**How to Cite**:
Ahmed, A., Al-Mukhtar, E. J., & Ghaleb, R. A. (2022). Antiproliferative activity of moringa oleifera's leaves ethanolic extract and docetaxel on lncap cell line and on the level of tumor necrosis factor-related apoptosis-inducing ligand. *International Journal of Health Sciences*, *6*(S1), 13569–13578. https://doi.org/10.53730/ijhs.v6nS1.8605

# Antiproliferative activity of *moringa oleifera's* leaves ethanolic extract and docetaxel on lncap cell line and on the level of tumor necrosis factor-related apoptosis-inducing ligand

**Aymen Ahmed**
Department of Pharmacology, College of Medicine, University of Babylon, Iraq
Corresponding author email: Ph.aymenahmed2@gmail.com

**Prof. Dr. Entisar J. Al-Mukhtar**
Department of Pharmacology, College of Medicine, University of Babylon, Iraq

**Prof. Dr. Rana A. Ghaleb**
Department of anatomy and histology, College of Medicine, University of Babylon, Iraq

***Abstract*---**Background: Cancer is abnormal cells growth that is responsible for the majority of global deaths and cancer is expected to rank as leading cause of death and the single most important barrier to increasing life expectancy in every country of the world in the 21st century. According to the estimates from the world health organization in 2015, cancer is the first or second leading cause of death before age 70 years in 91 of 172 countries, and it ranks third or fourth in an additional 22 countries. Many anti-cancer drugs in current use such as paclitaxel, vincristine, and vinblastine are naturally derived agents. The aim of this study is to determine the effects of ethanolic leaves extract of *Moringa oleifera* and docetaxel each separately and in combinations on the proliferation of LNCaP cell line, and on the level of tumor necrosis factor related apoptosis induced ligand (TRAIL). Methods: This work was performed in the laboratory of cancer research at college of medicine\ university of Babylon during the period from January 2021 to October 2021. The study was approved by the ethics committee in the college of medicine at university of Babylon. Results: The ethanolic extract of *Moringa oleifera*'s leaves and docetaxel cause significant (P ≤0.01) reduction in the viability of LNCaP cell line in dose and time depended manner, also the of level TRAIL was highly significantly (P ≤ 0.001) increased. The combination of ethanolic extract of *Moringa oleifera*'s leaves and IC50 of docetaxel


*Manuscript submitted: 18 March 2022, Manuscript revised: 9 April 2022, Accepted for publication: 27 May 2022*

enhanced the antiproliferative effect of docetaxel against LNCaP cell line in different incubation period (24-48 hours). Conclusion: Ethanolic extract of *Moringa oleifera*'s leaves reduce the viability of LNCaP cell line and increase TRAIL level in these cells in a dose depending manner. Ethanolic extract of *Moringa oleifera*'s leaves enhanced the antiproliferative effect of docetaxel against LNCaP cell line.

***Keywords*---***Moringa oleifera* leaves, Tumor necrosis factor-related apoptosis-inducing ligand, LNCaP cell line, MTT assay.

## Introduction

Many of anti-cancer drugs in current use such as paclitaxel, vincristine, and vinblastine are naturally derived agents. Several wild edible plant species have played a prominent role in traditional medicine. *Moringa oleifera* Lam. (*M. oleifera*) is one of the most common plants found in Southeast Asia that has been widely used in medicine. It is a valuable plant as it is rich in vitamins, protein, carbohydrate, fatty acid, fiber and phytochemical components (Tragulpak *et al.*, 2017).

Tumor necrosis factor -related apoptosis-inducing ligand (TRAIL) is a member of the TNF family that induces apoptosis in a variety of cancer cells, The TNF family consists of at least 13 homologous proteins, which play crucial roles in a wide range of biological processes, including apoptosis, immunity, inflammation, and development TRAIL selectively induces apoptosis of tumor cells but with no or less effect on normal cells (Lamhamedi- *et al.*, 2003). Death receptor 5 (DR5/TRAIL-R2) is an apoptosis-inducing membrane receptor for TRAIL (TRAIL/Apo2L) (Shiraishi *et al.*, 2005). Aim of this study is to evaluate the Effect of Ethanolic *Moringa oleifera's* leaves extract on LNCAP cell viability.

