

In vitro evaluation of cytotoxic impact of Adenium obesum crude extract on EJ138 bladder carcinoma cells

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Abstract

Bladder cancer is a prevalent neoplasm that exhibits higher incidence rates in males than females. The most common clinical manifestations of bladder cancer are hematuria, reduced urine flow, and urinary frequency. Plant-derived compounds have emerged as promising candidates for anti-tumor therapy. *Adenium obesum* extract has demonstrated various biological activities, such as antimicrobial, antioxidant, anticancer, antiviral, immunomodulatory, anti-malarial, and anti-trypanosomal effects. The aim of this study was to examine the cytotoxic effects of *Adenium obesum* crude extract (0.25, 0.5, and 1 µg/mL) on the bladder cancer cell line EJ138 in vitro. Cell viability was assessed by MTT assays, Neutral red uptake, and NO assays. Oxidative stress was evaluated by GSH and catalase assays. Apoptosis was detected by a comet assay. The results showed that *Adenium obesum* decreased EJ138 cell viability in a concentration-dependent manner. NO production also declined with increasing concentrations of *Adenium obesum*, except at 0.25. GSH and catalase assays indicated oxidative stress induction in EJ138 cells. A comet assay revealed significant apoptosis induction in a concentration-dependent pattern. These findings imply that *Adenium obesum* possesses potent anti-cancer properties and may be a potential source of anti-tumor agents.

1. Introduction

Cancer arises from genetic and epigenetic changes in a cell that alter gene expression and disrupt the normal regulation of cell division and differentiation (1). This causes an imbalance between cell proliferation and death, leading to tumor formation. Malignant cancer differs from benign tumors by its ability to invade locally, spread to lymph nodes, and metastasize distally (2). Behavioral and lifestyle factors, such as avoiding smoking, increasing fruit and vegetable intake, and controlling infections, can lower cancer risk. Other factors, such as reducing sun exposure, physical inactivity, alcohol, and red meat consumption, may also prevent cancer (3). Bladder cancer is the ninth most common cancer globally, more prevalent in men in Southern and Western Europe, North America, and some North African or Western Asian countries. (4)

Bladder cancer is a global health concern, with a higher incidence in males than females (5). It can be fatal without proper diagnosis and treatment. Hematuria is the main symptom of bladder cancer, which can be effectively treated by tumor excision, immunotherapy, chemotherapy, or radical cystectomy, depending on the stage of the disease (6, 7). Natural substances found in plants, fruits, and vegetables have demonstrated anti-cancer capabilities through a variety of methods, including altering the metabolism of carcinogens, improving DNA repair, boosting immunity, and triggering apoptosis (8). The Apocynaceae family includes the medicinal plant *Adenium obesum*, which thrives on rocky and sandy soils (9). There are 53 biological substances present, some of which are hazardous. The plant contains a variety of chemical substances, including terpenoids, pregnanes, terpenoids with prenylation, flavonoids, cardiac glycosides, and sugars. Additionally, it has antibacterial, antiviral, anticancer, antioxidant, and immunomodulatory properties. It also contains anti-trypanosomal and anti-malarial properties (9–12).

A. obesum has shown cytotoxicity in different cancer cell lines, such as MCF-7, HEPG2, and HeLa (12). Cell culture, which involves growing isolated cells from tissues in a suitable medium under in vitro conditions (13), is a useful method for studying the anti-cancer effects of plant extracts. Cell viability is essential for all cell culture experiments. It can be the main outcome in toxicity assays or a measure of the relationship between cell number and cell behavior, providing more insight into cellular activity such as anabolism (14). This research aims to evaluate the potential anticancer effect of the crude extract of the *Adenium obesum* plant on EJ138 cell lines under two-dimensional (2D) culture conditions, with the objective of identifying a potential therapeutic strategy. By utilizing two-dimensional (2D) culture conditions, we aim to investigate the effects of *A. obesum* extract on cell proliferation and survival. The results of this study may provide valuable insights into the potential use of *A. obesum* extract as a therapeutic agent for the treatment of bladder cancer.

2. Materials and Methods

This study was conducted as an experimental trial using bladder cancer cells (EJ138) obtained from the cell bank of the Pasteur Institute.

2.1. Preparation and Extraction of *Adenium obesum* Plant Extract

The preparation of the plant extract was carried out following the protocol described by Hastuti et al. (15). Specifically, the plant's leaves were carefully separated, washed thoroughly, and finely chopped into small pieces. A total of 0.5 gr of the chopped leaves were mixed with 1000 µl of extraction buffer containing Tris-HCL 100 mM, glycerol 20%, and phenylmethylsulfonyl fluoride (PMSF) 1 mM. It is worth noting that, instead of the originally recommended 4% mercaptoethanol, 1mM phenylmethylsulfonyl fluoride (PMSF) was used to preserve the non-denatured proteins and ensure that their native conformation and activity were retained.

2.2. Cell culture

EJ138 cells were cultured in 25 mL flasks containing DMEM culture medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution. The flasks were maintained in a controlled environment with a 37°C temperature, 5% CO₂, and 80% humidity. The culture medium was replenished three times per week to ensure optimal growth conditions for the cells.

