The Cytotoxicity Effect for the Crude Extract of (*Cyperus Esculentus*) Tubers on Human Breast Cancer Cell Line (MCF-7) in Vitro

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Abstract

The study aimed to identify the aqueous and alcoholic extract of *Cyperus esculentus* tubers in affecting human breast cancer (MCF-7) Michigan Cancer Foundation,preliminary diagnosis chemical groups in these extracts showed the presence of (Tannins, Terpenoids, Resins, Steroids, Flavonoids, Alkaloids, Glycosides and Phenols). The effect of cellular toxicity of crude aqueous and alcoholic extract of *Cyperus esculentus* tubers in vitro was tested in human breast cancer cells (MCF-7) through the use of six concentrations (midterm scares) (31.25,62.5,125,250,500,1000) µg /mL and for periods of exposure (24, 48, 72) hours. Using the MTT Test. The study found that there is a toxic effect of the crude aqueous and alcoholic extracts of the grain of *Cyperus esculentus* tubers in cancer cells (MCF-7) and of all the concentrations used, The highest percentage and a significant difference ($P \leq 0.01$) for the average of the inhibitory average of cancer cell growth (IR) was 68, 73 (%) for the aqueous extract and alcoholic extract, respectively, at the highest concentration of 1000 µg / mL and after 72 hours of exposure.

Key word : *Cyperus esculents, cytotoxicity, Inhibitory Rate*

Introduction

Cancer refers to a large number of diseases that affect all parts of the body and these diseases are called malignant tumors [(1)] and cancer is the second leading cause of death in the world. In 2018, 8.8 million people died, and nearly one out of 6 deaths worldwide is attributed to it [(2)]. Cancer occurs as a result of the abnormal division of cells and without control, which is able to spread to the rest of the body, and this division continues even after the catalyst is interrupted, This increase in division and reproduction leads to the formation of tumors, and these are of two types benign tumors and malignant tumors [(3)].Therefore, many countries of the world have paid great attention to their medicinal plants as the natural source of pharmaceutical. [(4)] The genus (*Cyperus esculentus* L) is considered a medicinal plant and it is a perennial herbaceous plant used in the field of folk medicine in many countries, including Africa, Asia,It is one of the genera of the Cyperaceae family known to have many active compounds and anti-carcinogenic substances, The plant is used to treat many diseases, such as problems and disorders of the digestive system, such as irritation of the colon and diarrhea.. less as an anti-oxidant by containing vitamin E and C and is used to reduce the incidence of Heart attacks Thrombosis [(5)] .That the alcoholic extract of the tubers of *Cyperus esculentus* plant can be used to treat convulsions. Many studies also showed that the aqueous extracts of *Cyperus esculentus* tubers contain anti-cancer compounds and active substances, including sterol. Anti-cancer, The secondary metabolic compounds present in the *Cyperus esculentus* tubers play the therapeutic role of cancer because of its therapeutic properties through its effect on killing or stopping cancer cells [(6)].

Materials and Methods

Preparing crude aqueous and alcoholic extracts for the seeds of the *Cyperus esculentus* plant, Cyperus esculentus tubers were obtained from the local herbalists in Karbala province during the month of December

2019, Washed, dried, and cleaned, It was milled by Mill device and keeping the powder in clean plastic. Preparing the aqueous and alcoholic crude extracts by methods of soaking and stirring according to (14) with a weight of 50 g of the plant powder and adding 250 ml of solvent to it). Magnetic stirrer at room temperature for 3 days, after which the filter was filtered with gauze and then with filter paper (Whatman NO.1), Then the leachate was dried to obtain the dry powder, from which the required concentrations were prepared. Prepare the crude alcoholic extract of the plant in the same methods, but using ethyl alcohol at a concentration of 70% instead of the distilled distilled water .After weighing the extract, it was divided into several sections and kept in glass bottles under 4 ° C. Dissolve 0.1 g of dry extract in 10 ml of serum free media (serum free media) to prepare the original stock and sterilize by filtering with filter paper with holes. 0.22µm

Cellular toxic effects:

A multi-hole tissue culture dish (96-Microtiter plates). The experiment included three stages:

Cells Seeding

the cells were harvested using Trypsin-Versen (T-V) solution. 20 ml of the culture medium provided with serum are added to each container (depending on the cell type) and mix well. After that, the cells returned using a blood cell count (Haemocytometer) using Trypan blue tincture (1%), (0.1) ml was taken by means of a micro-pipette from the cells suspension and distributed to pit the dish containing (1 x 104) cells/pit. The pit surface was then covered with sterile transparent adhesive paper for this purpose and the dish was stirred gently, then incubated at a temperature of 37 ° C until the next day to allow the cell attachment. Treatment of **(exposing) cancer cells with plant extract**

The MCF-7 cancerous carcinoma line (pass 35) was obtained from the center of the Faculty of Nursing at the University of Babylon, the secondary farms (passage or subculture) lattended six diluted of the abstracted agency(31.25), 62.5, 250, 500, 1000 µg / mL.)