## Methods

### Plant sample and extract preparation

Leaves of *Moringa oleifera* was taken fresh where it collected from local nursery and then grinded to fine powder and stored in tight dry container for further use. Extraction in this work was prepared by Soxhlet extraction method as
Described previously (Lin et al., 1999). A 200 g of *M. oleifera*'s leaves was soaked and macerated in 1 liter of ethanol 70 % and the ethanol was evaporated, filtered, and dried at oven in (40 °C) and extract were stored in refrigerator at (2-8 °C) for future experimental use.

### Cell line and cell culture

LNCaP cell line was procured from cancer laboratory in medicine college. Cells were cultured and maintained in RPMI-1640 medium supplemented with 10% Fetal Bovine Serum (FBS), 1% of streptomycin penicillin. Cell line was grown in humidified incubator at 37 °C for two period of incubation (24-48 hrs.).

**MTT (**3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], cell viability assay was used in this study to evaluate cell viability.

**Study design**
In-vitro cell line study

**Evaluate the Effect of Ethanolic *M. oleifera's* Leaves Extract on LNCaP Cell Line.**

This experiment was done by measure the effect of ethanolic ***M.oleifera's*** leaves extract on LNCaP cells line viability after exposure to serial concentration of extract (31.25-1000 µg/ml) and for (24-48hour)of incubation in compared with control.

**Evaluate the effect of docetaxel on LNCaP cells line.**

This experiment was done by measure the effect of docetaxel on LNCaP cells viability after exposure to different concentration of docetaxel (31.25-1000 µg/ml) in compare with control.

**Evaluate the Effect of Different Concentrations from Ethanolic *M. oleifera's* Leaves Extract and IC50 of Docetaxel as Combination on LNCaP Cells**

This experiment done to measure the effect of ethanolic leaves extract in different serial concentrations (31.25-1000 µg/ml) and IC 50 concentration of docetaxel as combination on LNCaP cells viability after incubation period (24-48hour) in compared with control.

**Evaluate the Effect of Ethanolic *M. oleifera's leaves* Extract and Docetaxel either alone or in Combination on Level of TRAIL in LNCaP Cell.**

This done through used the Elisa kit from ELabscience company, this ELISA kit applies to the in vitro quantitative determination of Human TRAIL concentrations in biological fluids.

**Results**

**Effect of Ethanolic extract of *Moringa oleifera*'s Leaves on the viability of LNCaP cell line according to the period of incubation**

Results showed significant ($P \leq 0.01$) reduction in the viability of LNCaP cell at concentration ≥500 µg/ml after 24 hour of incubation and highly significant $P \leq 0.001$ at concentration ≥1000 µg/ml after 48 hours incubation as shown in figure (1)

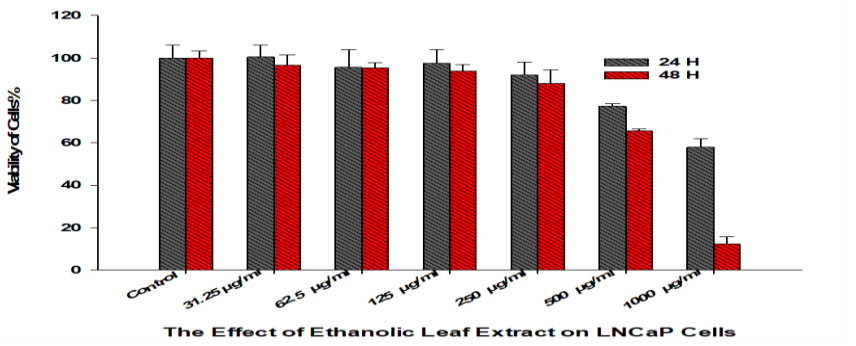


Figure (1) Effect of ethanolic extract of *Moringa oleifera*'s leaves on the viability of LNCaP cell line according to the period of incubation

**Effect of Docetaxel on LNCaP Cells Line**

Results showed that different concentrations of docetaxel (31.25- 1000µg/ml) cause highly significant reduction (P ≤0.001) in the viability of LNCaP cells in comparison to the control group, and this reduction was directly proportion to the concentration of docetaxel and duration of exposure (24 or 48 hour) as shown in figure (2).

