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Original Reserach Article

Evaluation of the Anticancer Activity of the Methanolic Extract of *Cordia myxa* in Prostate Cancer Cells

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Abstract: ***Background*:** Prostate cancer (PCa) is the second most prevalent cancer among men globally. Medicinal plants have emerged as a promising source of anticancer compounds. ***Aim*:** The aim of the present study was to evaluate the methanol extract from the Iraqi plant *Cordia myxa* as an anti-prostate cancer cell agent. ***Methodology*:** Fresh *Cordia myxa* leaves were collected and a methanolic extract was prepared. Phytochemical screening was performed on the extract. The MTT assay and calculation of IC_{50} values were used to assess the cytotoxic effects of the *Cordia myxa* methanolic extract on human prostate cancer cell lines **(**PC3). Acridine Orange staining**,** DNA fragmentation**,** and Annexin V-FITC/PI flow cytometry assays were also employed to identify the induction of apoptosis. ***Results*:** Phytochemical screening of the extract revealed the presence of flavonoids, alkaloids, tannins, saponins, terpenoids and phenolic compounds. The *Cordia myxa* methanolic extract exhibited dose- and time-dependent cytotoxicity against PC3 cells, with increased exposure time leading to enhanced cytotoxic effects. The findings also demonstrated that the treated prostate cancer cells underwent apoptosis. Flow cytometry confirmed that *Cordia myxa* extract induced significant apoptosis (48.2%) in human PC3 cells. ***Conclusion*:** The methanolic extract of *Cordia myxa* was observed to exert potent anticancer activity against prostate cancer cells, primarily through the induction of apoptosis. These findings represent a crucial step towards understanding the therapeutic potential of *Cordia myxa* as a natural treatment for prostate cancer, thereby paving the way for the development of novel, more effective, and less toxic therapies.

Keywords: *Cordia Myxa*, Prostate Cancer, MTT Test, Medicinal Plants.

INTRODUCTION

The second most frequently diagnosed cancer in men and the second most common cause of cancer deaths globally is prostate cancer (PCa) [1]. Over the past years, epidemiological data have demonstrated and continue to demonstrate a spectacular increase in PCa incidence particularly in developing nations. The most common reasons behind this growth are the global ageing population, and the growing advancement in diagnostic techniques and the change of life styles and diet [2]. Prostate cancer-related mortality rates are likely to continue increasing in the next four decades (up to 2040) and become a challenge to health care systems worldwide. This necessitates more investment into multi-component diagnostic, therapeutic and palliative care interventions and the need to develop better and less toxic forms of treatment [3]. Although treatment options are now available, including surgery, radiotherapy and chemotherapy, these procedures often have considerable side effects, and have a profound impact on the quality of life of the patient. Side effects can be urinary incontinence, erectile dysfunction, and cardiovascular issues. Besides this, drug resistance is another big problem; as time drags on cancerous cells end up becoming resistant to the action of conventional treatments leading to the recurrence and advancement of the disease [4]. The critical need of the present is to identify effective new therapies that show high activity and low toxicity. In the context of this, natural products from the sea have received great attention as a source of compounds with unparalleled chemical diversity and a wide range of biological activity that make them excellent sources for drug discovery in the field of cancer

[5]. Further, the use of nanotechnology, as in the case of nanocomposite fibres, provides a novel approach towards targeted drug delivery. The purpose of this method is to achieve maximum therapeutic activity with minimum toxicity to the rest of the body by targeting the active compounds directly to the cancerous cells [6].

Medicinal plants are a rich source of anticancer compounds and their multi-targeted mechanism of action against cancer has been attracting significant interest. These include promoting programmed cell death (apoptosis), reducing the proliferation of cancer cells, blocking the formation of new blood vessels (angiogenesis) to supply the tumor and altering the immune response in the body to fight against malignant cells. For example, some studies have shown that the use of metal oxide nanoparticles can greatly increase the anticancer properties of different plant extracts and both materials may be used together, as synergistic drugs [7]. Furthermore, plant-derived immunomodulatory phytochemicals have the potential to support the immune system's defense against cancer cells, providing a comprehensive and integrated approach to treatment [8].

