Cytotoxic activity of methanolic extract of Nerium Oleander Naturally Grown in Iraq

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Abstract

Background: Cancer cell lines have several applications in the scientific community, including as a model to learn more about the disease and find novel ways to treat it. This paper aims to assess the anti-cancer properties of Nerium oleander in relation to PC3 (PC-3), a human prostate cancer cell line. Method: Whole methanolic extract of Nerium oleander leaves was used to treat PC3 (PC-3) human prostate cancer cells. After 24 hours of cell line seeding at 1 104cells/well in 96-well plates, cells were treated with tested substances at a range of doses to detect anticancer activity using the MTT assay. After 72 hours of treatment, the cells were washed with PBS, the medium was replaced with 28 L of a 2 mg/mL solution of MTT, and the samples were kept in an incubator at 37 °C for 2.5 hours. After the MTT solution was taken away, the crystals in the wells were broken up by adding 130 L of DMSO (Dimethyl Sulphoxide) and shaking the plates for 15 minutes at 37 °C. A microplate reader was used to measure the absorbance at 492 nm. Conclusion: The development of prostate cancer tumours was slowed by using a plant extract from the Nerium oleander plant, demonstrating its potent anticancer properties. Our findings indicate that whole-plant extracts have the potential to serve as anticancer medicines.

Keywords: *Nerium Oleander, PC3, cancer cell line, anticancer Activity.*

INTRODUCTION

Many regions of the world still rely heavily on plant-based natural resources for human nutrition and healthcare. People's interest in foraging for food in the wild is piqued during times of food shortage and hunger, but consuming wild items is also becoming trendy in today's culture [1]. Synthetic medications, with their instantaneous effect, quickly surpassed phytomedicine in popularity[2]. In some regions, the use of medicinal plants is still stigmatised as a form of witchcraft and superstition because of the lack of scientific knowledge required to describe and predict the healing action of plants[3,4]. The flexibility of plants to accommodate different ecosystems is buffered by the production of diverse secondary metabolites with various biochemical modifications that contribute to the assortment of these molecules in nature.

These modifications are facilitated by the expression of enzymes with arms to link and modify a vast number of building blocks and perform a variety of chemical changes leading to the desired metabolites[5-7]. Studies in natural product chemistry showed that these metabolites express a wide range of bioactivities like antioxidant, antiviral, cytotoxic [8], antimicrobial [9], enzyme modulation [10], anti-inflammatory, anti-aging, hormone regulation, analgesic, and anticoagulant. These activities were credited to different classes of secondary metabolites, including plant phenolics, terpenoids, steroids, and alkaloids[11]. Many studies in drug discovery focused on isolating immense bioactive metabolites that provide lead molecules for new drug generations [12]. The most used traditional medicine and complementary and alternative therapies are medicinal herbs and acupuncture. Traditional and alternative medicine are playing a bigger part in the transformation of the health care systems in many nations today[13]. Given their wide variety of chemical structure and often low amount and variability, analysing phytochemicals in plants is a difficult undertaking. In the same species[14-17].Alkaloids, Terpenoids, saponins, glycosides, carbohydrates and tannins were detected in the phytochemical analysis of the plant . A lack of phenolic substances, flavonoids, and phlobatanins was found [18-23].A number of cardiac glycosides related to digitalis are found in the plant [24]. Among the most common components of oleandrin are the glycosides, gentiobiosyl, neriine, oleandrin, Cardenolides, and odoroside [25]. There are a number of additional pharmacologically active chemicals found in the plant as well, such as folinerin, rosagenin, rutin, and oleandomycin. Investigating the Nerium oleander plant revealed promising results from a phytochemical screen of the leaves (of alkaloids, terpenoids, saponins, glycosides, carbohydrates and tannin). Also, examine the cytotoxic impact of Iraqi Nerium oleander against PC3 (PC-3), a human prostate cancer cell line generated from an Iraqi cancer patient. Due to the fact that it is a first for the Iraqi people, it has gained special significance.

Figure 1: Nerium oleander L plant cultivated in Iraq.

Materials and Methods

Plant material

In January of 2022, we gathered Nerium oleander leaves from the Makishifa salah Aldin. At the University of Baghdad's Department of Biology, in the College of Sciences, is Professor Dr. Sukaena Abass, who confirmed the plant's identity and authenticity. After a thorough washing and drying in the shade, the leaves were crushed into a powder in a mechanical grinder.

Experimental work

At room temperature, one hundred grammes of the plant powder was soaked in one thousand five hundred millilitres of methanol, with

intermittent shaking. At the end of the three days, anything that could be dissolved in methanol was filtered out. A rotary evaporator was used to remove all of the moisture from the filtrate while operating at very low pressure. Once the residue was processed, it turned out to be a dark green colour. Dry the leftover residue off and check it for cytotoxic action in the lab.

Preliminary phytochemical examination of crude extracts

Standard protocols for phytochemical analysis were applied to crude extracts, fractions, and powder specimens from the medicinal plants under investigation with the purpose of screening and identifying bioactive chemical ingredients.