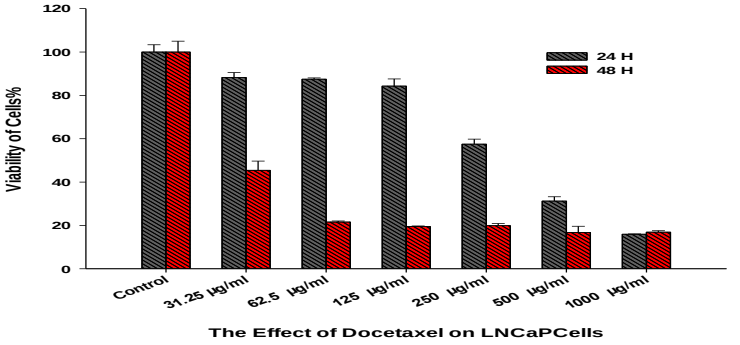


Figure (2): Effect of docetaxel on the viability of LNCaP cell line

**Effect of the Combination from Different Concentration of Ethanolic *Moringa oleifera*'s Leaves Extract plus IC50 of Docetaxel on LNCaP cell line.**

Regarding the effect of the combination of *M. oleifera*'s leaves extract and IC50 of docetaxel results showed highly significant reduction (P ≤ 0.001) in viability of LNCaP cell at the concentration ≥250 µg/ml after 24 hours of incubation, while there was a significant reduction (P ≤0.01) in viability of LNCaP cell after 48 hour of incubation at the concentration ≥31.25 µg/ml as shown in figure (3).

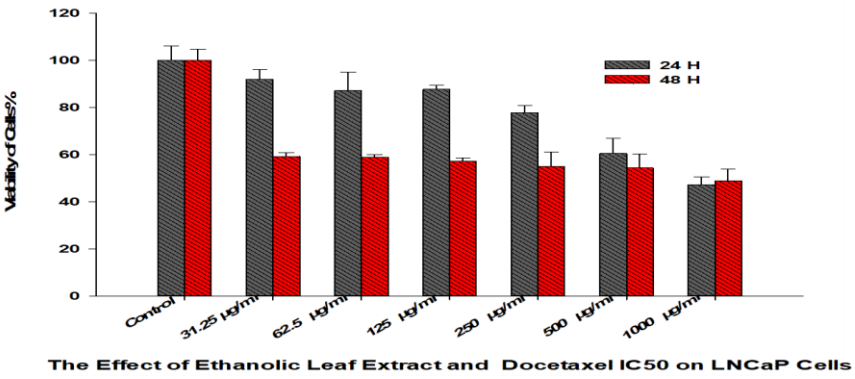


Figure (3): Effect of *Moringa oleifera*'s leaves ethanolic extract and docetaxel IC50 on LNCaP cell line.

**Level of Tumor Necrosis Factor Related Apoptosis Induced Ligand in LNCaP Cell Line Treated with combination of different concertation from ethanolic extract of *Moringa oleifera's* Leaves plus IC50 of docetaxel**

Result showed that the level of TRAIL was high significantly increased ($P \leq 0.001$) after the treatment of LNCaP cell line with a combination containing different concentration of ethanolic Leaves extract of *M. oleifera* plus IC50 of docetaxel and this increase was concentration dependent as shown in figure (4).

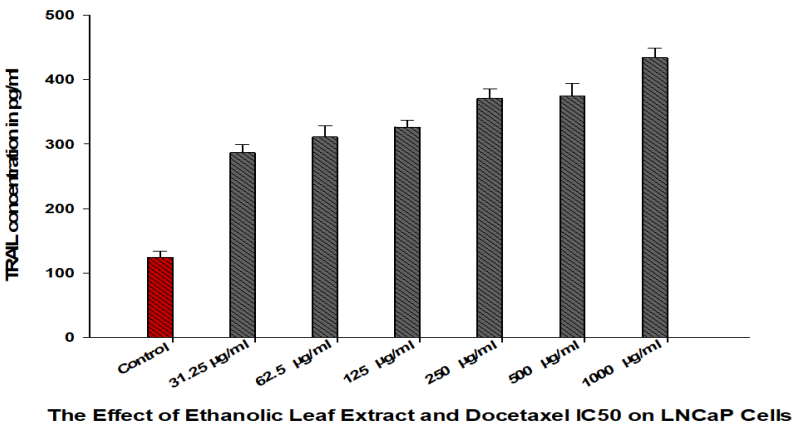


Figure (4) Effect of the combination of ethanolic ***Moringa oleifera's*** leaves extract plus docetaxel IC50 on LNCaP cell line

**Level of tumor necrosis factor related apoptosis inducing ligand in docetaxel treated LNCaP cell line**

According to the result of the current study the level of TRAIL was high significantly increased ($P \leq 0.001$) in a concentration dependent manner after the treatment of LNCaP cell line with different concentrations of docetaxel in comparison to the control group as shown in figure (5).