Cordia myxa L., a deciduous tree of the Boraginaceae family, is widely known and used in traditional medicine in various parts of the world, especially in the Middle East and Indian subcontinent, attributed to its established anti-inflammatory and analgesic effects [9]. It has been used for centuries, for various ailments, by providing a therapeutic value that has not been lost over time. Its leaves and fruits have been found to contain a wide array of bioactive compounds, with detailed metabolic profiling using advanced techniques like Ultra-Performance Liquid Chromatography-Electrospray Ionization/Mass Spectrometry (UPLC-ESI/MS-MS). These encompass different phenolic acids and flavonoids [10] which are well-known for their strong antioxidant activities as well as their inhibitory effects towards enzymes involved in disease progress, especially relevant in cancer treatment [11]. In addition, preliminary pharmacological studies have confirmed the therapeutic potential of the extracts of *Cordia myxa* as alternative drugs that are cost-effective and efficacious, thus opening up new research lines for their development and use in phytotherapy [12]. The Cordia genus has been the subject of thorough studies that have identified a wide range of secondary metabolites, including terpenoids and alkaloids that collectively affect the Cordia genus in a wide range of ways [13]. These compounds are important in plant defense and have shown to possess considerable pharmacological properties, one of which is considerable anticancer properties. Similar studies have been carried out on related species, *Cordia dichotoma*, which show a significant cytotoxic activity against various human cancer cell lines. Extensive phytochemical analysis employing state-of-the-art techniques, such as High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GCMS), has been carried out to meticulously identify the active compounds responsible for these effects [14]. In particular, the methanolic extract of *Cordia dichotoma* leaves was found to induce apoptosis and reduce oxidative stress of human prostate carcinoma cell lines (PC3) indicating the involvement of multiple cellular signaling pathways.

Moreover, recent computational studies, such as network pharmacology analysis, molecular docking, and molecular dynamics simulation, have helped to understand the multi-target effects of *Cordia myxa* in liver Cancer. Additionally, the extract has shown dose dependent antitumor activity in breast cancer cell lines, which further increased its possibility of being a broad spectrum anticancer agent [15]. Therefore, the present study aims to evaluate of methanol extract of *Cordia myxa* in prostate cancer cells. The purpose of this investigation is to try to solve the pressing need of effective phytotherapeutic interventions in the fight against this widespread disease. On the whole, this research is a useful addition to the knowledge of how much *Cordia myxa* can be used as a natural therapeutic agent to treat prostate cancer, and it will possibly result in the creation of new and even more efficient therapies in the future.

MATERIALS AND METHODS

Collecting and Identifying Plant Materials

Fresh leaves of *Cordia myxa* L. were carefully harvested in the months of March and April 2024 in the area of Abu Al-Khaseeb located in Basrah Governorate, Iraq (30°26′N 47°50′E). To guarantee proper documentation of collection site, geographical coordinates were recorded using Global Positioning System (GPS). The plant material was collected and authenticated by a qualified botanist from the Department of Biology, College of Science at University of Basrah.

Preparation of Methanolic Extract

The freshly collected leaves were washed thoroughly under running tap water to remove dust and other debris and then washed with distilled water. Then, the leaves were washed by distilled water, air-dried at room temperature (25±2 °C) in a shaded well-ventilated area for two weeks until they achieve constant weight. The dried leaves were then finely powdered using a mechanical grinding machine. About 200 g of the powdered plant material was macerated for 72 hours in 1000 mL of 80% methanol (analytical grade, Sigma-Aldrich, Germany) at room temperature with an occasional shaking. Then the mixture was filtered with Whatman No.1 filter paper. The filtrate was concentrated using a rotary evaporator (Büchi Rotavapor R-210, Switzerland) under reduced pressure at 40 °C to get crude methanolic extract. The extract obtained is dark green and viscous and then lyophilized to obtain the dry powder and stored at -20 °C until use [16].

Phytochemical Screening

The preliminary phytochemical screening was done on methanolic extract of *Cordia myxa* L. to

determine the presence of major secondary metabolites such as flavonoids, alkaloids, tannins, saponins, terpenoids and phenolic compounds. This was done using standard qualitative tests according to standard procedures [17].

Cell Culture and Maintenance

RPMI-1640 medium (Gibco, USA) was used for the cells which contained 10% fetal bovine serum (FBS, Gibco, USA), 100 U/mL penicillin and 100 µg/mL streptomycin (Sigma-Aldrich, Germany). Cells were cultured at 37 °C under 5% CO_2 atmosphere. Cells were seeded at plating densities suitable for each of the assays and left to adhere overnight for experiments [18].

In Vitro Study of Anticancer Activity Assay

The effect of *Cordia myxa* L. methanolic extract on prostate cancer cell lines was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The cells plated in a 96 well plate were exposed to different concentrations of the extract for 24, 48 and 72 hours. Specifically, cells were treated with various concentrations of the extract ranging from (0-500) µg/mL. After the treatment MTT solution was added to each well and allowed to incubated stay for 4 hours. The formazan crystals that were formed were then dissolved in dimethyl sulfoxide (DMSO), and the absorbance was determined at 570 nm with the use of a microplate reader (Bio-Rad, USA). The percentage cell viability was determined based on that of untreated control. Appropriate software was used to calculate the half-maximal inhibitory concentration (IC_{50}) value [19].