In order to conduct the alkaloid test, 0.5 to 0.6 g of each plant extract and fraction was combined with 8 ml of 1% HCl, heated, and filtered. Two millilitres of the filtrate was tested for the presence of alkaloids by treating it with Mayer's and Dragendorff's reagents one at a time and observing the resulting turbidity and precipitate development.

Checking for Saponins A total of 0.5 g of each plant extract and fraction was weighed out and dissolved in boiling water in a test tube, where it was allowed to cool before being rapidly agitated to produce a froth.

The plant powder, amounting to 2.0 g, was reconstituted with distilled water and then cooked in a test tube in a boiling water bath. The filter was diluted with distilled water (5 ml) to make 10 ml, and the mixture was agitated until a stable, persistent foam formed. The foam was mixed with three drops of olive oil and vigorously stirred to generate an emulsion, a feature of saponins.

Flavonoids Test: Fatty materials were extracted from 0.5 g of each plant extract and fraction by shaking them with petroleum ether (lipid layer). 20 cc of 80% ethanol was used to dissolve the defatted residue, which was then filtered. For these experiments, we employed the filtrate:

(a) Mixing 3 ml of the filtrate with 4 ml of 1% aluminium chloride in methanol in a test tube allowed us to examine the resulting colour. Evidence for flavonoids is provided by the production of a characteristic yellow colour.

(b) In a test tube, three millilitres of the filtrate was combined with four millilitres of 1% potassium hydroxide, and the resultant colour was examined. The presence of flavonoids was indicated by a bright yellow hue.

Tannins Test: Filtered 10 ml of distilled water was used to dilute and sterilise all plant extracts and fractions. A ferric chloride (FeCl3) solution in water at the concentration of 1% was used to treat the filtrate. Presence of tannins is indicated by the appearance of a dark green, blue, or black hue.

Cell cultures Maintenance

PC-3 Foetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 g/mL) were added to RPMI-1640 media to facilitate cell growth. Cells were cultivated at 37 degrees Celsius, and when they reached 80% confluence, they were divided using Trypsin-EDTA and reseeded twice weekly [26].

Cytotoxicity Assays

The MTT test, performed on 96-well plates, was used to evaluate the cytotoxic potential of Nerium oleander [27,28]. One x 104 cells per well was the seeding density for the cell lines. The cells were treated with x-substance after 24 hours, when a confluent monolayer had formed. After 24, 48, and 72 hours of treatment, the medium was removed and replaced with 28

litres of a 2 mg/mL solution of MTT, and the cells were incubated for 2.5 hours at 37 °C to evaluate cell viability. By adding 130 L of DMSO (Dimethyl Sulphoxide) and shaking the plate at 37 °C for 15 minutes, we were able to dissolve any remaining crystals in the wells [29]. Absorbance at 492 nm was measured using microplate readers, and for accuracy, triplicate experiments were performed. In order to calculate the cytotoxicity %, we utilised the following formula (the rate at which cell growth was inhibited) [30,31]:-

Where A represents the standard and B the samples' optical densities [32].

Cytotoxicity = A-B/A *100, Viability=100-cytotoxcity

Where A and B are the optical density of control and the optical density of test

After 24 hours of growth at 37 °C, the cells' morphology was examined under an inverted microscope after being seeded at a density of 1 105 cells mL1 into 24-well micro-titration plates. After that, C- SUBSTANCE was allowed to sit in contact with the cells for a whole day. Following exposure, crystal violet stain was applied, and the plates were incubated at 37 °C for 10-15 minutes. We used mild soap and running water to carefully remove the stain and any traces of colour. At 100x magnification, the cells were seen using an inverted microscope, and the accompanying digital camera was used to record the observations [33-34].

Reagents and Chemicals

Table 1: Reagents and Chemicals Used for the Analysis of Cytotoxic Activity

No.	Country / Company /Items
1	Germany / Capricorn/ EDTA
2	USA / Santacruz Biotechnology/ DMSO
3	Germany / Capricorn / RPMI 1640
4	USA / Bio-World / MTT stain
5	Germany / Capricorn / Fetal bovine serum

Instruments

Table 2: Instruments Used for the Assay of Cytotoxic Activity

No.	Country / Company /Items
1	Belgium / Cypress Diagnostics/ CO_2 incubator
2	USA / Gennex Lab/ Microtiter reader
3	Korea / K & K Scientific Supplier/ Laminar flow hood
4	Belgium / Cypress Diagnostics / Micropipette
5	USA / Santa Cruz Biotechnology / Cell culture plates

Statistical analysis:

The obtained data was subjected to an unpaired t-test in GraphPad Prism 6 [35]. All readings were taken three times to ensure accuracy and were presented as th e mean SD [36,37].

