Evaluation the cytotoxic effect of *Ficus carica* extracts on cancer and normal cell lines *in vitro*

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Abstract

In this study, extracts were prepared from *Ficus carica* fruits and latexes, and then estimate the cytotoxic activity of these extracts on cancer and normal cell lines. Brest cancer (Ahmed, Murtudha, Jabriyah, 2013 (AMJ13)) and Brain cancer (A human glioblastoma multiforme (ANGM)) cancer cell lines and Rat Embryo Fibroblast (REF) normal cell line were used in the *in vitro* study. Normal cell line was measured after only 72 hours of exposure while cancer cell line exposure times were estimated after (24, 48, and 72) hours under sterile conditions in a micro titration plate. Anti-cancer property of *Ficus carica* extracts on cancer cells has been reported and there was no effect on normal cells. In this work, the AMJ13, ANGM and REF cell line were treated with six concentrations of two type of *F. carica* extract (31.25, 62.5, 125, 250, 500 and 1000 µg/mL). The anticancer effect of *F. carica* extracts was measured using the microculture tetrazolium assay (MTT). The result obtained showed concentration and time dependent cytotoxic effect also the type of cell line effect on the result, the higher concentrations (1000 µg/mL) of extracts gave significantly ($p<0.05$) higher cytotoxic effect on cancer cell lines at 48 hours of exposure.

Keywords:Anticancer; MTT; *Ficus carica* L.; Cell line

Introduction

The frequency of cancer is continuously rising in both developed and developing nations, making it one of the top causes of illness and mortality globally (Al-Khuzaay *et al*., 2019). The pressing need for efficient preventive, treatment, and therapeutic approaches to tackle this complicated illness is highlighted by the expanding worldwide burden (Al-Khuzaay *et al*., 2019). However, there is growing interest in complementary and alternative medicines, especially those derived from natural sources, as orthodox cancer treatments like radiation, chemotherapy, and surgery are frequently linked to serious side effects and limits. Historically, medicinal plants have been essential natural resources for enhancing health outcomes, serving as the basis for herbal therapy. The Philippines, a biodiversity hotspot, has a long

history of using herbal remedies, which makes its indigenous ethno botanical knowledge very important (Susaya-Garcia *et al*., 2018). These traditional methods yield important ethno botanical data that can direct scientific investigation. Traditional medicinal claims are frequently founded on decades of empirical observation, providing a useful foundation for finding plants with therapeutic potential and bioactive chemicals.

Examining these assertions offers the chance to find new therapeutic agents and mechanisms, improving our knowledge of natural products and their potential uses in contemporary medicine (Alaman *et al*., 2020).

The fig (*Ficus carica*) is a seasonal fruit that may have originated in the Middle East and is now a major crop all over the world. The Mediterranean basin's natural ecosystems are home to the common fig. Customers can purchase dried figs at any time of year, wherever in the world. The fig tree belongs to the Moraceae family, which includes mulberries. Items made from figs are great examples of natural items that are utilized in traditional medicine and as food (Abdel-Rahman *et al*. 2021).

Good examples of raw materials that are used extensively in both traditional medicine and as food are fig products. Fig root is used to cure ringworm and leukoderma in traditional medicine. The antipyretic, purgative, and aphrodisiac qualities of fig tree fruit have been demonstrated to be effective in the treatment of inflammation and paralysis. (Abdel-Rahman *et al*. 2021)

Many studies on *F. carica* have verified the existence of a wide range of bioactive substances, including volatile substances like hydrocarbons and aliphatic alcohols, phenolic compounds, phytosterols, organic acids, anthocyanin composition, triterpenoids, and coumarins. The majority of *F. carica* cultivars have high levels of phenolic compounds, organic acids, and volatile chemicals. Numerous investigations on *F. carica* have shown the existence of a wide range of bioactive substances, including organic acids, triterpenoids, coumarins, phenolic compounds, phytosterols, anthocyanin content, and volatile substances including aliphatic alcohols and hydrocarbons. The majority of *F. carica* cultivars have high levels of phenolic compounds, organic acids, and volatile chemicals. (Alis *et al*., 2011)

This study was used to measure the cytotoxic effect of latex extract and the aqueous extract of fruits of *F. carica* on cancer cell lines (AMJ13 and ANGM) and normal cell lines (REF).