2.3. MTT Assay

The MTT assay was performed as follows: 3×10^4 cells were seeded in a 96-well plate with 100 µL of DMEM supplemented with 10% FBS and incubated overnight for cell attachment. The medium was aspirated, and fresh DMEM without FBS but containing *Adenium obesum* crude extract at different concentrations (0.25, 0.5, and 1 µg/mL) was added to each well. The plate was incubated for another 24 hours. Afterward, 5 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 hours to

allow the formation of formazan crystals. The medium was discarded, the wells were washed with PBS, and 100 µL of DMSO was added to each well and incubated in the dark for 3–4 hours to dissolve the formazan crystals. The absorbance was measured at 570 nm for each well. The medium served as the blank sample, and untreated cells with toxins served as the control.(16, 17). To ensure the reliability of the results, the assay was performed in triplicate, and the cell viability was calculated using the following formula:

Cell viability percentage = (OD of treated cells - OD of blank) / (OD of control - OD of blank) × 100.

2.4. Neutral Red Uptake (NRU)

The NRU assay was performed as follows: 3×10^4 cells were seeded in a 96-well plate with 100 µL of DMEM supplemented with 10% FBS and incubated overnight for cell attachment. The medium was aspirated, and fresh DMEM without FBS but containing *Adenium obesum* crude extract at different concentrations (0.25, 0.5, and 1 µg/mL) was added to each well. The plate was incubated for another 24 hours. Afterward, 5 µL of neutral red solution (5 mg/mL) was added to each well and incubated for 1 hour in the incubator. The solution was discarded, the wells were washed with PBS, and 100 µL of fixing buffer (formaldehyde 37% + calcium chloride 10%) was added to each well. After 1 minute, 100 µL of solubilization buffer (ethanol 50% + acetic acid 1%) was introduced to each well. The plate was incubated in the dark on a shaker for 20 minutes. Finally, the absorbance was measured at 540 nm for each well to determine the extent of cell death.(18, 19). To ensure the accuracy of the results, the NRU assay was performed in triplicate. The cell death was calculated using the following formula:

Cell inhibition percentage = $(1 - (\text{OD of treated cells} - \text{OD of blank}) / (\text{OD of control} - \text{OD of blank})) \times 100$.

2.5. Alkaline Comet Assay

The alkaline comet assay, a sensitive, cost-effective, and rapid technique for detecting DNA fragmentation in individual cells (21), was employed to determine the mode of cell death (apoptosis or necrosis) induced by the crude extract (20) from the *Adenium obesum* plant. The assay was performed as follows: 12×10^4 cells were seeded in a 24-well plate with 300 microliters of serum-free medium and incubated overnight for cell attachment. The medium was aspirated, and fresh medium without serum but containing various concentrations (0.25, 0.5, and 1 micrograms per milliliter) of the crude extract was added to each well. The plate was incubated for another 24 hours. Afterward, the cells were detached with trypsin and PBS, transferred to 1.5 ml microtubes, and centrifuged at 1500 rpm and 4°C for five minutes twice, discarding the supernatant. The cells were resuspended in 200 microliters of PBS and dissociated with a sampler and an insulin syringe. A cell layer was formed on each slide by coating it with one percent NMA 1% and combining it with one percent LMA 1% at a ratio of 1:2. The slides were covered with a coverslip and cooled for ten minutes at 4°C. The coverslip was removed, and the slides were lysed in cold-fresh lysis buffer for 16–18 hours at 4°C. The slides were washed twice with electrophoresis buffer for 20 minutes each and incubated in cold-fresh electrophoresis buffer in the refrigerator for 40 minutes to denature the DNA. Electrophoresis was conducted for 45 minutes at 25

volts and 300 milliampere-hours below 20 degrees in the dark. The slides were neutralized with a neutralization buffer for five minutes.

For cell staining, 100 microliters of ethidium bromide solution (20 micrograms per milliliter) were added to each slide and allowed to incubate at room temperature for ten minutes. The slides were subsequently washed twice with distilled water for ten minutes and examined using an inverted fluorescent microscope to capture multiple images from each sample. Finally, the data were subjected to statistical analysis.

2.6. NO Assay

The investigation involves the creation of a cellular suspension and the assessment of the cell count per cubic millimeter. The suspension was used to seed each well of a 96-well plate with 3×10^4 cells in 100 μL of medium supplemented with serum. Cell attachment and growth were observed overnight at 37°C , 5% CO_2 , and roughly 80% humidity. The old media and dead cells were aspirated in order to examine the impact of *adenium obesum* extract on the cells. The extract was then given to the wells at different concentrations (0.25, 0.5, and 1 micrograms per milliliter) in medium without serum and cultured overnight under the same conditions. The medium from each well was then transferred to brand-new microtubes, and they were centrifuged for 5 minutes at 1500 rpm and 4°C . A 96-well plate was prepared by taking the supernatant and adding an equal amount of Griess reagent (0.4 grams per milliliter in PBS, pH 7.4) to each well. A plate reader (Biotek, USA) was used to measure the optical density of each well on the plate at 540 nm after it had been incubated at room temperature for 10 minutes(22). Finally, a sodium nitrite standard curve was used to calculate the concentration of nitrite in the control and treated cells, which was then represented in micromoles per milliliter.

