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Modulation of TRAIL-Induced Apoptosis by *Moringa Oleifera* Seed Extract and Docetaxel in LNCaP Cells

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Abstract

Background: Cancer continues to be one of the leading causes of worldwide mortality, which has led to a quest for new therapeutic agents, including natural products from plants. *Moringa oleifera* is a medicinal plant, and TRAIL shows an affinity to apoptosis in cancer cells**. Objective**: To evaluate the individual and combined effects of an ethanolic extract of *Moringa oleifera* seeds and the chemotherapeutic agent docetaxel on the viability and TRAIL expression of the LNCaP human prostate cancer cell line**. Methodology:** Ethanolic extract was obtained from the seeds of *Moringa oleifera* by Soxhlet's apparatus. LNCaP cells were then exposed to different doses of extract, docetaxel and/or the combination. MTT assay detected cell viability after 24 and 48 h. The levels of TRAIL were measured with an ELISA kit**. Results:** The *Moringa oleifera* seed extract and docetaxel significantly induced dose- and time-dependent reductions in LNCaP cell viability ($P \leq 0.001$) along with significant increases in TRAIL levels. The IC 25 of the extract in combination with IC 50 dose of docetaxel exhibited synergistic potentiation of antiproliferative activity, accompanied by a highly remarkable enhancement in TRAIL expression ($P \leq 0.001$)**. Conclusion:** *Moringa oleifera* seeds ethanolic extract has a potent antiproliferative and pro-apoptotic activity in LNCaP cells, which is partly mediated through TRAIL up-regulation. In addition, it has a synergistic effect with docetaxel and can be used as an adjuvant therapy for prostate cancer**.** [*Bangladesh Journal of Infectious Diseases, December 2025; 12(2):222-227*]

Keywords: Moringa oleifera; Apoptosis; Docetaxel; apoptosis enhancement; LNCaP Cells**.**

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Introduction

Natural sources have historically been a rich source of therapeutic agents, supplying as a row materials to be used as a part of a formulas from which many major cancer chemotherapeutic drugs, including paclitaxel, vincristine, and vinblastine, were first identified from natural products[1]. From the wide range of plant species used in traditional medicine, one of the most remarkable has been Moringa oleifera Lam., which is native to Southeast Asia[2]. This species is known for its nutritional value,

which contains a number of vitamins, proteins, carbohydrates, fatty acids, and dietary fiber as well as several bioactive phytochemicals[3].

The Tumour Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL) starts programmed cell death, mostly in cancer cells. This is called TRAIL-induced apoptosis. many researches showed that *Moringa oleifera* seed extract improves TRAIL-induced apoptosis. This makes it a possible drug for cancer treatment. The extract contains bioactive chemicals that make cancer cells more sensitive to TRAIL. This makes them more likely to die while normal cells stay alive. This targeted intervention lessens the bad effects that are often linked to normal treatments. Moringa oleifera may also work better because it is an antioxidant and an anti-inflammatory[5]. This shows that it could be used as an extra ingredient in cancer treatments that target TRAIL pathways.

One of the best-characterized family of signaling molecules in cancer involves members of the Tumor Necrosis Factor (TNF) superfamily, which consists of at least 13 homologous proteins that regulate important aspects of biology such as apoptosis, immunity and inflammation and development. One well-known member of this family is the Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL), which has received great attention, as it can specifically trigger apoptosis in a variety of cancer cells while showing minimal or no cytotoxic effects towards normal non-transformed cells[5]. TRAIL appoptic signal is transduced by the specific membrane receptors, including Death Receptor 5 (DR5/ TRAIL-R2)[6].

Because of the high content and potential therapeutic properties of phytochemicals in *Moringa oleifera* and the intermediate role TRAIL pathway plays in apoptosis, we aimed to determine if an ethanolic extract from *Moringa oleifera* seeds has effects on LNCaP human prostate cancer cell line viability. The study was aimed to study the effects of an ethanolic extract of *Moringa oleifera* seeds and the chemotherapy drug docetaxel on the LNCaP human prostate cancer cell line's ability to live and its TRAIL expression.