Materials And Methods

Aqueous extraction procedure for *F. carica* fruits

Fresh fruits of *F. carica* were collected from Wasit, Al-Suwaira farms - Iraq. The fruits were shade dried and grinded into powder and kept in tight containers protected completely from light. 100 g of dried powder fruits were dissolved and boiled in distilled water (250 ml) for six hours, then filtered using filter paper, and then it dried by heated in oven at 50º C and kept until it was used at 4º C. (Khodarahmi *et al*., 2011).

Extraction procedure for *F. carica* latex

F. carica latex was collected drop-by-drop without squeezing over summer months from unripe fruits of fig trees and 1 ml of the latex was put into in eppendorf tubes. The latex was filtered using Whatman No. 1 and centrifuged (at 13000 rpm/4 °C) to separate the polymeric gum from the aqueous filtrate part. Further purification of the aqueous part was subsequently attained by filtration using a 5 µm disposable filter membrane, and then it dried by heated in oven at 50º C and kept until it was (Ghanbari *et al*., 2019).

Briefly, 10 g of both extracts was dissolved in 100 ml of phosphate-buffered saline (PBS), then filtering the suspension using 0.2 µl Millipore filter to get stock solution of 1000 µg/ml. The stock solutions were then appropriately diluted with the medium to prepare various concentrations (31.25, 62.5, 125, 250, 500 and 1000 µg/ml) (Khodarahmi *et al*., 2011).

Cell Growth Assay

Using an in vitro MTT test, the effects of *F. carica* aqueous fruits and latexes extracts on cancer cell lines (AMJ13 and ANGM) and normal cell lines (REF) were assessed. The Iraqi Centre for Cancer and Medical Genetic Research (ICCMGR) protocol was used to prepare the solutions.

Cytotoxicity Assays

In brief, 180 µL of the cells were seeded in 96-well microplates and incubated for 24 hours following two to three subcultures. Upon the addition of 20 µL of various sample concentrations, the microplates were incubated for a further 48 hours under the same conditions. The positive control was taxol. While the blank wells contained only 200 µL of the RPMI medium, the first column of the microplate, which included 180 µL of the cell suspension and 20 µL of RPMI, was considered the negative control and 20 µL of each dilution was added to the 96-well microplate containing 180 µL of the cell suspensions Each of the wells was then incubated with 20 µL of MTT solution for three hours in order to assess the cell survival. To dissolve the

formazan crystals, 200 µL of DMSO was then added to each well's medium and pipetted up and down (Basim and Kasim, 2023).

A microplate reader was used to measure the absorbance at (570 nm wave length), by comparing the absorbance of cells treated with extracts to that of untreated control cells, the percentage of live cells was ascertained. The following formulas were used to measure the percentage of cytotoxicity, or the inhibition rate of cell growth (Al-Shammari *et al*., 2020).

= [(optical density (OD) of control - optical density (OD) of test)/ optical density (OD) of control] × 100

Statically analysis

The Statistical Analysis System (SAS) (2018) program was utilized to identify the impact of the different elements in the study parameters. In this study, the means were significantly compared using the least significant difference (LSD) test.

Results and Discussion

Two types of *F. carica* extracts were used in this study; including latexes extract and aqueous extract of fruits to determine their anticancer effect against cancer cell lines.

- **latex extraction:**

The *F. carica* latex produced a light brown, sticky substance that dried to powder.

- **Fruit aqueous extract**

F. carica fruits produced a dark brown, sticky substance that turned to powder when dried, providing a 15% yield.

Effect of *F. carica* extracts on cancer and normal cell lines

F. carica extracts' effects on normal and cancer cell lines for 24, 48, and 72 hours, the ANGM, AMJ13, and REF cell lines were subjected to varying concentrations of *F. carica* extracts (31.25, 62.5, 125, 250, 500, and 1000) µg/ml. The ELISA reader was used to measure the cell lines' optical density (OD) at 570 nm.

- **Effect of *F. carica* extracts on AMJ-13 cells**

The effect of treating AMJ-13 cells with aqueous extract of fruits and latex after 24 hours of exposure are shown in Figure (1), the results revealed that the aqueous extract of latex had the greatest growth inhibition effect on AMJ13 cell line than the aqueous extract of fruits after 24 hours of exposure.

Also, the results in Figure (2) revealed that the latex extract had the greatest growth inhibition effect on AMJ13 cell line than the aqueous extract

of fruits after having been exposed for 48 hours. The viability of AMJ13 cells was affected by the two varieties of *F. carica* extracts in a time dependent manner. The cytotoxic effect is considerable at all concentration ($p<0.05$).