2.7. GSH

The assay was performed as follows: a cellular suspension was prepared, and the cell density was estimated. Subsequently, 5×10^5 cells were seeded in a 24-well plate and incubated overnight for cell attachment and growth. The medium and dead cells were aspirated, and the cells were exposed to various concentrations of *Adenium obesum* extract (0.25, 0.5, and 1 micrograms per milliliter) in a serum-free medium overnight. Afterward, the treated cells were collected, washed with PBS, and detached with trypsin. The cell pellets were centrifuged, frozen, lysed, sonicated, and centrifuged again, and the supernatants were collected for further analysis. The protein concentration was quantified by the Bradford method. A portion of the sample was mixed with TCA and incubated at 4°C for 2 hours. After centrifugation, a portion of the sample was transferred to a 96-well plate and mixed with lysis buffer, Tris buffer, and DTNB. The absorbance of each well was measured at 412 nm using a plate reader(23, 24). Subsequently, the level of glutathione reductase in EJ138 cells was computed using the following equation:

$$\text{GSH} = (\text{OD} \times 1.98) / (\text{mg of sample}).$$

2.8. Catalase

The assay was performed as follows: a cellular suspension was prepared, and the cell density was estimated. Subsequently, 5×10^5 cells were seeded in a 24-well plate with 500 microliters of medium supplemented with 10% serum and incubated overnight for cell attachment and growth. The medium and dead cells were aspirated, and the cells were exposed to various concentrations of *Adenium obesum* extract (0.25, 0.5, and 1 micrograms per milliliter) in a serum-free medium overnight. Afterward, the cells from each well were collected and washed twice with PBS (pH = 7.4). Trypsin was used to detach and collect the remaining cells. The cells were centrifuged at $500 \times g$ for 5 minutes at 4°C , frozen, lysed, incubated at room temperature for 30 minutes, sonicated for 10–15 minutes at 4°C , and centrifuged at $2000 \times g$ for 15 minutes. The supernatant was collected and used for further analysis (23). The protein concentration was determined by the Bradford method (24). Next, 5 microliters of the sample were mixed with 50 microliters of lysis buffer, 20 microliters of distilled water, and 30 microliters of 15% H_2O_2 . The mixture was shaken thoroughly and incubated at 37°C for 2 minutes. Then, 100 microliters of potassium dichromate solution (0.1 M in glacial acetic acid) were added to each sample, and the mixture was heated in boiling water for 5–10 minutes. The green color indicated enzyme activity. Next, 100 microliters of the sample were transferred to a 96-well plate, and the absorbance of each well was measured at 570 nm using a plate reader (Biotek, USA) (24, 25). The level of catalase activity in EJ138 cells was calculated using the following equation:

$$\text{Catalase} = (\text{OD} \times 36.49) / (\text{mg of sample}).$$

2.9. Statistical Analysis

The statistical analysis was performed by repeating each of the tests three times and reporting the results as mean $\pm$ SD. The obtained data were analyzed using GraphPad Prism 8 software (La Jolla, CA, USA). EJ138 cells were treated with different concentrations of raw plant extract, and the results were examined using one-way analysis of variance (ANOVA) and Tukey's test, with the control group serving as the reference. A significance level of $P < 0.05$ was considered.

3. Results

3.1. The outcomes of the analysis concerning the deleterious impact of crude botanical extract on the growth of EJ138 cells, utilizing the MTT assay, are as follows

The present study investigated the cytotoxic effects of crude extract on bladder cancer cells (EJ138) using the MTT assay. The crude extract was tested at various concentrations (0.25, 0.5, and 1 microgram per milliliter) under in vitro conditions. The survival percentage of EJ138 cells at each concentration was determined, and the results showed that the crude extract significantly inhibited cancer cells at all concentrations compared to the control group. Specifically, the survival percentages of EJ138 cells at 0.25, 0.5, and 1 microgram per milliliter of crude extract were 64.3%, 51.1%, and 39.9%, respectively. Although no significant difference was observed among the different concentrations, the data imply that the raw venom is capable of inducing cytotoxicity in EJ138 cells. Figure 1 illustrates the inhibitory effects of crude extract on EJ138 cells.

3.2. The results of the inhibitory effects of raw plant extract on EJ138 cells using the Neutral Red Uptake assay are as follows:

The present study evaluated the cell inhibition percentage induced by raw *Adenium obesum* plant extract at various concentrations (0.25, 0.5, and 1 microgram per milliliter) on EJ138 cells using the neutralred uptake assay. The data indicated that the cell inhibition percentage at these concentrations was 28.5%, 40.6%, and 49.7%, respectively. The cell inhibition percentage was significant at all concentrations compared to the control group, which containing only cells and medium. However, there was no significant difference observed among the different concentrations (Figure 2). These findings corroborate the MTT assay results, implying that raw *Adenium obesum* plant extract can induce cytotoxicity in EJ138 cells. Figure 2 illustrates the impact of the plant extract on EJ138 cell inhibition.