Methodology

Preparation of *Moringa oleifera* Seed Extract: Seeds of *Moringa oleifera* were collected fresh by purchasing from a local herb vendor. Using a fast blender, convert raw seeds to powder for easy consumption. Stored in sealed container in dry place for future use." The extraction was carried out using the Soxhlet procedure as previously described by Lin et al[6]. Extract preparation: Two hundred grams of ground M. oleifera seed powder were macerated in 1 L of 70% ethanol. After that, the ethanol was evaporated and the solution was filtered and dried at room temperature. The resultant extract was kept at -20°C until used for experimentation.

Cell Line and Culture Conditions: The LNCaP prostate cancer cell line, which is human, was acquired from the medical college's laboratory for cancer research. Amphotericin-B was also used while the cells were cultivated in RPMI-1640 media that also included 10% FBS, 100 µg/mL of streptomycin, and 100 U/mL of penicillin. The cultures were kept at 37°C in a humidified environment with 5% CO_2.

Cell Viability Assessment: Cell viability was measured by using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, which is a common colorimeter to detect the cellular metabolic activity.

Experimental Design: This research was performed as an in vitro study in the LNCaP cell line. The design of the experiment included the following assessments:

Exploring the Impact of M. oleifera Seed Extract on LNCaP Cells Evaluation: To investigate the effect of ethanolic seed extract from M. oleifera on the viability of LNCaP cells, a study was performed. This was performed by incubating the cells with different extract concentrations (31.25–1000 µg/ml) for 24 to 48 hours and then assessing the cell viability. The results were compared to those of controls that had not been treated.

Assessment of Docetaxel Impact on LNCaP Cells: Docetaxel treatment on LNCaP cell viability was investigated in the present study. The cells were incubated with different concentrations of docetaxel (31.25-1000 µg/ml) and compared with the control group.

Combined M. oleifera Extract and Docetaxel Cytotoxicity Analysis: In this section, we followed up to determine the synergistic effect of ESEE and docetaxel in LNCaP cells. Cells were also exposed to different concentrations (31.25-1000 µg/ml) of the seed extract with an IC50 value (concentration produce fifty percent inhibition) of docetaxel as

well. The cell viability was determined at 24 and 48 h of incubation relative to the control.

Analysis of TRAIL Levels: The impact of ethanolic M. oleifera seed extract and docetaxel, either alone or in combination, was determined by measuring the TRAIL levels in LNCaP cells. Measure human TRAIL at different time points by using in vitro Quantitative ELISA (Enzyme-Linked Immunosorbent Assay) Kit (EIAab, China) as per the manufacturer's protocol.

Results

Impact of M.oleifera Seed Extract on the Viability of LNCaP Cells: The impact of ethanolic seed extract of M.oleifera on LNCaP cell line viability over various incubation periods was tested. Significant decrease in LNCaP viability was observed ($P \leq 0.001$). This inhibitory effect was evident at a concentration of 500 µg/ml or more, after 24 h of incubation, and in those of ≥250 µg/ml even after 48 h (Figure I).

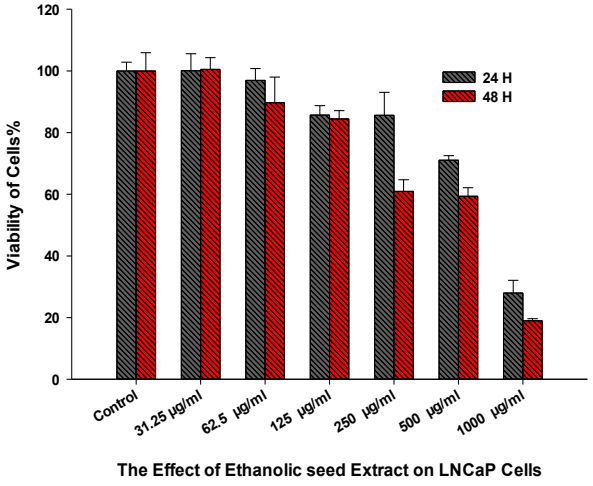

Figure I: Relationship between incubation time and the effect of an ethanolic extract of Moringa oleifera seeds on LNCaP Cell Viability