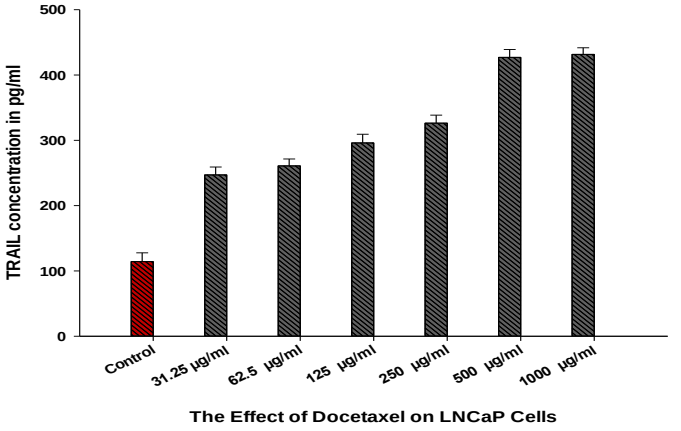


Figure (5) Effect of docetaxel on the level of TRAIL in the LNCaP cell line

**Level of tumor necrosis factor related apoptosis induced ligand in LNCaP cell line treated with ethanolic Leaves extract of *Moringa oleifera*.**

According to the result of the current study the level of TRAIL was high significantly increased (P ≤0.001) in a concentration dependent manner after the treatment of LNCaP cell line with different concentrations of ethanolic extract of *M. oleifera's* Leaves in comparison to the control group as shown in figure (6).

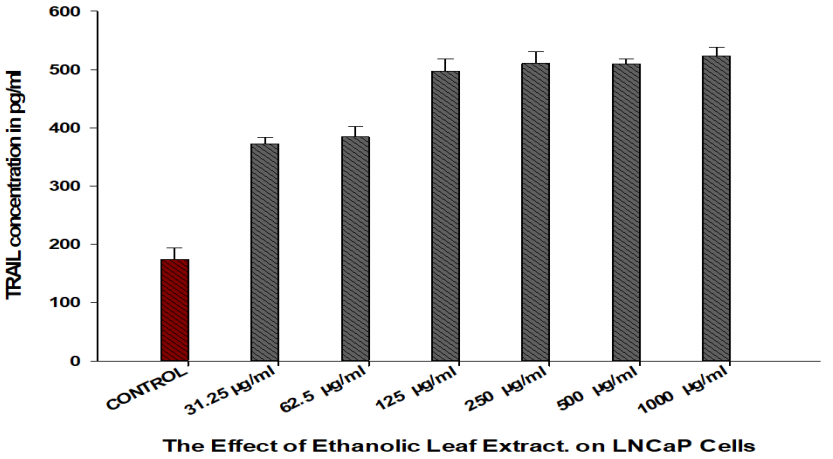


Figure (6): Effect of ethanolic extract of *Moringa oleifera's* Leaves on the level of TRAIL in the LNCaP cell line

**Discussion**

Natural materials or plant-derived bioactive compounds have already gained increasing attention in cancer chemotherapy since they are supposed to be more biologically responsive and more co-evolved with their target sites and having less toxicity to normal cells (Cragg and Newman, 2005; Chaudhary *et al.*, 2019).

Further, there are multitudes of evidences that confirmed the anticancer properties of natural product-derived drugs as an alternative mode of promoting cell death. Based on these shreds of facts, many researchers are now focusing their investigations on the plants' potential to deliver natural products that can become beneficial to the pharmaceutical industry (Iqbal *et al.*, 2017; Kumar *et al.*, 2018a). *Moringa oleifera* plant is often found worldwide and have many medicinal properties including anti-inflammatory, antifungal, antibacterial and anticancer activities (Karim *et al.*, 2016; Elsayed *et al.*, 2016; Kou *et al.*, 2018).