Results and Discussion

The first stage in creating the finished natural product from its basic elements is extraction. Which specific extraction method is used to remove a chemical is determined by the nature of the substance. After employing two extraction techniques, one at a lower temperature and the other at a higher one, for the extraction of a single plant component, the optimal solvent and extraction technique were determined by comparing the percentage yield

obtained from each technique and analysing the crude extract for constituents using thin-layer chromatography. The selectivity, solubility, cost, and safety of a solvent in light of the law of similarity and intermiscibility are all factors that should be taken into account when selecting a solvent for solvent extraction (like dissolves like) There will be better mixing between the solute and solvent if the solute's polarity is close to that of the solvent. The major drawbacks of this extraction process are the lengthy extraction time and the poor extraction effectiveness. Thermolabile substances might be extracted using this method which requires two days of macerating leaves In the event of pharmacological action, this approach was opted for.

Cytotoxic Effect of Nerium oleander Against PC3 human prostate cancer cell line

Nerium oleander's cytotoxic activity on cancer cells was investigated. The capacity of Nerium oleander extracts to slow the growth of the PC-3 prostate cancer cell line was studied to determine the plant's antiproliferative efficacy. This research confirmed previous findings that Nerium oleander has cytotoxic effects on the PC-3 cell line as in Figures (3.1-3.3).

Figure 2: Cytotoxic effect of Nerium oleander in PC-3 cells.

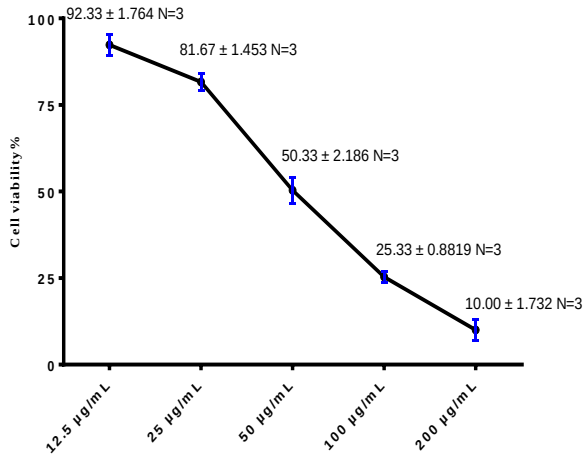


Figure 3: Control untreated PC-3 cells

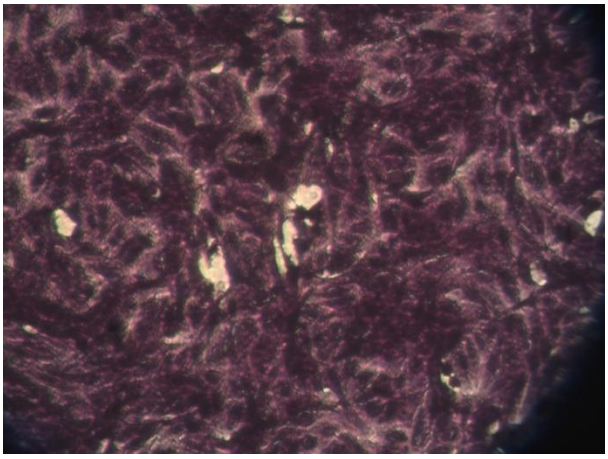
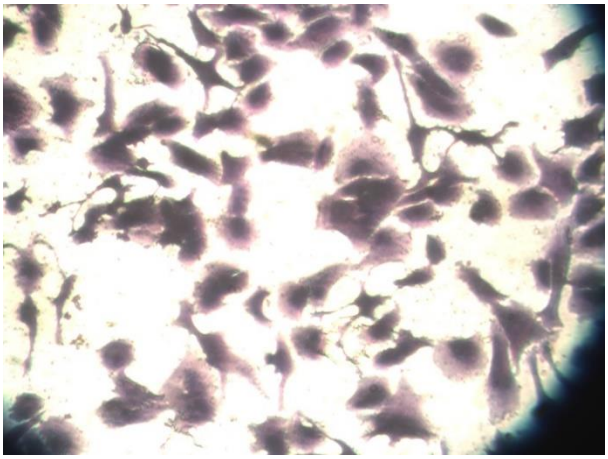


Figure 4: changes in PC-3 cells after being treated with Nerium oleander



Nerium oleander's cytotoxic activity on cancer cells was investigated. Researchers looked examined the Nerium oleander's potential to slow the growth of cancer cells to determine whether or not it has anticancer properties. The results of the investigation showed exceptionally significant cytotoxic activity against the human cancer cell lines, as shown in figures (2,4) accordingly. The evidence suggests that Nerium oleander may be able to prevent cell line growth in a concentration-dependent way. Historically, people have turned to medicinal plants for treatment because of the wide range of health benefits they provide, including protection

against cancer, inflammation, bacterial infection, and cardiovascular disease. One of the main motivations for studying natural chemicals from plants and the sea is the rise of cancer patients who have developed resistance to conventional cancer treatments. It is becoming increasingly accepted that the beneficial effects of plants are caused by a complex interplay of the composite mixture of compounds present in the entire plant (additive/synergistic and/or antagonistic), rather than constituent single agents alone, despite the fact that many compounds isolated from plants are being put through rigorous anticancer testing.

Conflict of interest: Non

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