Prepare the cells suspension by treating a 25 cm tissue culture vessel with a trypsin solution - fersin (20 ml of trypsin solution 1 g: 100 ml of 10: PBS ml of fersin), then add 20 ml of the new serum-free culture medium 16.4) SFM g of RPMI-1640 media. : 15 ml of sodium carbonate: 0.5 ml 0.25: Streptomycin ml of Gentamycin and Nystatin complete the volume to 1 liter of distilled water (then mix the cells suspension well and transfer 0.2 µL to the flat bottom tissue culture calibration using an automatic pipette, leaving the plates until the cells adhere In the pits. Then the old medium in the pits was removed, and 0.2µl of the previously prepared concentrations of the extract was added and at 3 replicates per concentration, as well as the control replicates) only a culture medium.

Detection of the toxic effect Cytotoxicity Assay

The MTT test was used to detect the cytotoxic effect of extracts of Tuber plant extracts on. According to the following steps:

After the end of each incubation period, the dishes were taken and the contents were poured, then (28) µµL of MTT pigment prepared at a concentration of 2 mg/ml was added to each pit of the pits dish and incubated at a degree of (37) C° / for two hours.

The pigment was removed and the Dimethyl sulphoxide (DMSO) solution was added (130 µµL / pit).The dishes were placed for a period of (15) minutes with a microshaker, then it read the optical density at the wavelength (492) nm using the ELISA microplate spectrophotometer, taking into consideration that the MTT work should be away from light. Cancer cell growth rate (Inhibitory Rate / I.R) was calculated according to the formula

$$IR\% = \frac{A - B}{A} \times 100$$

where:

IR = percentage of inhibition rate

A = optical density of negative control.

B = optical density of the test group

Results

Primer (inductive) chemical detection of secondary metabolism compounds in the extracts of

tuber plants.

The results of the chemical disclosures of the aqueous and alcoholic extracts of the tubers of the dried plant showed the results shown in Table (1):

Table (1) Primer (inductive) chemical detection of aqueous and alcoholic extracts of the tuber plant

Chemical group	Crude alcohol (ethyl) of Cyperus esculentus tubers	Crude aqueous extract of Cyperus esculentus tubers
Tannins	+	+
Terpenoids	+	+
Resins	+	+
Steroids	+	+
Saponins	-	-
Flavonoids	+	+
Alkaloids	+	+
Glycosides	+	+
Phenols	+	+

(+) the positive of a diagnosis .

(-) the negative of a diagnosis

Toxic effect of crude aqueous extract of *Cyperus esculentus* tubers on MCF-7 cancer cell line during exposure periods 24, 72,48 hours ,

The results of the toxicity test shown in Figure 1 on MCF-7 cells of the crude aqueous extract of *Cyperus esculentus* tubers showed a variation in the percentage of cell inhibition depending on the concentration used and the periods of exposure, The inhibitory effect of the aqueous extract appeared from the lowest concentration (31.25) µg / ml up to high concentrations, with the highest inhibition rate of the extract at concentration (1000) µg / ml and for all periods of exposure (72,48,24) hours which was (68.75) ± 3.82, 59.86 ± 1.45, 52.78 ± 1.54 (respectively compared to other concentrations and significant difference with P3.0 probability level)). Whereas, the lowest inhibition rate of the aqueous extract was at concentration (31.25) µg / m at 48 hours (and it reached) (17.46 ± 0.77).