Acridine Orange Staining for Apoptosis Detection

To qualitatively analyze morphologic changes typical of apoptosis in prostate cancer cells, Acridine Orange/Ethidium Bromide (AO/EB) dual staining was employed. Cells were seeded in 6-well plates at a density of 5×10^4 cells/well and left to adhere overnight. After treating the cells with the extract at the half-maximal inhibitory concentration (IC_{50}) for 24, 48, and 72 hours, cells were harvested by trypsinization and washed twice with phosphate-buffered saline (PBS). A cell suspension was prepared at a density of 1×10^5 cells/mL. A 100 µL aliquot of the cell suspension was combined with 10 µL of staining solution (PBS containing 100 µg/mL each of acridine orange and ethidium bromide) and mixed. The mixture was incubated in the dark at room temperature for 5 minutes. The stained cells were then examined immediately under a fluorescent microscope (Olympus BX51, Japan) equipped with a digital camera. Cells were categorized based on their fluorescence and nuclear morphology: viable cells showed uniform green fluorescence with intact nuclei; early apoptotic cells exhibited bright green fluorescence with evident chromatin condensation or nuclear fragmentation; late apoptotic cells displayed bright orange-red fluorescence with condensed or fragmented nuclei due to the uptake of ethidium bromide; and necrotic cells showed uniform orange-red fluorescence with damaged membrane integrity and non-fragmented nuclei [20].

DNA Fragmentation Assay

DNA fragmentation is detected by using agarose gel electrophoresis (AGGE), which is also a characteristic of Apoptosis. *Cordia myxa* L. methanolic extract was seeded in 100 mm culture dishes at the half-maximal inhibitory concentration (IC_{50}) for 48 h and with prostate cancer cells. Cells were then collected, washed twice in cold PBS and lysed with a DNA lysis buffer (10 mM Tris-HCl pH 8.0; 10 mM EDTA; 0.5% Triton X-100). The cell lysate was then subjected to centrifugation at 13,000 rpm for 15 minutes at 4 °C, to separate out the intact DNA from the fragmented DNA. The supernatant fraction after removal of the fragmented DNA was carefully collected and DNA was precipitated by adding an equal volume of isopropanol and 0.1 volume of 3 M sodium acetate (pH 5.2) and placed on -20 °C for 1 hour. Following centrifugation at 13,000 rpm for 15 min at 4 °C, the DNA pellet was washed with 70% ethanol, air dried and dissolved in 20 µL TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA) with 100 µg/mL RNase A. The DNA samples were then incubated at 37 °C for 30 minutes. DNA samples were then loaded with 6X DNA loading dye and run on a 1.5% agarose gel with 0.5 µg/mL ethidium bromide. Electrophoresis was carried out at 80 V for 90 minutes. DNA ladders were then seen, and pictures were taken, under UV transillumination by a gel documentation system (Bio-Rad, USA). A characteristic ladder pattern was observed showing fragmentation of DNA and thus confirming the induction of apoptosis [21].

Annexin V-FITC/PI Flow Cytometry Assay

Annexin V-FITC/ Propidium Iodide (PI) Apoptosis Detection Kit (Sigma-Aldrich, Germany) was used to calculate the percentage of apoptotic cells based on the instructions of the manufacturer. In short PC3 cells were placed into 6-well plates, and the *Cordia myxa* extract was added at the IC_{50} level to the cells over a period of 48 hours. Cells were treated and then harvested, washed with cold PBS twice and resuspended in 1X Binding Buffer. Subsequently 5 µL Annexin V-FITC and 5 µL PI were added to the cell suspension and the mixture was incubated at room temperature in the dark after 15 minutes. A flow cytometer (BD FACSCalibur, USA) was used to analyze the samples. Each sample was recorded at least 10,000 events. FlowJo software was used to analyze the data to distinguish viable (Annexin V-/PI-), early apoptotic (Annexin V+/PI-), late apoptotic (Annexin V+/PI+) and necrotic (Annexin V-/PI+) cells [22].

Statistical Analysis

Each experiment was repeated three times and results presented as mean ± standard deviation (SD). The data were analyzed statistically using graph pad prism software (version 9.0, GraphPad Software Inc., USA). One-way analysis of variance (ANOVA) followed by

Tukey's post-hoc test was used to determine significant differences between groups. Statistically significance was set at $p < 0.05$.