3.3. The results of the investigation on the level of nitric oxide released (NO) into the medium of EJ138 cells after treatment with the crude Adenium obesum plant extract are as follows:

Nitric oxide (NO) generation in EJ138 cells was shown to be proportionally reduced by the unprocessed plant extract, as shown by a typical NO concentration curve. NO levels were 7.13, 6.04, and 5.72 micromolar per milliliter, respectively, at doses of 0.25, 0.5, and 1 microgram per milliliter of the extract. At 0.5 and 1 micrograms of extract per milliliter, but not at 0.25 micrograms per milliliter, significant decreases in NO were noted. The extract's concentration appears to have an impact on how much NO is produced by EJ138 cells. In Figure 3

3.4. The results of measuring the level of reduced glutathione in EJ138 cells treated with raw plant extract are as follows:

The present study investigated the impact of raw plant extract on the level of reduced glutathione in EJ138 cells. The results showed a concentration-dependent increase in the level of glutathione compared to the control group. At concentrations of 0.25, 0.5, and 1 microgram per milliliter of the unprocessed plant extract, the glutathione levels were measured at 0.53, 0.64, and 0.80 micrograms of GSH per milligram of protein ($\mu\text{gGSH}/\text{mg protein}$), respectively.

These findings suggest that the raw plant extract can significantly increase the production level of glutathione in EJ138 cells at all tested concentrations, as demonstrated by Figure 4.

3.5. The outcomes of assessing the catalytic activity of the enzyme catalase in EJ138 cells exposed to unprocessed botanical extract are as follows:

The present study investigated the effect of raw plant extract on catalase activity in EJ138 cells. The results indicated a concentration-dependent increase in catalase activity compared to the control group. Specifically, at 0.25, 0.5, and 1 microgram per milliliter of the extract, the catalase level was 1.87, 2.98, and 3.15 micromoles of hydrogen peroxide decomposed per minute per milligram of protein, respectively.

The increase in catalase activity was statistically significant at 0.5 and 1 microgram per milliliter of the extract, but not at 0.25 microgram per milliliter. These findings imply that the extract has the potential to increase catalase activity in EJ138 cells, depending on the concentration.

3.6. The results of the Alkaline Comet assay to investigate the cytotoxic effect of raw plant extract on the growth of EJ138 cells are as follows:

In this study, an alkaline comet assay was used to investigate the DNA damage induced by different concentrations of raw plant extract (0.25, 0.5, and 1 microgram per milliliter) on EJ138 cells. The results showed that the percentage of apoptosis by raw plant extract at concentrations of 0.25, 0.5, and 1 microgram per milliliter was 45%, 51%, and 58%, respectively. Furthermore, the results demonstrated that the raw plant extract induced apoptosis in the cells depicted in figure7 and resulted in cancer cell death in the above concentrations (Figure 6).

4. Discussion

The present study investigated the cellular toxicity of *Adenium obesum* plant extract on EJ138 bladder cancer cells under in vitro conditions. The cytotoxic effect of *Adenium obesum* on EJ138 cell viability was determined by MTT, NRU, and NO assays. The oxidative stress induction by *Adenium obesum* in EJ138 cells was measured by GSH and catalase tests. The induction of apoptosis by *Adenium obesum* in EJ138 cells was detected by the COMET assay. The biochemical analysis of *Adenium obesum* led to the isolation and identification of 53 bioactive compounds, some of which exhibit toxic properties. The biochemical analysis of *Adenium obesum* led to the isolation and identification of 53 bioactive compounds, some of which exhibit toxic properties. The plant contains various chemical compounds such as carbohydrates, cardiac glycosides, flavonoids, prenylated flavonoids, terpenoids, and more (9). Previous studies have demonstrated that *Adenium obesum* possesses antimicrobial, antioxidant, anticancer, antiviral, and immunomodulatory activities (9–11). Other studies have revealed its anti-malarial and anti-trypanosomal properties (12). The biochemical properties of *Adenium obesum* may be utilized as a novel drug for cancer treatment and prevention.

The MTT test, a cellular toxicity indicator, was used in the study to assess the impact of *Adenium obesum* plant extract on malignant cell survival, and the neutral red uptake assay was used to confirm the findings (26). Following treatment with *Adenium obesum* extract, the EJ138 cell viability was shown to decline in a concentration-dependent manner according to the MTT and neutral red tests. Cells of bladder cancer were destroyed by the extract, which was extremely poisonous. These experiments showed that *Adenium obesum* extracts at different doses (0.25, 0.5, and 1 microgram per milliliter) significantly decreased the survival rate of EJ138 cells. The results align with the findings of Al-Mudhaffer et al., who observed a decrease in cell viability linked to the concentration of *Adenium obesum* extract when utilizing the MTT assay on MCF-7, HFB4, HEPG2, and HEK293 cell lines indicating a potential cytotoxic effect of the extract (27). However, differences in concentrations and results compared to our study may be attributed to factors such as the composition of the plant, extraction methods, and the

specific cell lines used. The findings from the research conducted by Qasim Ali et al., examining the cytotoxic impact of the ethanol extract from various components of *Adenium obesum* on the MCF-7 cell line, align with our results, demonstrating a reduction in cancer cell viability dependent on concentration (28). Nevertheless, the concentrations employed in their study differed from ours, which could be due to factors akin to those mentioned in the preceding paragraph.