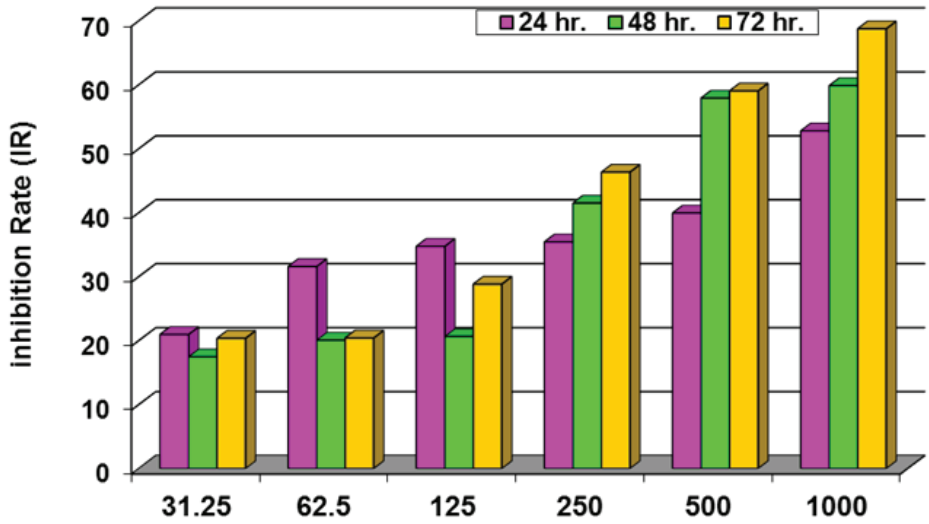

Figure 1. effect of aqueous extract of *Cyperus esculents* tubers plant on the percentage of inhibition of human breast cancer cell linesMCF-7

Toxic effect of crude (ethyl) extract of *Cyperus esculentus* tubers on MCF-7 cancer cell line

during exposure periods 24, 72,48 hours

The crude alcoholic extract did not differ from the aqueous extract in its effect on MCF-7 cancer cells, where it is observed in Figure (2) the toxic effect of ethanol extract and significant difference P≤0.01) began at concentration 31.25µg / ml and for all exposure times (24,48, 72) hour by inhibition rate (16.77 ± 6.99, 44.50 ± 3.75, 49.67 ± 6.63), respectively, The rate of inhibition of cells increased upwards by increasing the concentration and exposure period of the cells to the extract where the highest inhibition ratio (73.16 ± 3.54) for the alcoholic extract at the concentration reached 1000 µg / ml within 72 hours of exposure

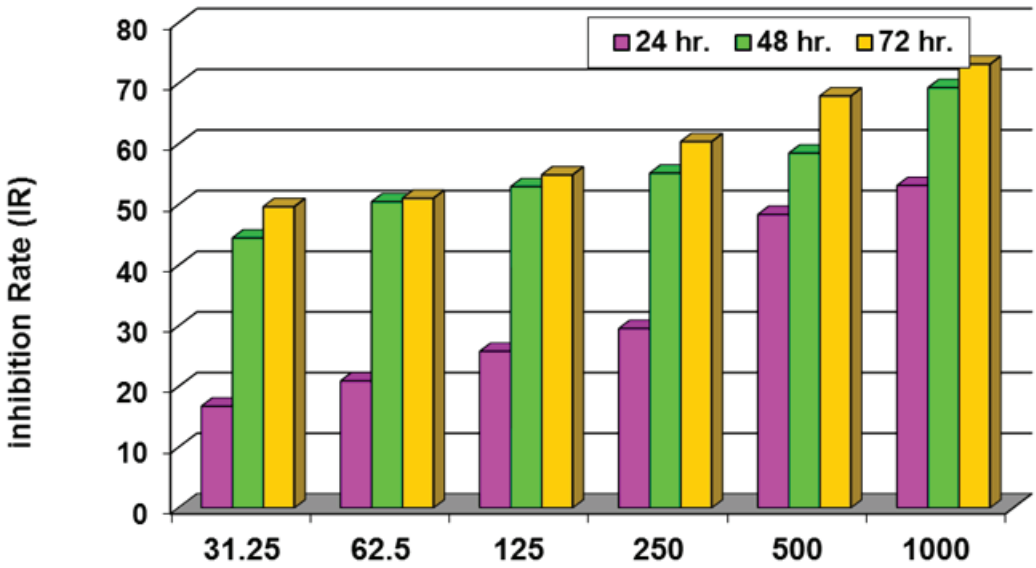

Figure 2. effect of alcoholic extract of *Cyperus esculents* tubers plant on the percentage of inhibition of human breast cancer cell lines (MCF-7)

Comparison of the effect of crude alcoholic and aqueous extracts of *Cyperus esculentus* plant tubers on cancerous cellular line MCF-7))

The results shown in Figures (3.4.5) when comparing the effect of crude alcoholic and aqueous extract on the cellular line of breast cancer showed that the alcoholic extract was most effective in inhibiting the growth of MCF-7 cancer cells at periods of exposure (48,72),It is noticed in Figure (3) that the alcoholic extract at concentration 500 µg / ml gave the highest average of inhibition of cells during a period of 24 hours, and reached 48.38 ± 3.07)) compared to (39.94 ± 3.77) for the aqueous extract with a significant difference (P≤0.01) .. But when comparing the effect of aqueous and alcoholic extracts during a period of (48) hours of exposure, Figure (4) shows that the alcoholic extract gave higher cell inhibition rates than the aqueous extract and for all concentrations, As the inhibition ratio increased with increasing the concentration, the concentration (1000 (/g / ml) gave an inhibition ratio of 69.28 ± 1.83) compared to (59.86 ± 1.45) for the aqueous extract. As for the passage of (72) hours of exposure, the inhibitory ratio of the aqueous and alcoholic extracts increased compared to the inhibitory ratio of them in a period of (48) hours, Figure(5.).