The greatest percentage of inhibition, 74.14%, was achieved by the maximum concentration (1000 µg/ml) after 48 hours of exposure.

After 72 hours of treatment, Figure (3) indicates that the latex extract had the strongest growth inhibitory effect on the AMJ13 cell line. After 72 hours, the latex extract at the maximum concentration (1000 µg/ml) had a growth inhibition percentage of 62.98%.

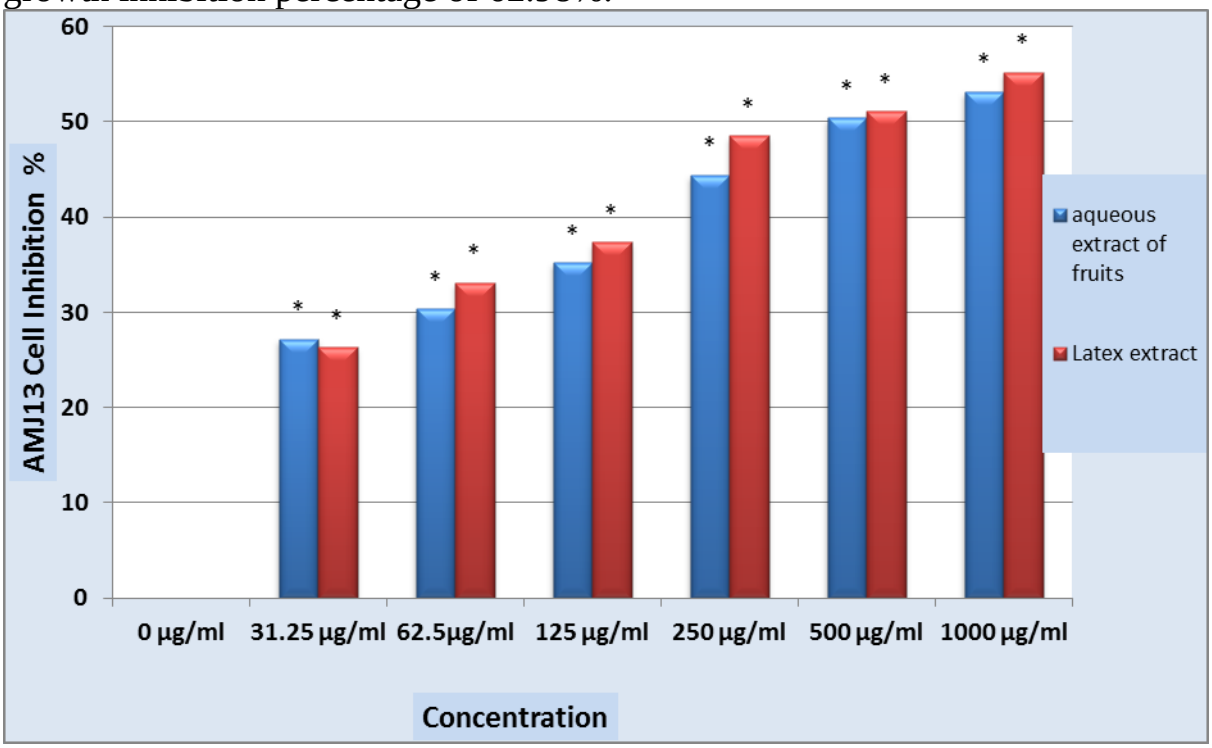

Figure 1. A comparison of inhibition rates (IR) of latex extract and aqueous extract of fruits of *F. carica* on AMJ13 cell line after 24 hrs. of exposure.