RESULTS

Phytochemical Screening of *Cordia myxa* L. Methanolic Extract

Preliminary phytochemical screening of *Cordia myxa* L. methanol extract showed the presence of various bioactive secondary metabolites. Results of the qualitative analysis showed the presence of flavonoids, alkaloids, tannins, saponins, terpenoids and phenolic compounds. The findings are summarized in (Table 1) below with details given on the nature of the tests, observations and interpretations made.

Table 1: Detailed phytochemical constituents identified in the methanolic extract of *Cordia myxa* L., including specific tests, observations, and results

Phytochemical Constituent	Test Employed	Observation	Result
Flavonoids	Shinoda Test	Formation of magenta to red color	Present
	Alkaline Reagent Test	Increased yellow coloration upon NaOH addition	Present
Alkaloids	Mayer's Test	Creamy white precipitate	Present
	Dragendorff's Test	Orange-brown precipitate	Present
Tannins	Ferric Chloride Test	Bluish-black or greenish-black coloration	Present
	Gelatin Test	Formation of white precipitate	Present
Saponins	Foam Test	Persistent foam formation (1 cm for 15 min)	Present
Terpenoids	Salkowski Test	Reddish-brown ring at interface	Present
Phenolic Comp.	Ferric Chloride Test	Dark blue or black coloration	Present

Cytotoxic Effect of *Cordia myxa* L. Methanolic Extract on PC3 Cells

MTT assay was used to measure the *Cordia myxa* L. methanolic extract's cytotoxic activity against human prostate cancer (PC3). The extract was administered to the cells at different concentrations (0 – 500 µg/mL) for 24, 48 and 72 h. The results (Table 2 & Figure 1) reveal a dose- and time-dependent decrease in cell viability. The percentage of viable cells in the different extracts compared with control cells (without extract) diminished significantly as the concentration of extract increased. Likewise, a sustained exposure to the extract resulted in greater cytotoxic activity.

Table 2: Cell viability of PC3 cells treated with *Cordia myxa* L. methanolic extract at different concentrations and time points (Mean ± SD)

Concentration (µg/mL)	Cell Viability (24h) %	Cell Viability (48h) %	Cell Viability (72h) %
0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
50	88.5 ± 2.1	78.2 ± 2.4	65.4 ± 2.8
100	75.2 ± 2.5	62.1 ± 2.8	48.7 ± 3.1
200	60.4 ± 3.1	45.3 ± 3.4	32.1 ± 3.2
300	48.1 ± 2.8	32.7 ± 2.9	21.5 ± 2.5
400	39.5 ± 2.4	25.4 ± 2.1	15.2 ± 1.8
500	32.8 ± 1.9	18.9 ± 1.5	10.4 ± 1.2

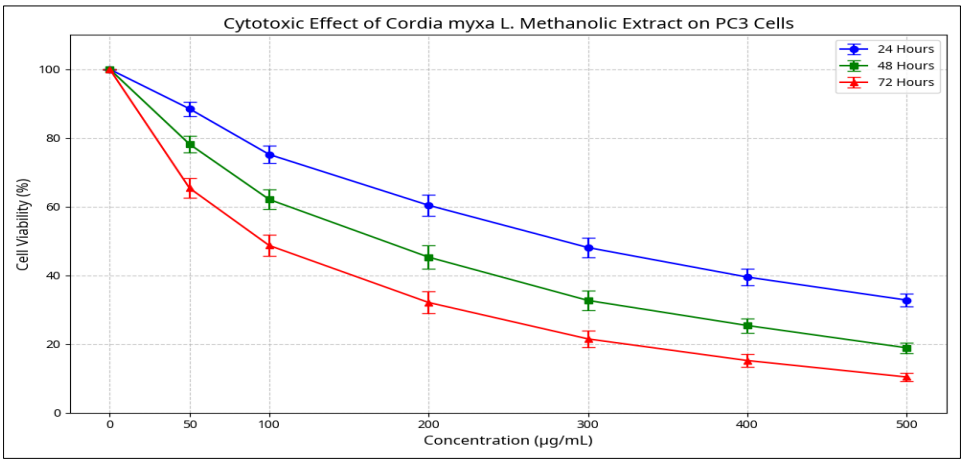

Figure 1: Dose- and time-dependent cytotoxic effect of *Cordia myxa* L. methanolic extract on PC3 cell viability as determined by MTT assay. Data are presented as mean ± SD of three independent experiments

These half-maximal inhibitory concentration (IC_{50}) values, obtained from the dose-response curves, also help to quantify the potency of the extract in its ability to cause a cytotoxic effect. The IC_{50} values were found to be decreasing with the increase in exposure time, which means that efficacy was improved with time (Table 3).