Effect of Docetaxel on LNCaP Cell Viability: The study also examined the impact of docetaxel on the LNCaP cell line. Treatment with docetaxel at concentrations ranging from 31.25 to 1000 µg/ml led to a significant, dose- and time-dependent decrease in LNCaP cell viability when compared to the untreated control group. The relationship between drug concentration, duration of exposure (24 and 48 hours), and cell viability is depicted in Figure II.

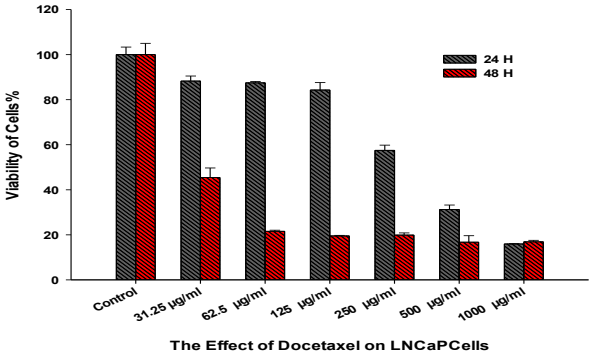

Figure II: The effect of docetaxel treatment on the viability of the LNCaP cell line.

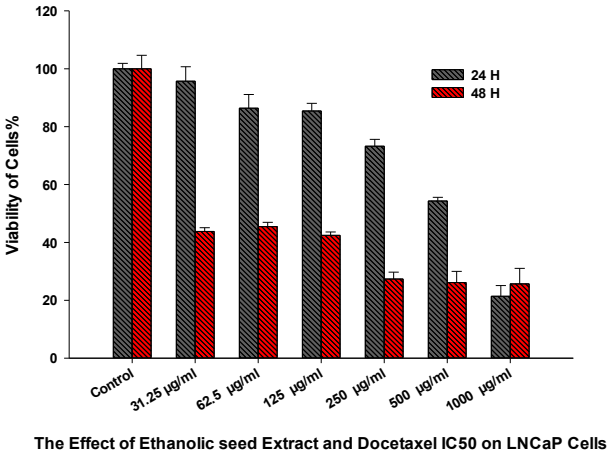

Figure 3: Combined effect of the ethanolic extract of M.oleifera seeds and the IC50 of docetaxel on the LNCaP cell line

Docetaxel and Moringa oleifera seed extract Promote LNCaP Cell Growth in a Synergistic Manner: The LNCaP cell line was exposed to a mixture of docetaxel at its IC50 concentration and an ethanolic seed extract of Moringa oleifera to test if they would have a synergistic effect. The combination of these two treatments led to obvious cell death. After 24 h incubation, the decrease of LNCaP cell viability was significant ($P \leq 0.05$) with extract concentrations ≥62.5 µg/ml. At a concentration of 31.25 µg/ml and above, significantly greater decrease ($P \leq 0.001$) in cell viability was detected after incubation of cells for 48 h with the extracts compared to controls (Figure III).

TRAIL protein Levels in *Moringa oleifera* Seed Extract and Docetaxel Co-treated LNCaP cells: At the molecular level, we determined the levels of TRAIL expression in LNCaP cells. Significant ($P \leq 0.001$) elevation of TRAIL levels was observed against treatment with the ethanolic combination *M. oleifera* seed extract and the IC50 value of

docetaxel. This elevation was concentration dependent for the *M. oleifera* extract (Figure IV).