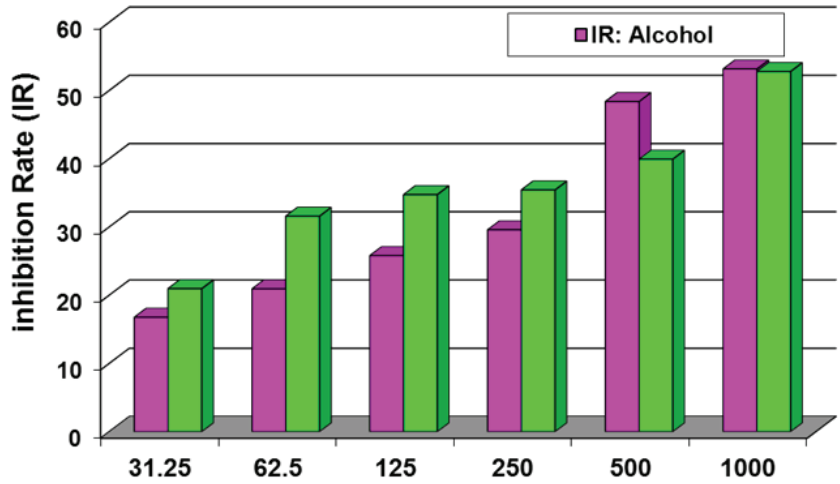

Figure 3. Comparison of the effect of aqueous andalcoholicextracts of *Cyperus esculents* tuberson the percentage of Inhibition rate of human breast cancer cell lines(MCF-(7 after24 hours of treatment.

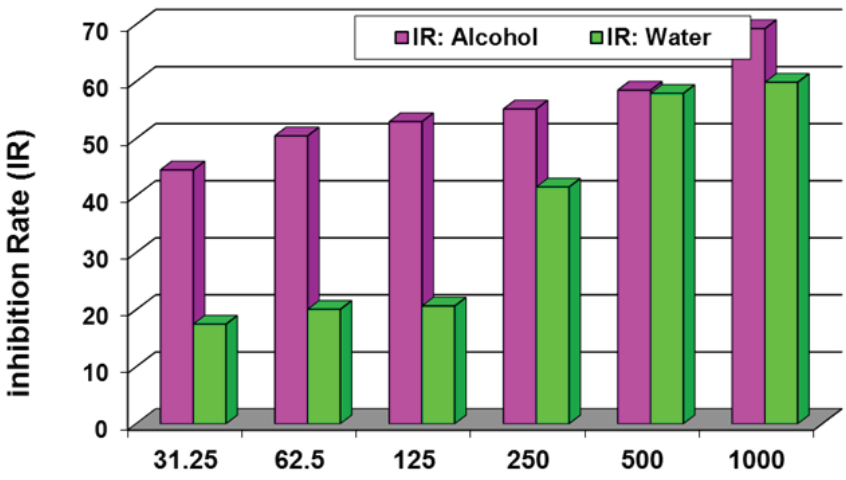

Figure 4. Comparison of the effect of aqueous and alcoholic extracts of *Cyperus esculents* tuberson the percentage of Inhibition rate of human breast cancer cell lines(MCF-(7 after 48 hours of treatment.