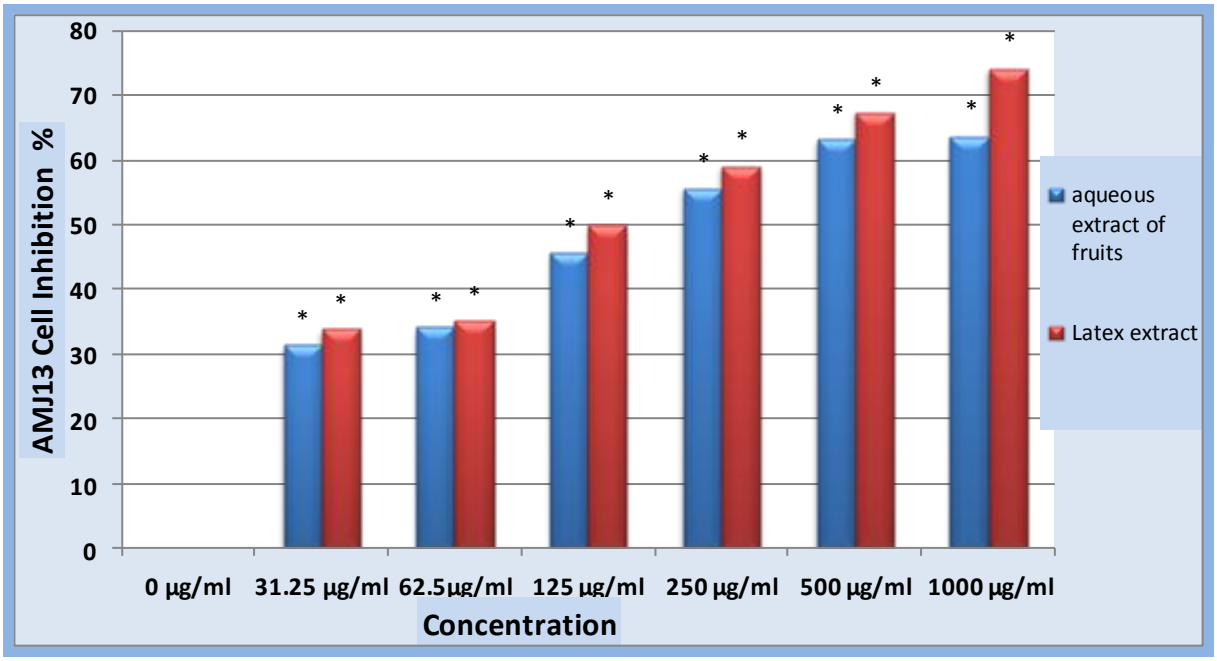

Figure 2. A comparison of inhibition rates (IR) of latex extract and aqueous extract of fruits of *F. carica* on AMJ13 cell line after 48 hrs. of exposure.