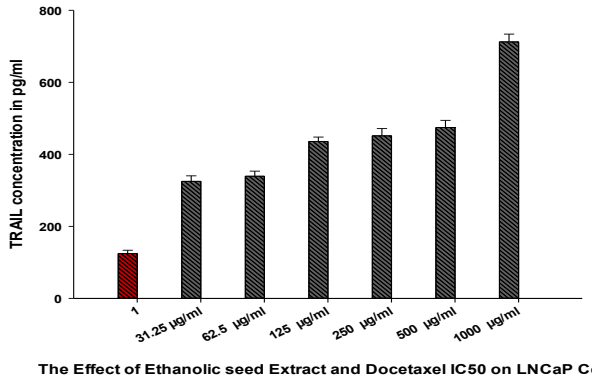

Figure IV: The effect of the combined treatment of ethanolic *Moringa oleifera* seed extract and the IC50 of docetaxel on TRAIL levels in the LNCaP cell line

Levels of TRAIL in LNCaP cells treated with docetaxel: To examine the effect of docetaxel on TRAIL expression, LNCaP cells were exposed to different concentrations of docetaxel. Results It was observed that TRAIL levels were significantly higher ($P \leq 0.001$) in treated cells than untreated control group (Figure V).

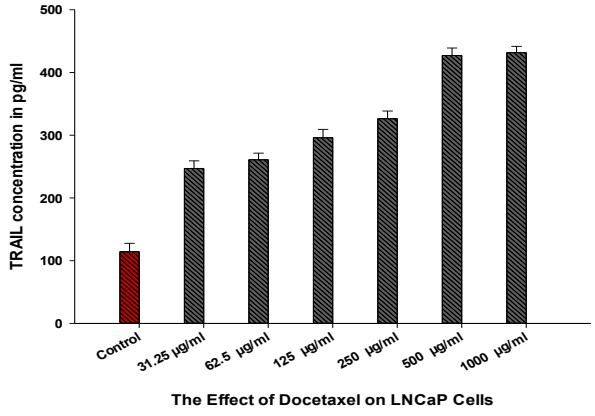

Figure V: The impact of docetaxel on TRAIL levels in the LNCaP cell line

TRAIL Concentration in LNCaP Cells Treated with Moringa oleifera Seed Extract: At last, the effect of the ethanolic *Moringa oleifera* seed extract per se was tested on TRAIL levels. When LNCaP cells were treated with the extract, there was a significant (TRAIL; P=0.001) increase in levels compared to control (Figure VI).

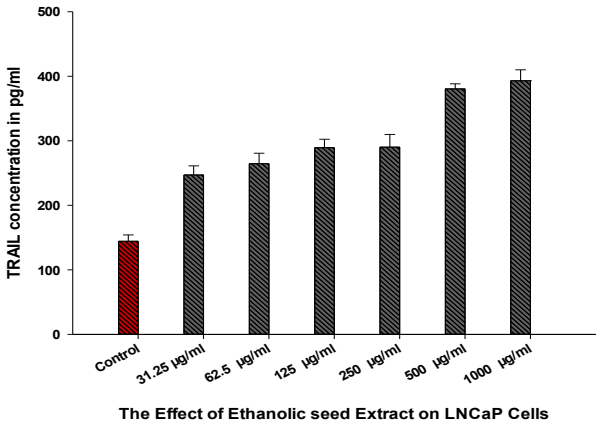

Figure VI: The impact of ethanolic extract from *Moringa oleifera* seeds on TRAIL levels in the LNCaP Cell Line

Discussion

A significant development is the search for bioactive plant-derived compounds for cancer chemotherapy. These natural agents are generally considered to possess better biocompatibility and lower toxicity to normal tissues than synthetic therapeutics, and such a view has indeed been validated by abundant research[7]. As such, there is a growing interest among the scientific community to scrutinize and validate new drug entities of plant origin to augment the armamentarium for anticancer therapy[8,9].

The plant *Moringa oleifera* is found abundantly around the world and is a well-recognized plant with a broad spectrum of medicinal properties, such as anti-inflammatory, antimicrobial, and antitumor effects[10,11]. This study provides a refinement of the work and seeks to evaluate the mechanistic responses to an ethanolic extract from *Moringa oleifera* seeds used here.

