, a preliminary screening using several cancer cell lines and solvent extract was conducted. The screening results indicated the methanol extract had positive effects on the well-known ovarian cancer cell line OVCAR-3. The effect was associated with the induction of apoptosis and was dose dependant. Research on prospective treatments can have a big impact on ovarian cancer because it is a serious health concern. Subsequently, additional research was carried out with a particular OVCAR-3 cell line with methanol extract. This approach allowed for more in-depth and targeted research on the selected cell line while also conserving resources and time.

The outcomes of different solvent extraction differed because of their chemical compositions. The cytotoxicity assay results showed the particular lichen employed in this investigation has both cytotoxic and apoptotic effects on cell line. The loss of viability of cell lines as proved by morphological changes were examined by confocal microscopy. Studies using flow cytometry suggested the species caused apoptosis in cancer cell lines and had no harmful effects on in-vitro cultured untreated cells.

The study employed cell proliferation assays to assess the impact of the *A. ornatoides* extract on the growth of cancer cells. Meticulously designed experiments were conducted to investigate the extract's ability to inhibit cancer cell proliferation. Results consistently demonstrated the potent anticancer properties of the extract, suggesting its potential as an effective agent in impeding the uncontrolled growth of cancer cells. Understanding the extract's influence on programmed cell death, apoptosis assays were conducted. The induction of apoptosis in cancer cells is a critical aspect of anticancer therapies. The study revealed *A. ornatoides* extract possesses the ability to induce apoptosis, providing further evidence of its potential as a therapeutic agent for cancer treatment. Reactive oxygen species (ROS) play a pivotal role in oxidative stress, a phenomenon linked to various diseases, including cancer. The research included anti-ROS evaluations to determine the extract's capacity to counteract oxidative stress. The findings indicated *A. ornatoides* extract exhibits anti-ROS properties, suggesting its role in mitigating oxidative stress. Ensuring the safety of therapeutic candidates is paramount for their development. Toxicological assessments were conducted to evaluate the safety profile of the *A.*

ornatoides extract. Importantly, the results revealed a favourable safety profile, laying the foundation for further development and clinical applications.

The comprehensive findings of this study collectively highlight the multifaceted therapeutic potential of the *A. ornatoides* extract. Its ability to inhibit cancer cell proliferation, induce apoptosis, the extract as a promising candidate for a range of therapeutic applications. The favourable safety profile established through toxicological assessments is a crucial factor in the development of therapeutic agents. With a safe profile, the *A. ornatoides* extract holds promise for further clinical investigations. The absence of significant toxic effects supports the feasibility of advancing the extract into clinical trials, bringing us closer to realizing its therapeutic potential in real-world applications. While the current study provides compelling evidence of the *A. ornatoides* extract's therapeutic potential, further research is warranted. Elucidating the underlying mechanisms of action and optimizing dosages for clinical applications are essential next steps. Future studies should focus on exploring the extract's interactions at the molecular level, identifying specific pathways affected, and conducting preclinical trials to validate its efficacy in vivo. This research significantly contributes to the growing body of knowledge regarding natural compounds with therapeutic potential. The *A. ornatoides* extract's multifaceted properties make it a valuable resource in the development of novel therapies. By elucidating its anticancer effects, this study lays the groundwork for future investigations aimed at harnessing the full therapeutic potential of this natural compound.