Based on the results of a study by Al-Shahri, which parallels our own, the cytotoxic impact of the ethanol extract from *Adenium obesum* leaves on the A549 lung cancer cell line was assessed. The findings indicated a decrease in cell survival rate with escalating concentration, aligning with our own results (29). Nonetheless, disparities in the concentrations utilized in Al-Shahri's study compared to ours have been previously noted.

The results of the study conducted by Abuelfarh et al., who investigated the cytotoxic effects of silver nanoparticles synthesized from *Adenium obesum* leaf extract on the MCF-7 cell line, are also consistent with our findings. The survival rate of cancer cells decreased in a concentration-dependent manner (30). However, the concentrations and IC50 values used in their study were different from ours, which may be due to the use of nanoparticles, the method of extract preparation, and the cell line used in the experiment.

Nitric oxide regulates molecular signaling and physiological and pathophysiological processes such as vascular functions, neural functions, and cytotoxic functions at high concentrations (31). Nitric oxide can induce mitochondrial apoptosis, leading to chromatin condensation, DNA fragmentation, and caspase activation (32). NO regulates apoptosis in various cells, but its effects depend on the NO amount. It has both inhibitory and inducible effects on apoptosis. Nitric oxide inhibits apoptosis in some cells, such as leukocytes, hepatocytes, trophoblasts, and endothelial cells (33). In the present study, nitrite, the main product of NO metabolism, was determined in EJ138 cells after treatment with *Adenium obesum* extract. The findings revealed that the extract led to a reduction in nitrite production, which was notably lower than the control. However, we noted a substantial decrease in NO levels in cells in a concentration-dependent fashion. Thus, our study exhibited a noteworthy decline in NO levels in cells in a concentration-dependent pattern.

In this study, methods such as GSH assay and catalase measurement were used to investigate the ability of *Adenium obesum* crude extract to induce oxidative stress in MCF-7 cells (22). Glutathione (GSH) participates in various cellular processes, such as differentiation, proliferation, and apoptosis. Its disruption is associated with numerous human conditions, including cancer. GSH deficiency, or a low ratio of glutathione disulfide (GSSG)/GSH contributes to cancer progression via oxidative stress, whereas elevated GSH levels enhance antioxidant capacity. GSH in cancer cells modulates carcinogenic mechanisms, responsiveness to cytotoxic agents, radiation, and cytokines, DNA synthesis and replication, and cell death. The main function of GSH is to detoxify antibiotics and some intracellular compounds (34, 35).

In the present study, EJ138 cells exhibited a significant increase in GSH levels at higher concentrations following treatment with *Adenium obesum* extract. To the best of our knowledge, this is the first report of GSH levels in EJ138 cells treated with *Adenium obesum* extract. Gibelli et al. reported that modulation of GSH levels did not affect the apoptosis rate. Therefore, it can be inferred that GSH depletion in apoptosis is not solely due to oxidative stress and that GSH reduction is not sufficient to induce apoptosis (36), which is consistent with our results.

Catalase converts hydrogen peroxide into water and oxygen (37). Catalase, a hemoprotein, protects cells from hydrogen peroxide damage. Nitric oxide (NO) inhibits hydrogen peroxide consumption, and catalase and hydrogen peroxide consume NO, leading to cellular imbalance (38). The findings of this research indicate that the crude extract of the *Adenium obesum* plant considerably enhances the catalase enzyme production in EJ138 cancer cells. This outcome aligns with the results obtained from the original investigations examining nitric oxide and glutathione levels, which are herein reported for the first time.

The impact of *Adenium obesum* crude extract on the induction of apoptosis in EJ138 cells was investigated using the comet assay. Apoptosis is started by caspases, which are particular cysteine proteases. Caspase-3 activation is a crucial stage in apoptosis and is important for cellular functions such as DNA fragmentation, chromatin compaction, cell bursting, and shrinkage (39, 40). According to the study's findings, *Adenium obesum* crude extract causes EJ138 cells to undergo apoptosis in a concentration-dependent manner. This is in contrast to Ghasem Ali et al.'s findings, which showed that in MCF-7 cells, apoptosis increased after 12 hours but decreased after 24 hours in a concentration-dependent manner (28). Our findings, however, are consistent with those of Abolfarh et al., who assessed the cytotoxic impact of silver nanoparticles made from *Adenium obesum* leaf extract on MCF-7 cells and discovered a concentration-dependent rise in apoptosis (30). The use of nanoparticles, variations in the processing of the crude extract, and the cell line employed are only a few examples of the elements that might be responsible for the study's varying amounts. In his study, Mr. Alshahri examined the cytotoxic effects of *Adenium obesum* ethanol leaf extract on A549 lung cancer cells. His results showed a concentration-dependent increase in apoptosis, which is consistent with our findings (29).