Antiproliferative Effects of Moringa oleifera Seed Extract and Docetaxel: Our study presented here also demonstrated that the ethanolic extract of *M. oleifera* seed exerts strong antiproliferative activity against LNCaP cells. This suppression of cell viability was dose-and time-dependent and statistically significant. These results are consistent with a similar study by Khan et al[12], who also indicated that the antiproliferative effect of plants is followed by concentration as well as duration of extract exposure. In addition, a study in 2015 also attested that *M. oleifera* seed oil induced loss of viability for various cancer cell lines, exhibiting a concentration-dependent response, as documented in this report[13]. At the same time, DOC induced a

clear dose- and time- dependent decrease in LNCaP cell viability as well (P<0.001). This is in agreement with published literature, including a study carried out back in 2018, which proved that docetaxel significantly inhibited the proliferation of LNCaP cells[14].

Impact of ethanolic extract from Moringa oleifera seeds and docetaxel on TRAIL levels in the LNCaP Cell Line: Activation of apoptosis, also known as PCD (programmed cell death), is one of the key mechanisms responsible for eliminating cancer cells. Cancer cells often evade apoptosis, and therefore, their reactivation is the ultimate goal of any successful therapeutic modality[15-16]. Apoptosis marker levels of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) were measured in this study. A marked and concentration-dependent induction of TRAIL was also evident in the ethanolic *M. oleifera* seed extract-treated LNCaP, along with docetaxel. This implies that the two agents are perhaps capable of inciting the apoptotic cascade in this cell line.

These findings are in agreement with other preclinical works that proved the apoptotic effects of *M. oleifera* seed, leaf or stem extract on various tumor cells[17]. As an instance, Saini et al[18] also demonstrated that ethanolic extract of M. oleifera seeds had the apoptotic activity against HCT8 cells and supporting anti-apoptotic effects for this plant too.

Synergistic Effects of Combination Therapy: Naturally occurring molecules with cytotoxic and chemotherapeutic properties have been proven to be an interesting approach in improving the effectiveness of treatment, reducing side effects, and drug resistance as well[19]. We demonstrated that the extract of *M. oleifera* (seed) could synergize with docetaxel. The combination exhibited a pronounced cytotoxic effect compared with the free agents. In detail, a significant decrease in viability (P<0.05) was observed at 62.5 µg/ml and higher concentrations after 24 hours, while this reduction intensified results (P≤0.001) for all ≥31.25 µg/ml concentration values after 48 h.

This positive interaction matches the results of other researchers. For example, Sahruddin et al[20] revealed enhanced antitumour effect of gemcitabine in pancreatic cancer by *M. oleifera* extract. Similarly, Brown et al[21] found that the plant's extract potentiated the oncolytic activity of vesicular stomatitis virus against cervical cancer cells. According to a separate study by Tiloke et al[22], *M. oleifera* increases the cytotoxicity and cell death caused by doxorubicin in HeLa human cervix cancer cells.

Conclusion

In conclusion, the present study demonstrates anti-proliferative and pro-apoptotic activity of Moringa oleifera seed is ethanolic extracts in LNCaP cell lines. The extract shows a significant decrease in cell viability in a dose and time-dependent manner, and induces apoptosis through up-regulation of TRAIL. Moreover, the combinational role of *Moringa oleifera* seed extract with docetaxel implies a potential for improving conventional chemotherapeutic efficacy as well. These findings imply that Moringa oleifera is useful as an adjuvant in prostate cancer treatment for preventing cancer cells.

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None

Conflict of Interest
The author has no conflicts of interest to disclose

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Authors' contributions
Conception and design by Aymen Ahmed Al-Khafaji, Acquisition, analysis, and interpretation of data by Ali mohamed Al-Mastafa, Manuscript drafting and revising it critically by Ali mohamed Al-Mastafa and approval of the final version of the manuscript was done by Ali M. Abdulsahib and Sinan Forat Hussein.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
The Institutional Review Board granted the study ethical approval. Since this was a prospective study, every study participant provided formal informed consent. Each method followed the appropriate rules and regulations.

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