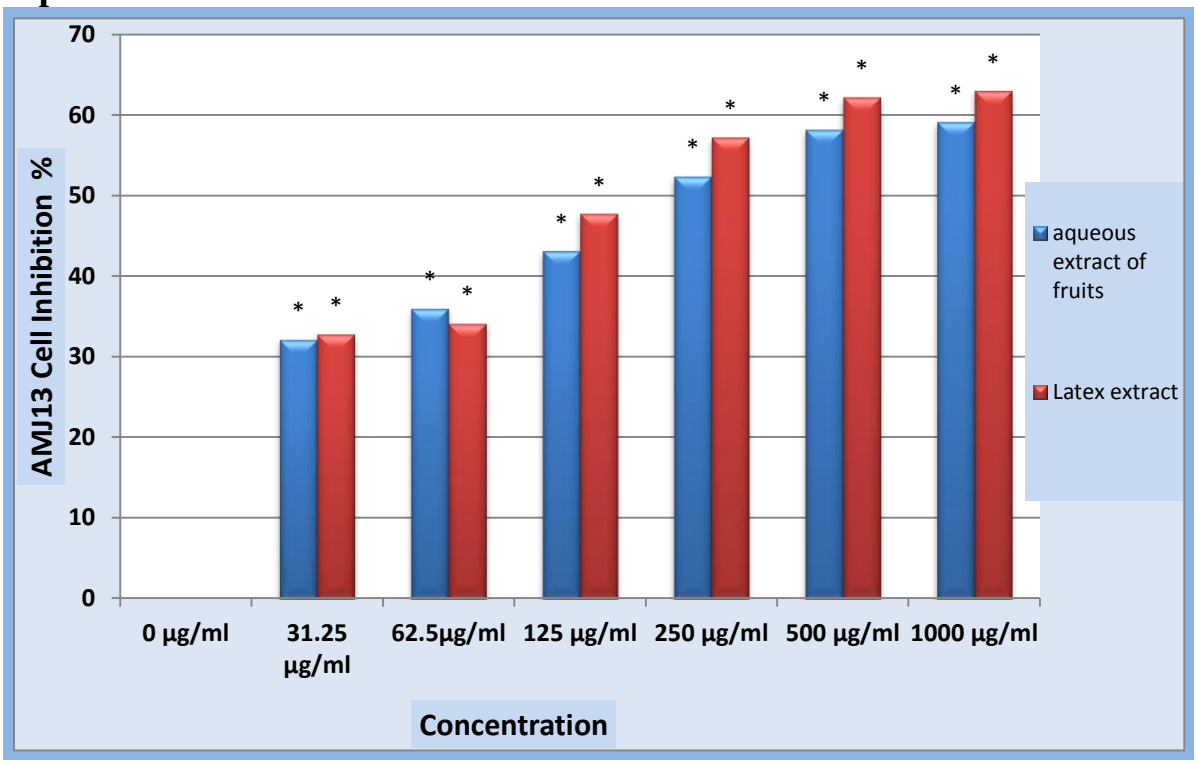

Figure 3. A comparison of inhibition rates (IR) of latex extract and aqueous extract of fruits of *F. carica* on AMJ13 cell line after 72 hrs. of exposure.

- **Effect of *F. carica* extracts on ANGM cells**

Figure (4) illustrates the results of treating ANGM cells with *F. carica* latex extract and fruits aqueous extract following a 24 hour exposure period. After 24 hours of exposure, the inhibition rate increased to 63.55% at a higher dose of latex extract.

Figure (5) shows that the latex extract had the greatest inhibition rate effect on ANGM cell line after 48 hrs. of exposure. The growth inhibition rate for the latex extract of *F. carica* at highest concentration was 83.34 % after 48 hrs. of exposure.

The results that shown in Figure (6) explain all concentrations of the two types of extracts have cytotoxic effect after 72 hrs. of exposure.

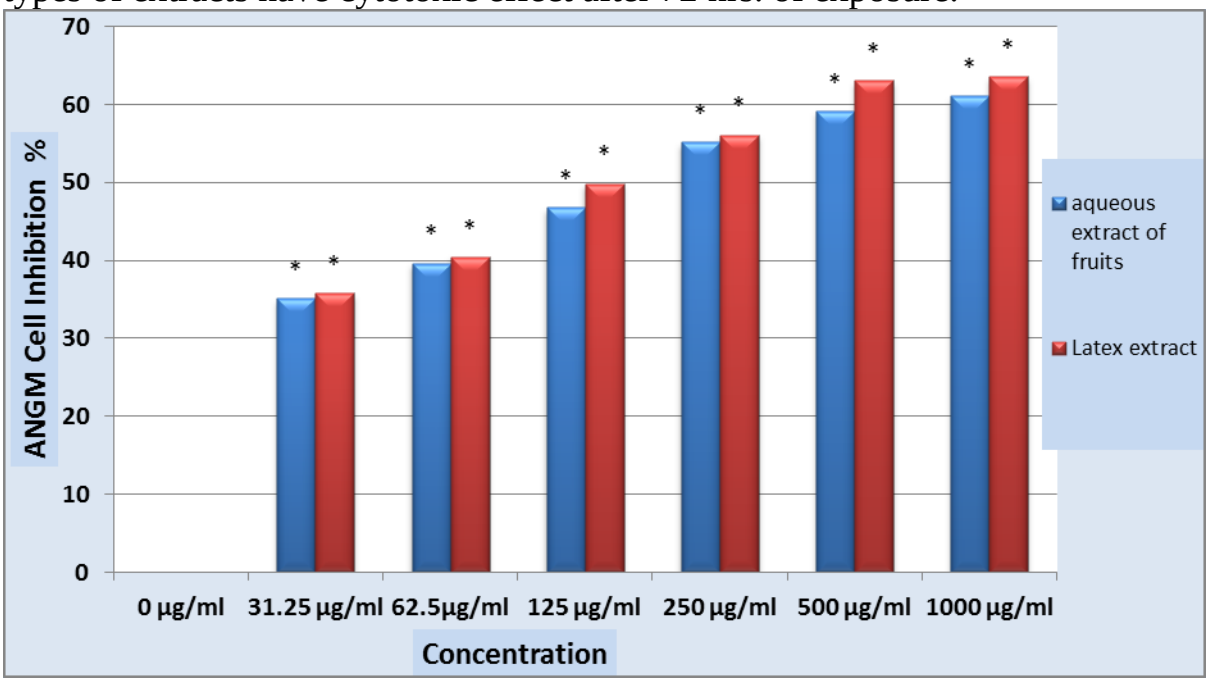

Figure 4. A comparison of inhibition rates (IR) of latex extract and aqueous extract of fruits of *F. carica* on ANGM cell line after 24 hrs. of exposure.