5. Conclusion

The results of our study illustrate that the extract from the green leaves of *A. obesum* can trigger apoptosis in EJ138 cells by elevating nitric oxide production. These findings imply that *A. obesum* may hold promise as a valuable reservoir of potent molecules with anticancer attributes. Continued investigation and identification of the specific compounds accountable for this effect could pave the way for the creation of innovative anticancer treatments.

Declarations

Authorship contribution statement

Seyed Mahmoud Moulai: Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – original draft. **Jamil Zargan:** Conceptualization, Data curation, Project administration, Resources, Supervision, Validation, Writing – review & editing **Ashkan Hajinourmohammadi:** Data curation, Visualization, Writing – review & editing. **Mohammad Sadegh Odeh Zadeh:** Conceptualization, Data curation, Writing – review & editing

Declaration of competing interest

The authors declare that there is no conflict of interest.

Data availability

The datasets produced and/or analyzed during the study can be obtained from the corresponding author upon reasonable request.

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Conflict of Interest

No conflict of interest associated with this work.

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Figures

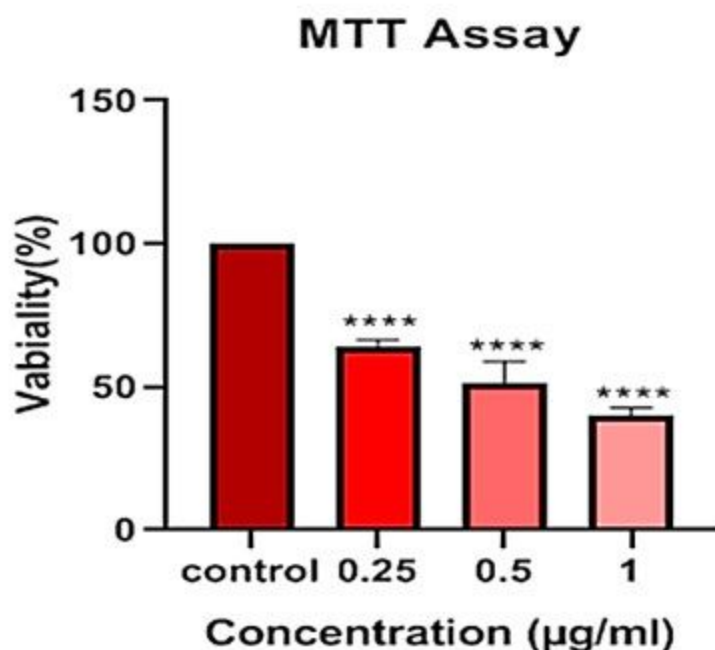


Figure 1

The percentage of survival of EJ138 cells after treatment with different concentrations of raw *Adenium obesum* extract. The concentrations were evaluated relative to the control group.

(****:p<0/0001)(ns:p>0/05)(*:p<0/05)(**:p<0/01)(***:p<0/001).

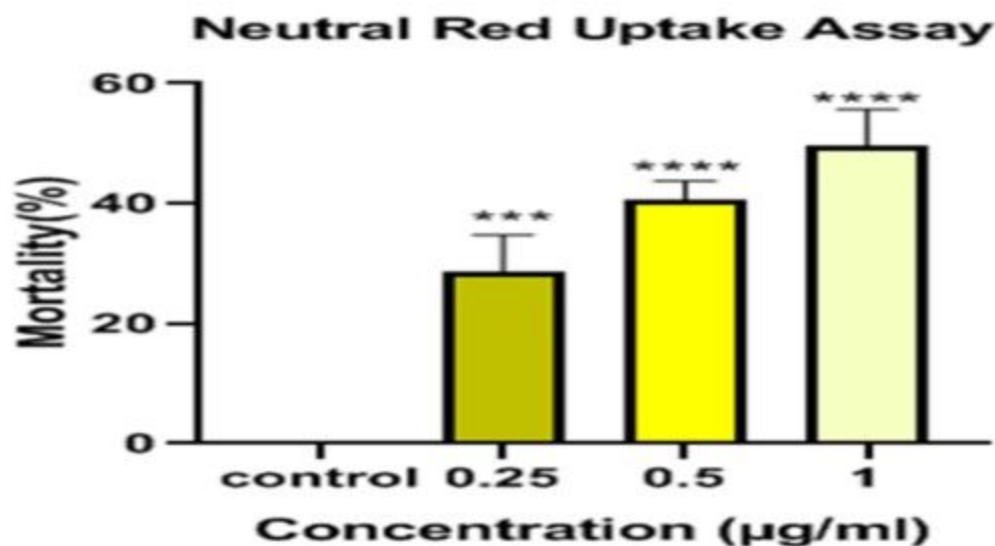


Figure 2

The percentage of cell viability of EJ138 cells, post-treatment with varying concentrations of crude *Adenium obesum* extract, relative to the control group.

(****:p<0/0001)(ns:p>0/05)(*:p<0/05)(**p<0/01)(****:p<0/001).