Table 3: IC_{50} values of *Cordia myxa* L. methanolic extract on PC3 cells at different time points

Time Point	IC_{50} (µg/mL)
24 hours	315.2
48 hours	210.8
72 hours	135.5

Apoptosis Induction by Acridine Orange Staining

The morphological changes as indicators of apoptosis in PC3 cells was examined by staining with Acridine Orange (AO) (Figure 2). The untreated control cells were seen to have green uniformly green nuclei with intact chromatin typical of viable cells. Cells in the same contrast treated with the extract especially at higher concentrations and longer incubation times, showed clear apoptotic characteristics. These were chromatin condensation, nuclear fragmentation and formation of apoptotic bodies which were bright orange-red fluorescent. This shift in the colour of fluorescence and nuclear morphology confirmed the induction of apoptosis of *Cordia myxa* L. extract in PC3 cells.

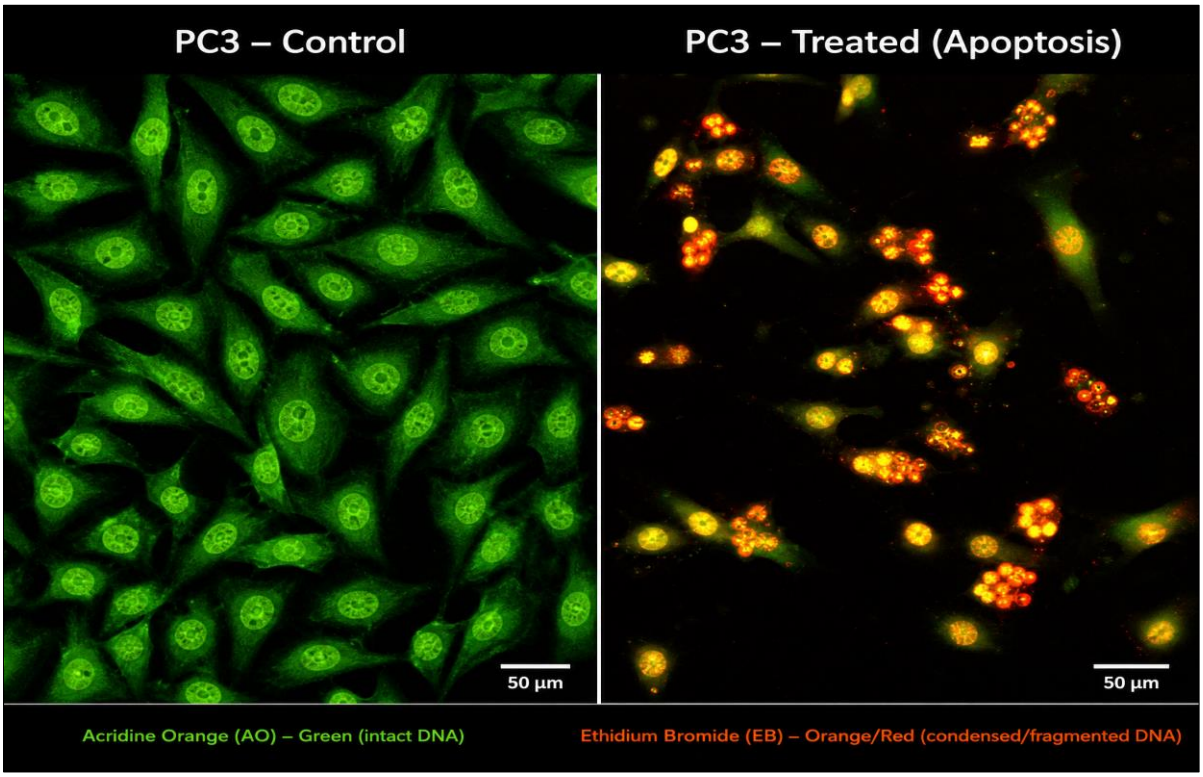

Figure 2: Representative fluorescence micrographs of PC3 cells stained with Acridine Orange/Ethidium Bromide. Left panel shows untreated control cells with intact green nuclei. Right panel shows cells treated with *Cordia myxa* L. extract, exhibiting apoptotic features such as chromatin condensation and orange-red apoptotic bodies.