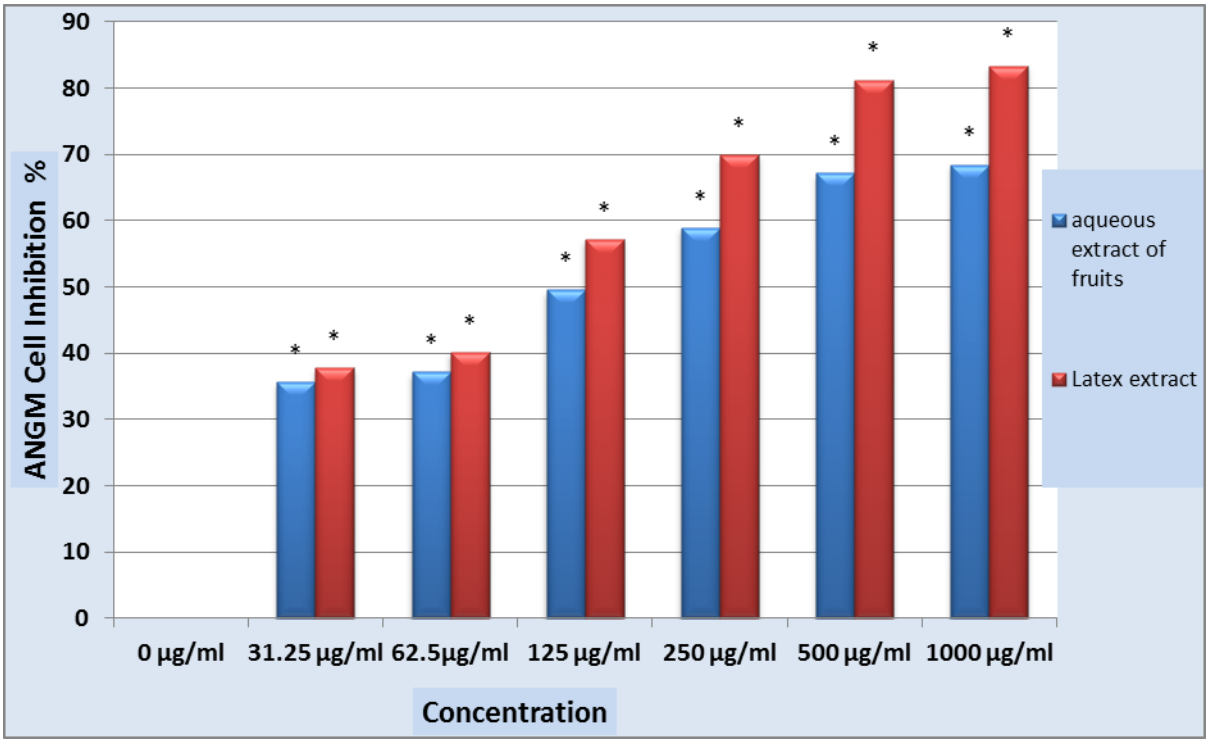

Figure 5. A comparison of inhibition rates (IR) of latex extract and aqueous extract of fruits of *F. carica* on ANGM cell line after 48 hrs. of exposure.