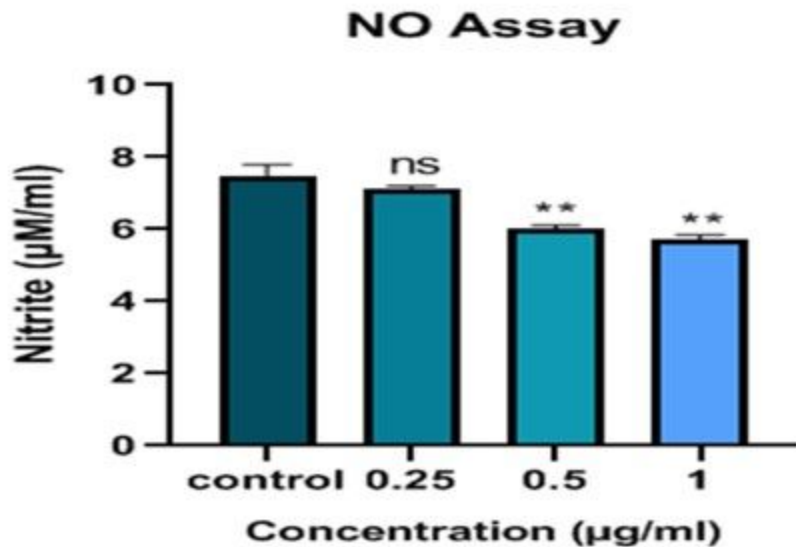


Figure 3

The level of nitric oxide produced by raw *Adenium obesum* extract in EJ138 cells. The concentrations were evaluated relative to the control group.

(****:p<0/0001)(ns:p>0/05)(*:p<0/05)(**p<0/01)(****:p<0/001).

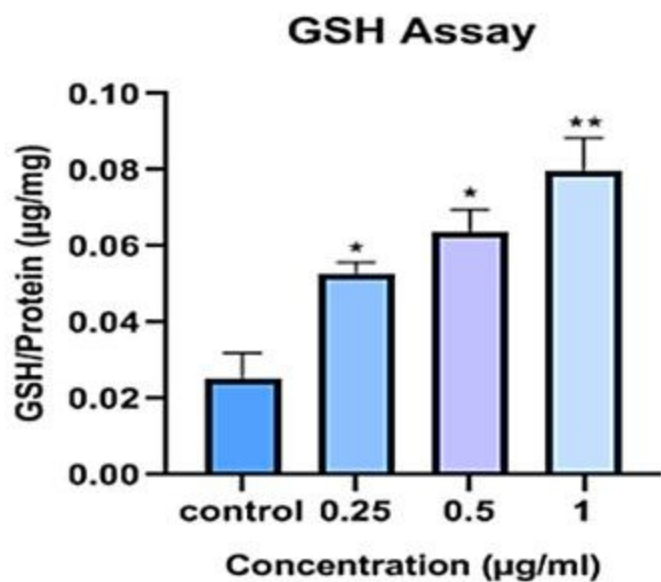


Figure 4

The level of glutathione produced by raw *Adenium obesum* extract in EJ138 cells. The concentrations were evaluated relative to the control group.

(****:p<0/0001)(ns:p>0/05)(*:p<0/05)(**p<0/01)(***:p<0/001).

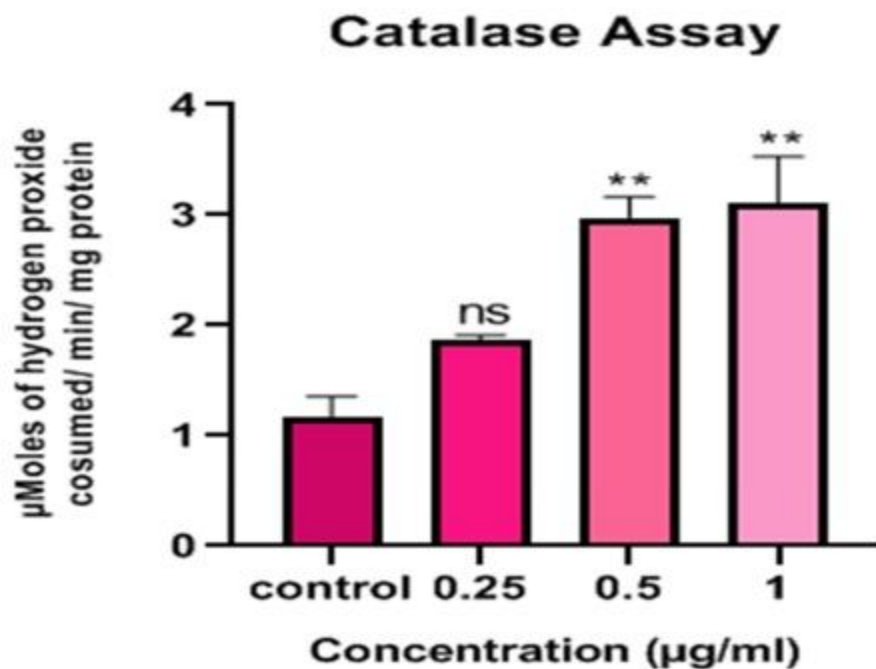


Figure 5

The level of catalase produced by raw *Adenium obesum* extract in EJ138 cells. The concentrations were evaluated relative to the control group.