DNA Fragmentation Analysis

The DNA fragmentation was also measured by agarose gel electrophoresis, which was used to further confirm the apoptotic mechanism. Assuming the presence of intact genomic DNA, DNA extracted from untreated PC3 cells showed a single band with high molecular weight. DNA of PC3 cells treated (for 48 hours) with *Cordia myxa* L. methanolic extract at the same IC_{50} concentrations however, exhibited a typical ladder pattern of DNA. This laddering directly reveals internucleosomal DNA cleavage, as it is a definite biochemical feature of DNA fragmentation during apoptosis, and shows that the extract actually causes apoptosis in prostate cancer cells by DNA fragmentation, as indicated by this biochemical characteristic (Figure 3).

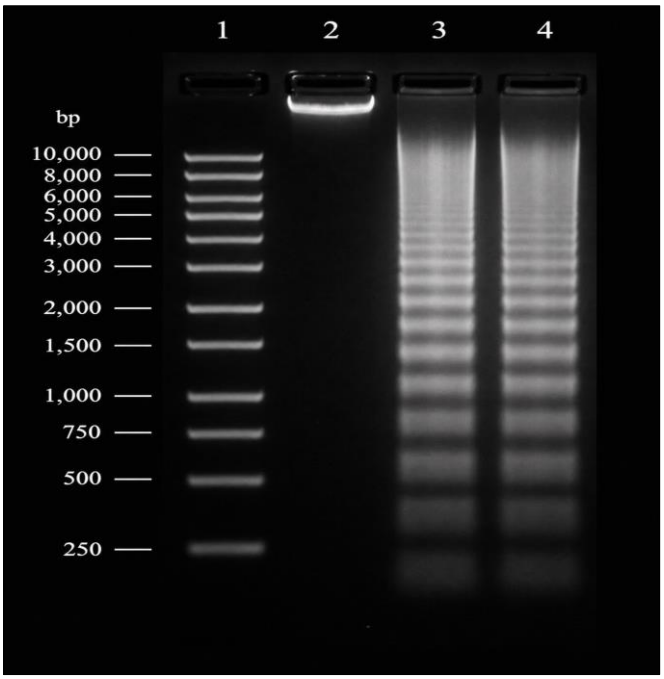

Figure 3: Agarose gel electrophoresis showing DNA fragmentation in PC3 cells. Lane 1: DNA ladder. Lane 2: Untreated control cells showing intact genomic DNA. Lanes 3 and 4: Cells treated with *Cordia myxa* L. extract, demonstrating a characteristic DNA ladder pattern indicative of apoptosis

Annexin V-FITC/PI Flow Cytometry Assay

The flow cytometry analysis using Annexin V-FITC/PI indicated that by the end of the 48-hour treatment, the PC3 cell viability was significantly lowered and that the incidence of apoptosis was also significantly increased, upon treating the PC3 cells with the methanolic extract of *Cordia myxa* in greater amounts (IC_{50}). In control group, most cells (94.2) were alive whereas this percentage significantly reduced to 48.5% in the treated group. On the other hand, the overall proportion of apoptotic cells (early and late) rose with the percentage of 4.6 in the control and 48.2 in the treated cells. In particular, early apoptotic cells (Annexin V+/PI-) constituted 26.4% and late apoptotic cells (Annexin V+/PI+) constituted 21.8%. The proportion of necrotic cells (Annexin V-/PI+) was quite low (3.3%), which means that the extract mostly induces a programmed cell death but not non-specific necrosis (Fig. 4).