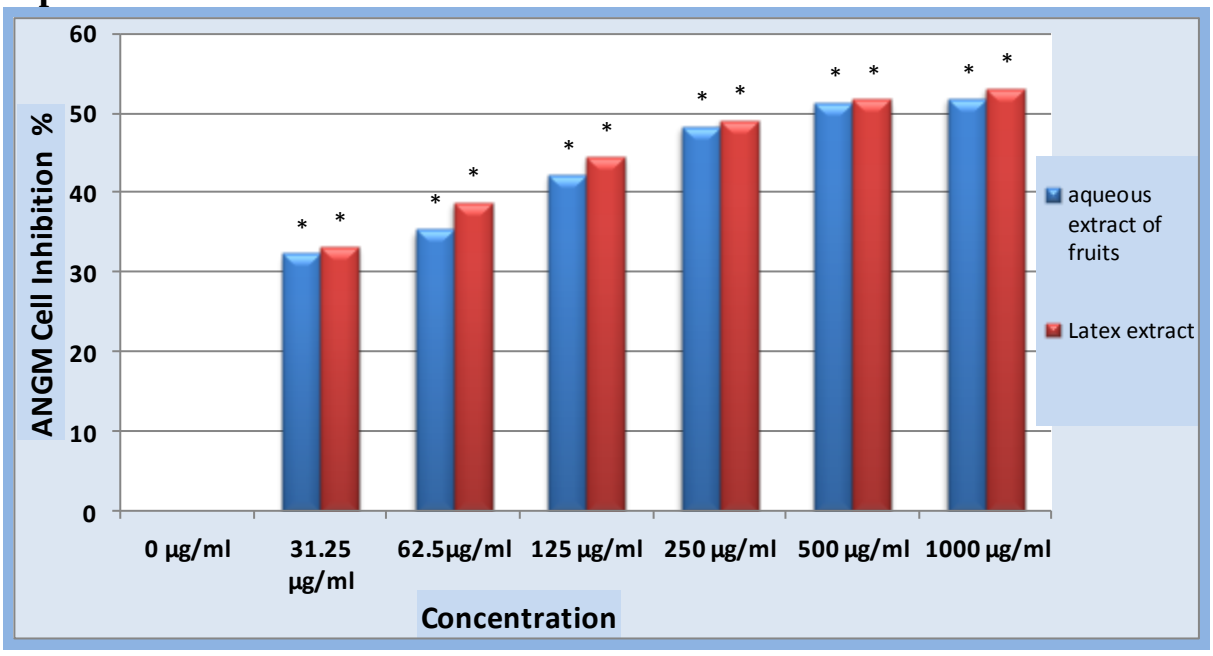

Figure 6. A comparison of inhibition rates (IR) of latex extract and aqueous extract of fruits of *F. carica* on ANGM cell line after 72 hrs. of exposure.

- **Effect of *F. carica* extracts on REF cells**

After 72 hours of exposure, normal cell line (REF) was treated with a latex extract and fruits aqueous extract. As seen in Figure (7), the results demonstrated that neither extract had a cytotoxic effect on the REF cell line.

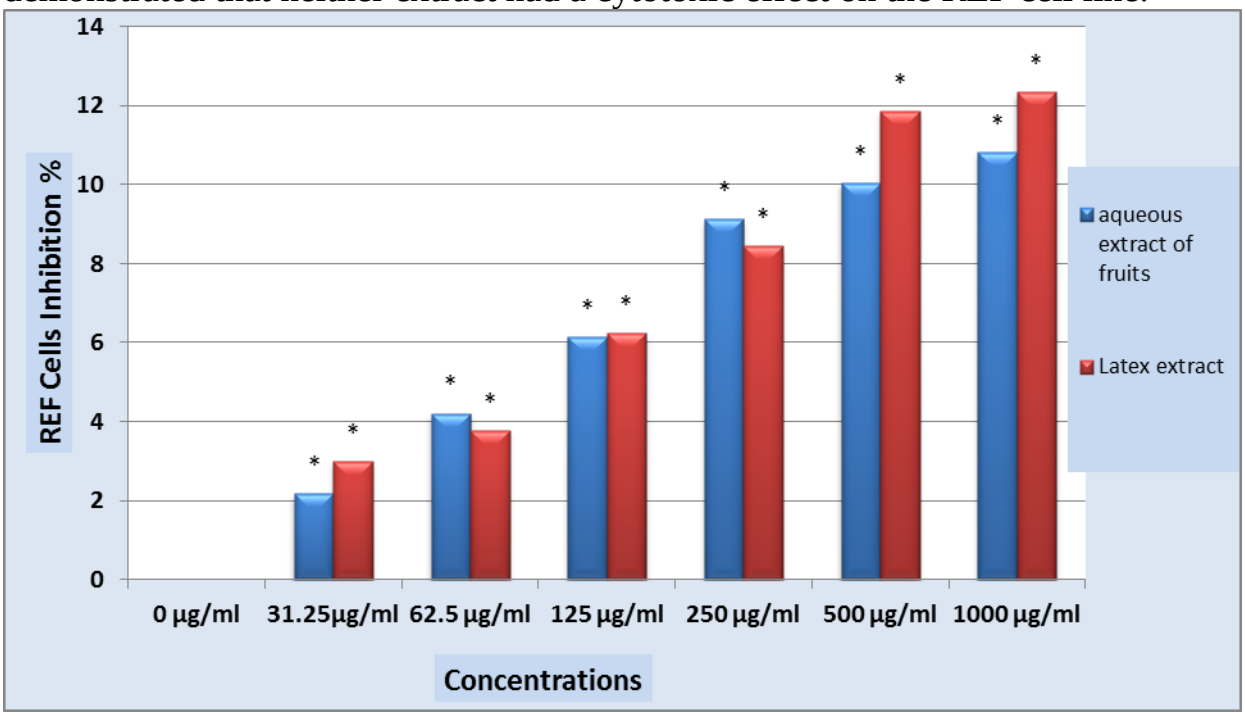

Figure 7. A comparison of inhibition rates (IR) of latex extract and aqueous extract of fruits of *F. carica* on REF cell line after 72 hrs. of exposure.