(****:p<0/0001)(ns:p>0/05)(*:p<0/05)(**:p<0/01)(***:p<0/001)

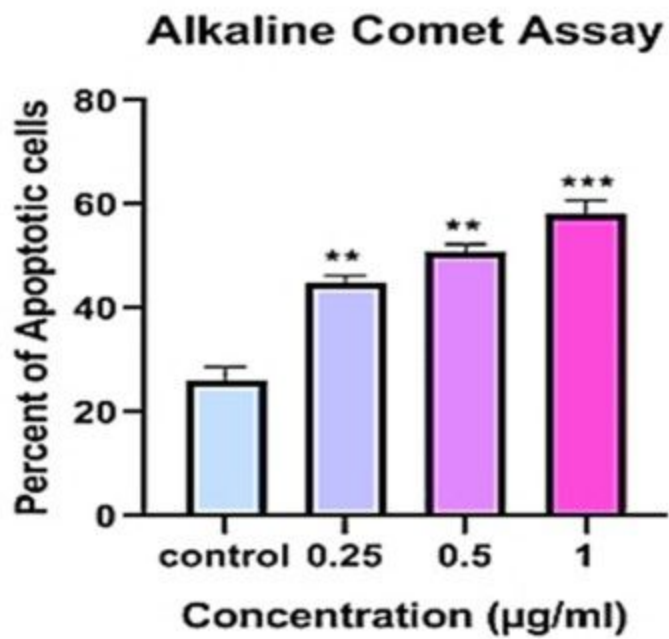


Figure 6

The level of apoptosis induced by the raw extract of *Adenium obesum* was evaluated in EJ138 cells compared to the control group

(****:p<0/0001)(ns:p>0/05)(*:p<0/05)(**:p<0/01)(***:p<0/001)

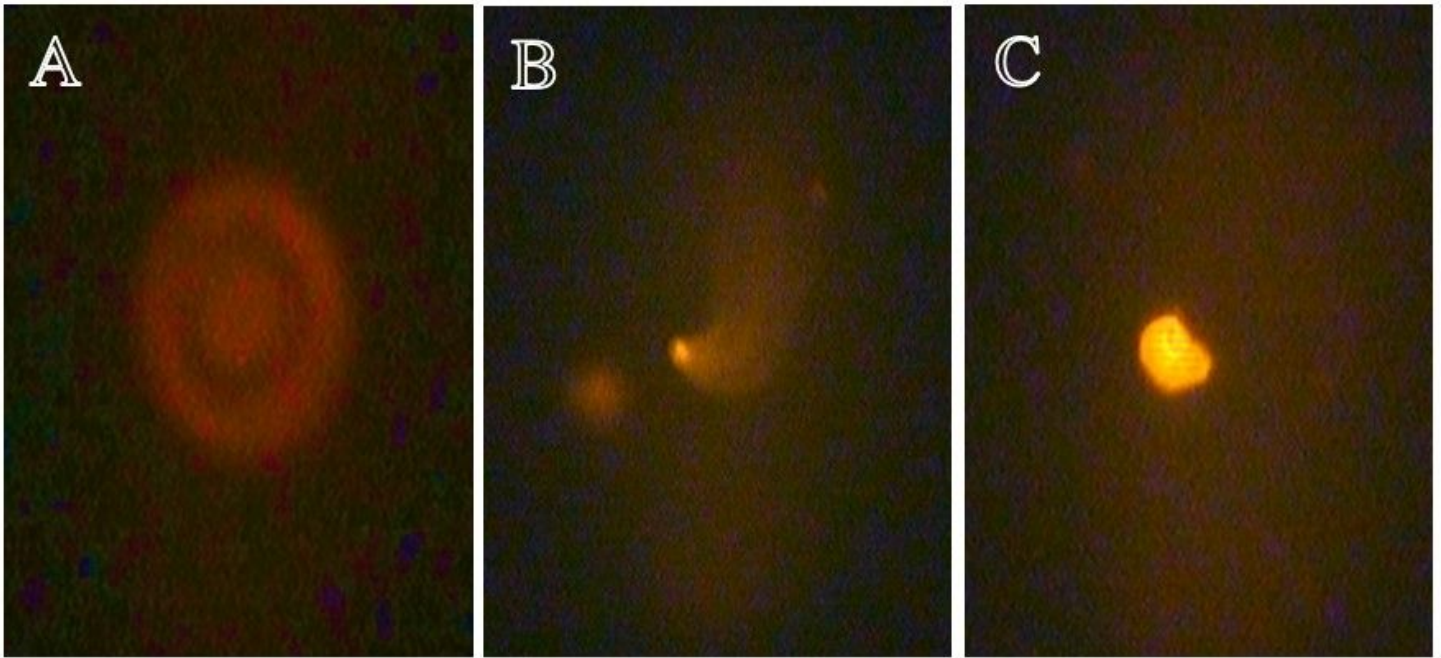


Figure 7

Representative images of DNA damage in EJ138 cells as detected by Comet assay. A) Image of intact cells with no DNA fragmentation. B) Image of apoptotic cells with comet-like tails of fragmented DNA. C) Image of necrotic cells with large amounts of DNA leakage