**Antiproliferative effect of ethanolic *Moringa oleifera*'s Leaves extract and docetaxel against LNCaP cell line**

The current study also found that ethanolic leaves extract of *Moringa oleifera* significantly reduced LNCaP cell viability (P≤ 0.01), and that this reduction was dose and time dependent. This finding is consistent with the findings of many other studies, including (Wang *et al.*, 2020) study, which discovered that the anti-proliferative effects of *Moringa oleifera* extracts were time- and concentration-dependent after incubation for various periods (12-24–48 hours). In addition to another study published in 2011, (Sreelatha *et al.*, 2011) conducted a semi-similar study to the present study by using the MTT reduction assay to determine the effect of *Moringa oleifera* leaves extracts on the extent of survival of KB tumor cells. Moringa oleifera leaves extract inhibited KB cell proliferation in a dose-dependent manner. Another study discovered that increasing the concentration of *Moringa oleifera* leaves extract (seeds or leaves) resulted in a significant dose-dependent increase in its anticancer effect (Berkovich *et al.*, 2013a). Based on the all above findings and research, it is possible that the ethanolic extract of *Moringa oleifera* leaves has antiproliferative properties.

**Effect of ethanolic extract of *Moringa oleifera*'s Leaves and docetaxel on the level of TRAIL in LNCaP cell line**

Apoptosis is a major process for cell death that plays an important role in many diverse processes ranging from development to stress responses (Levine *et al.*, 2001). The inactivation of apoptosis is a central cause to many cancers. Therefore, induction of apoptosis seems to be an effective strategy against tumor progression (Brown and S. Sreelatha *et al.* 2005). Screening of plants and plant derived products as potential inducers of apoptosis have become the major target in anticancer drug research. In the present study the TRAIL was used as indicator to the induction of apoptosis that occurred after the treatment of LNCaP cell line with ethanolic extract of *M. oleifera's* leaves and docetaxel. The highly significant concentration dependent increase in the level of TRAIL after the treatment of LNCaP cell line with each ethanolic extract of *M. oleifera's* leaves and docetaxel reported by the current study indicate the ability of this extract to induce apoptosis in the LNCaP cell line. There are several preclinical research works had confirmed the ability of seed, leaf and bark of *M. oleifera* to induce apoptosis in many cancer cells leading to their ultimate death (Adebayo, Balogun, and Arsad 2017).

**Effect of the combination of ethanolic extract of *Moringa oleifera's* Leaves plus docetaxel IC50 on LNCaP cell line**

The use of plant extracts, either alone or in association with other therapeutic agents, have high potential in the field of cancer therapy due to their safety, efficacy, reduced toxicity, and low propensity for the development of resistance (Fuel *et al.*, 2021). Many efforts have been made to study the synergistic effects of combined therapy between conventional and traditional herbal medicines. The ethanolic extract of *Moringa oleifera* leaves caused a highly significant (P≤ 0.001) reduction in cell viability of the LNCaP cell line starting at 250µ g/ml in a 24-hour exposure period and a significant (P ≤0.01) reduction in cell viability starting at 31.25µg/ml in a 48-hour exposure period in this study. This result of current study refers to the presence of an enhancement in the anti-proliferative effect of docetaxel when combined with an ethanolic extract of *Moring oleifera* (seeds and leaves). This finding is consistent with a recent study by (Sahruddin *et al.*, 2021), which found that combining gemcitabine with M oleifera extract improved the chemotherapeutic effect of gemcitabine on pancreatic cancer. The current study's findings are also consistent with the findings of(Brown *et al.*, 2020), who found that an ethanolic extract of *Moringa oleifera* synergized the antiproliferative effect of vesicular stomatitis virus, which has been used as oncolytic viral therapy against cervical cancer cells. In addition, another study found that *Moringa oleifera* acted synergistically with doxorubicin to induce cytotoxicity and increased apoptosis in human HeLa cervical cancer cells. Finaly

(Berkovich *et al.*, 2013b) which revealed to a dose-dependent significant increase in anticancer effect of *Moringa oleifera* leaves extract. which inhibits the growth of pancreatic cancer cells, the cells NF-κB signaling pathway, and increases the efficacy of chemotherapy in human pancreatic cancer cells with increase plant extract concentration.

**Conclusion**

Ethanolic extract of *M. oleifera's* leaves has a concentration and time dependent antiproliferative effect against LNCaP cell line, also it increased the level of TRAIL in LNCaP cell line. Ethanolic extract of *M. oleifera's* leaves enhanced the anticancer effect of docetaxel on LNCaP cell line.

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