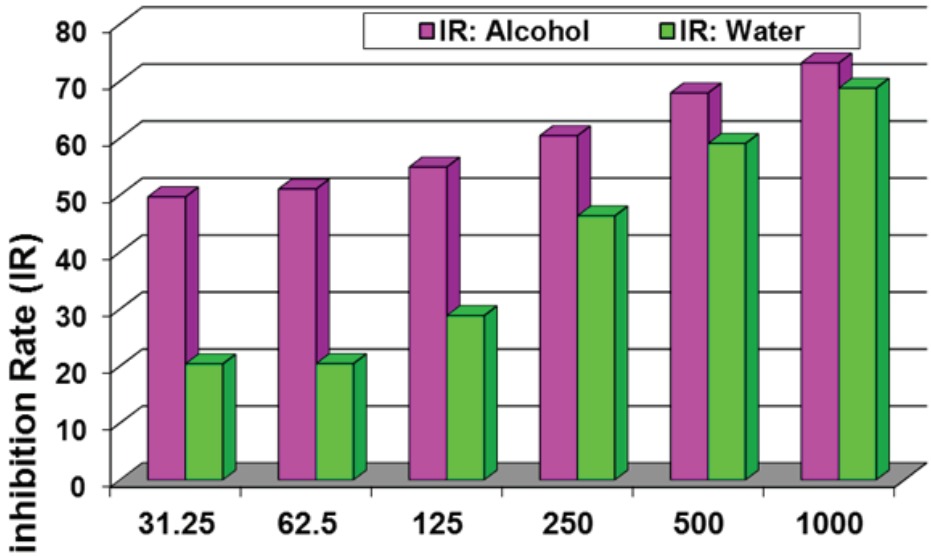

Figure 5. Comparison of the effect of aqueous and alcoholic extracts of *Cyperus esculents* tuberson the percentage of Inhibition rate of human breast cancer cell lines(MCF-7) after 72 hours of treatment

Discussion

Due to the importance of finding effective compounds against cancer and finding more types of plants that possess these compounds, and given the ability of crude extracts to inhibit the multiplication of cancer cells [7] , it was chosen that the *Cyperus esculentus* plant, which is one of the locally available medicinal plants, This is to identify the effects of crude aqueous and alcoholic extracts on human breast cancer cells (MCF-7) and the extent to which these extracts can be used as medical therapeutic substances against cancer in the future. The results of the current study when conducting confirmatory testing of the active compounds present in the alcoholic and aqueous extracts of the tuber plants showed the presence of most of the secondary metabolites that may together contribute to killing cancer cells more effectively as a result of the synergistic action among them, which may reduce the toxicity of the pure compounds used and that each one Among these plant secondary active compounds is an important role in inhibiting the growth of cancer cells outside and inside the body of the organism. It was also found through the results of the current study that the crude extracts of plant tubers played a role in killing cancer cells and inhibiting their growth and division outside the living body, which came similar to many studies, including [8].

The results showed that the toxic effect of *Cyperus esculentus* tubers on the human breast cancer cell line (MCF-7) depended mainly on the concentration used, exposure period and extract type, Alcoholic extract was the best in effect compared to the aqueous extract and for all exposure periods, This may be due to that the percentage of the active substance extracted with ethyl alcohol (70%) is greater than it is when using the aqueous extract and this is indicated by[9] and his group. A study conducted by[8] reached the presence of sterol in the extracts of tubers plant, which is one of the steroidal compounds that have anti-cancer effectsInterest has increased in recent years in flavonoids in which results of extensive research in the field of medicine and biology have shown their anti-cancer, anti-allergic, anti-viral, and anti-oxidant efficacy and other activities [10] Flavonoids are considered one of the most important active compounds extracted from medicinal plants Which is used in the treatment of many different diseases, most importantly cancer, because it possesses a high ability to act as antioxidants to resist the action of free radicals that are produced by pathological factors..

One of the first mechanisms studied was the antioxidant activity of these compounds. Phenolic compounds, including polyphenols, have multiple effects, including the antioxidant effects of cancer cell growth, due to their ability to scavenging the free radicals generated when normal cells turn into cancer cells. Flavonoids work to caught free radicals that lead to distortions of DNA, and thus mutations in tumor genes or inhibiting the appearance of tumors, Which is considered a precursor to the emergence of this disease [(11)] and is a substance (quercetin) of flavonoids called (Flavanols), which has proven its presence in the plant of dear love [(12). (13)] showed the ability of flavonoids to limit the spread of cancer cells in cellular lines through their association with estrogen receptor, and this enhances the ability of the *Cyperus esculentus* plant to inhibit the growth of cancer cells.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

Conclusion

The study found that there is a toxic effect of the crude aqueous and alcoholic extracts of the grain of *Cyperus esculentus* tubers in cancer cells (MCF-7) and of all the concentrations used, The highest percentage and a significant difference ($P \leq 0.01$) for the average of the inhibitory average of cancer cell growth (IR) was 68, 73 (%) for the aqueous extract and alcoholic extract, respectively, at the highest concentration of 1000 µg / mL and after 72 hours of exposure. The plant (Cyperus esculentus L) is one of the most promising medicinal plants that have an effective role in treating cancer through its inhibitory and toxic effect on cancer cells Mcf-7.

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