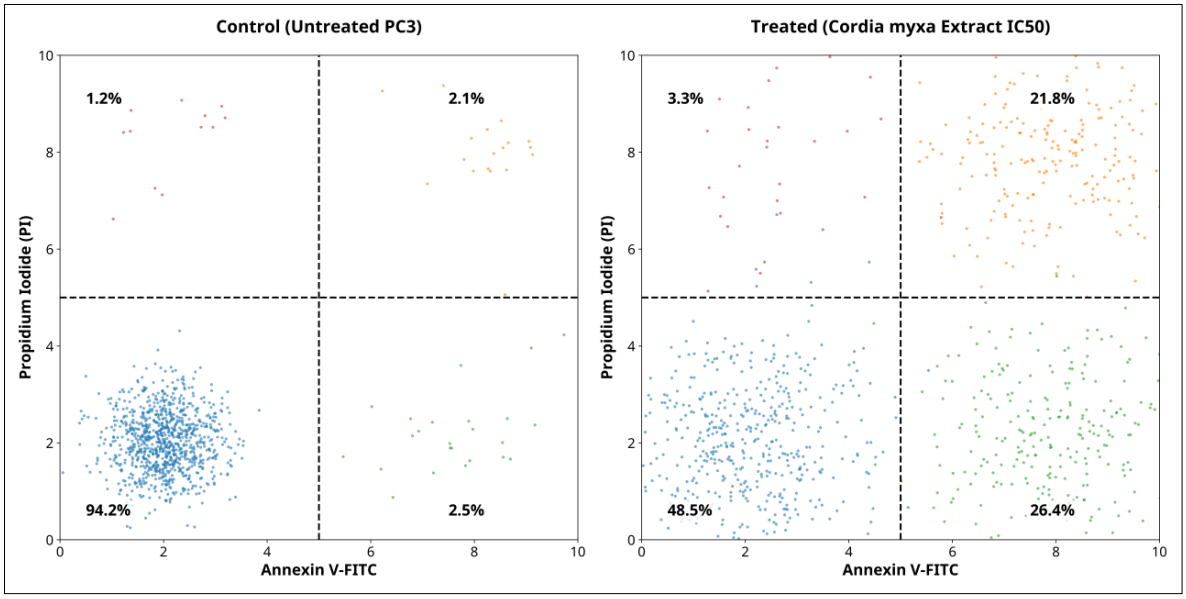

Fig. 4: The apoptosis process of PC3 cells was examined by means of the Annexin V-FITC on PI staining in the flow cytometer. (Left) Control cells, untreated; (Right) PC3 cells, which were treated with *Cordia myxa* methanolic extract at an IC_{50}, in 48 hours. The quadrants are: Viable (Lower Left), Early Apoptotic (Lower Right), Late Apoptotic (Upper Right) and Necrotic (Upper Left) cells

DISCUSSION

The present study aimed to thoroughly examine the in vitro anticancer activity of the methanolic extract of the *Cordia myxa* L. leaves of the Abu Al-Khaseeb region in the Basra Governorate of Iraq. Our data clearly shows that this extract has potent cytotoxic effects, induces programmed cell death (apoptosis) and causes DNA fragmentation in human prostate cancer cells (PC3). The findings are significant in the face of the rising incidences of prostate cancer worldwide, resulting in a large number of cancer deaths that demand identification of new, more effective and non-toxic therapeutic options [23]. The estimated global burden of prostate cancer till 2040 further emphasizes the need for new methods in the treatment of this condition, as these methods can ease the tremendous burden of prostate cancer on health care services throughout the world [24, 25].

Our initial phytochemical evaluation on the *Cordia myxa* L. methanolic extract indicated the presence of a wealth of bioactive secondary metabolites in the extract. These compounds were confirmed to be present in the extract based on the qualitative analysis, which revealed flavonoids, alkaloids, tannins, saponins, terpenoids and phenolic compounds. The complex phytochemical structure is found in reference with previous literature of Cordia genus which is widely known for its rich secondary metabolites resulting in a wide spectrum of biological activities [26]. The compounds identified are already well studied in the scientific literature for their strong pharmacological activity, including antioxidant, anti-inflammatory and in particular, anticancer activities. Marine natural products, for example, with unique chemical diversity as well, are actively being explored as the sources of anti-prostate cancer agents, thus reflecting the general scientific interest in natural compounds [27]. Such a variety of phytochemicals in our extract can be a good basis for the observed anticancer activities as many of the plant derived compounds are known to act on a number of cellular pathways associated with cancer initiation and progression [28].

The MTT assay results were promising as they showed a dose and time dependent decrease in cell viability when PC3 cells were treated with extract. Stable IC_{50} values (see Table 3) decreased slowly with increased exposure time with the half-maximal inhibitory concentration (IC_{50}) reducing by 315.2 µg/mL at 24 to 135.5 µg/mL at 72 hours. This pattern implies there might be the accumulation of cytotoxic effect with time, implying that the active compounds of the extract have a long-term anti-proliferation and anti-survival effect on PC3 cells. This could be supported as metal oxide nanoparticles are also used in the treatment of different kinds of carcinomas; and the combination of nanoparticles with various therapeutic agents usually leads to a higher effect of the specified cure and, therefore, a potential effect of synergism [29]. The results obtained in our study are similar to, or even better than, those found for other plant extracts in different cancer models, thus corroborating the potential use of *Cordia myxa* L. as a therapeutic agent [30].

In order to understand the underlying mechanism of observed cytotoxicity, we stained with Acridine Orange (AO) for its morphological changes indicative of apoptosis. In PC3-untreated cells, the nuclei of all these cells showed uninterrupted distribution of green color, which corresponds to healthy and viable cells. Conversely, cells exposed to the *Cordia myxa* L. extract showed significant characteristics of apoptosis, especially at higher concentrations and longer exposure periods. These involved strong chromatin condensation, distinct nuclear fragmentation and the development of apoptotic bodies with a characteristic bright orange-red fluorescence. The morphological changes are hallmarks of true programmed cell death, a very desirable mode of cell death in cancer therapeutic treatments, because it allows the orderly clearance of cancer cells to prevent an inflammatory response. Also the immunomodulatory phytochemicals in *Cordia myxa* extract have been seen to enhance the natural defence of the body against cancerous cells thus supporting its application in the management of cancer [29].