The leading cause of death in human history is cancer (Sharma *et al.*, 2023). Fruits from *F. carica* are often consumed as part of a Mediterranean diet to promote health. Because it contains a lot of polyphenols, flavonoids, and anthocyanins, F. carica latex is used as a therapy for a variety of diseases worldwide. Ficin, which comes in several forms, is a type of cysteine proteinase that causes cancer cells to undergo apoptosis. Furthermore, because F. carica latex contains a lot of polyphenolic components, the anticancer actions may be linked to antioxidant qualities.

(Boyacioglu *et al.*, 2021).

The MTT assay's final results demonstrated varying anticancer effects on the AMJ13, ANGM, and REF cell lines. The aqueous and latex extracts of fruits were found to have a cytotoxic effect on cancer cell lines, as shown in figures 1, 2, 3, 4, 5, and 6. However, the degree of this effect varied depending on the type of extract and the cell line. Figure (7) shows that neither of the two types of *F. carica* extracts had a cytotoxic effect on normal

cells (REF). According to the results, the latex extracts had the most cytotoxic effect on cancer cell lines.

The overall results obtained from this study on the cytotoxic effect of *F. carica* extracts on cancer cell lines refer to the fact that the highest cytotoxic effect of these extracts occurred on ANGM cancer cell line, which proves that ANGM cell line was very sensitive to the effect of *F. carica* extracts compared to AMJ13 cell lines at the same conditions.

Our results showed that *F. carica* extracts have cytotoxic effect and these results agree with the results of other studies, Khodarahmi and his collagenous (2011), confirmed that that latex, fruits and leaves of *F. carica* have cytotoxic effect against HeLa cell line.

Another study has demonstrated that *F. carica* extracts can dramatically reduce cell viability in a dose-and time-dependent manner (Mustapha *et al*., 2016). According to Rubnov and his collagenous (2001), Fig latex exhibits anti-tumoriferative properties in a number of cancer cell lines. According to Alaman and his collagenous (2020), fig fresh latex shows anticancer efficacy against both spontaneous and xenografted tumors in mice (Soltana *et al*. 2019).

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تقييم التأثير السمي الخلوي لمستخلصات نبات التين (*Ficus carica*)على خطوط الخلايا السرطانية والطبيعية

مستخلص البحث

في هذه الدراسة، تم تحضير مستخلصات حليب نبات التين و المستخلص المائي لثمار نبات التين *Ficus carica* (*F. carica*)، ثم تم تقدير النشاط السام لهذه المستخلصات على الخلايا السرطانية والطبيعية. استُخدمت في الدراسة المختبرية (2013) (AMJ13)، وخلايا سرطان الدماغ (ANGM) ، و الخلايا الطبيعة لأجنة الجرذ الليفية (REF). تم قياس الخلايا الطبيعية بعد 72 ساعة فقط من التعرض، بينما قُدِّرت أوقات تعرض الخلايا السرطانية بعد (24، 48، و72) ساعة في ظروف معقمة باستخدام صفيحة معايرة مجهرية. وقد أُشير إلى أن لمستخلصات نبات التين تأثير سمي خلوي على الخلايا السرطانية، ولم يُلاحظ أي تأثير على الخلايا الطبيعية. في هذا البحث، عولجت خطوط الخلايا AMJ13 وANGM وREF بستة تركيزات من نوعين من مستخلصات نبات التين (31.25، 62.5، 125، 250، 500، و1000 ميكروغرام/مل). قيس التأثير المضاد للسرطان لمستخلصات نبات التين باستخدام اختبار (MTT). أظهرت النتيجة تأثيراً ساماً للخلايا يعتمد على التركيز والوقت، حيث أظهرت التركيزات الأعلى (1000 ميكروغرام/مل) من المستخلصات تأثيراً ساماً للخلايا أعلى بشكل ملحوظ ($p<0.05$) على سلالات الخلايا السرطانية بعد 48 ساعة من التعرض.

الكلمات المفتاحية

مضاد للسرطان، اختبار MTT، نبات التين، خط خلوي