To further confirm the concept of the apoptotic mechanism, agarose gel electrophoresis was conducted to reveal DNA fragmentation which is a biochemical characteristic of apoptosis. Molecular weight of DNA extracted out of the untreated PC3 cells was observed to have one high molecular weight band showing position of intact genomic DNA. On the other hand, the DNA extracted from the PC3 cells treated with *Cordia myxa* L. methanolic extract (at IC_{50} for 48h) exhibited the typical ladder pattern. This multiplied form of internucleosomal DNA cleavage is a ladder made up of a few single bands, multiplication of which is done by a base pairs of approximately 180-200 base pairs. The direct evidence of DNA fragmentation means that we can say without doubt that the extract induces the cell death by apoptosis through activation of the intrinsic apoptotic pathways in prostate cancer cells. It is this mechanism that is often attacked by the conventional chemotherapeutic drugs, and the fact that it is also targeted by a natural product, especially from a plant that has been traditionally used as a medicine for its anti-inflammatory and analgesic properties is of great importance [31].

Cordia myxa L. is a multi-targeted plant, and this can be mainly explained by its complex phytochemical composition and the synergy among them. Its anticancer activity is thought to be due to the combined or additive effects of different compounds including flavonoids, alkaloids and phenolic compounds. Often more successful in targeting multiple dysregulated pathways in complex diseases such as cancer as opposed to single Compound therapies. A previous study of Cordia genus has been conducted on Cordia dichotoma

to prove its strong cytotoxic activity and through the phytochemical advanced screening the active compound was identified [32,33]. Moreover, computational studies such as network pharmacology, molecular docking, and molecular dynamic simulations have been instrumental to the understanding of the multi-targeting nature of *Cordia myxa's* active constituents in treating liver cancer, indicating that *Cordia myxa's* active constituents interact with different molecular targets, disrupting the survival and proliferation of cancer cells [34,35]. The extract has also shown dose-dependent anticancer activity in breast cancer cell lines, which confirms its broad spectrum anticancer activity [36].

These results indicate the strong pro-apoptotic effect of *Cordia myxa* extract on prostate cancer cells, which is probably explained by a high level of bioactive secondary metabolites, including flavonoids and phenolic compounds. It is known that these phytochemicals can prevent cell survival pathways and induce pro-apoptotic signaling cascades [37]. The significant shift of cells to Annexin V-positive quadrants indicates the externalization of phosphatidylserine, which is characteristic of early apoptosis and involves the remodelling of the plasma membrane [22]. Our findings are in agreement with earlier findings with the Cordia genus that yielded pronounced DNA fragmentation and caspase activation in different cancer cells, which further validates the potential of this plant as a promising source of natural chemicals that are likely to be used in the development of selective and less toxic chemotherapeutic products [38].

The present study offered a sound in vitro data concerning the anticancer activity of *Cordia myxa* L. extract, future research is required to isolate and characterize particular compounds with active effects from the extract. Additional exhaustive mechanistic studies like gene expression studies, protein profiling and cell cycle studies are suggested to obtain deeper understanding of the exact how molecular pathways altered by the extract. In addition, it is important to confirm these in vitro findings by in vivo experiments using an appropriate animal model of prostate cancer to be able to test the effectiveness and safety of the extract in a biological environment. Also, the phytochemical profile of *Cordia myxa* originating Abu Al-Khaseeb, Basrah could be specific to the area as there could be a comparison with the plant of other locations across the globe to identify differences in bioactivity.

CONCLUSION

Cordia myxa L. leaves extract from Abu Al-Khaseeb, Basrah, has exhibited a strong in vitro anticancer activity against the human prostate cancer cells (PC3). The extract's potent cytotoxic effects may be attributed to its wealth and diversity of phytochemical constituents, including flavonoids, alkaloids, tannins, saponins, terpenoids, and phenolic compounds. The overall results highlight the therapeutic value of *Cordia myxa* L. as a potential source for discovering new phytotherapeutic molecules that can be used in the treatment prostate cancer. More in-depth research is needed to isolate and identify the specific active compounds as well as to fully understand their precise molecular mechanisms of action to pave the way for possible clinical applications in cancer therapy.

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