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CYTOTOXICITY OF ALCOHOLIC EXTRACT OF QUINOA SEED AGAINST SOME CANCER CELL LINES

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ABSTRACT

Cancer is one of the deadly disease which recorded highly incidence in last decades, cancer term refers to more than 100 types of malignant tumors have different etiological and pathological features, Cancer is a disease that is associated with the abnormal proliferation and growth of living cells. A variety of approaches and methods are employed clinically for the treatment of cancer; however, each of these approaches or methods has some significant limitations especially adverse effects

Chenopodium quinoa (C.quinoa) is a plant with a complex composition that includes numerous of natural compounds, there were study on the antitumor properties of C.quiona and it is capable to induce cell apoptosis in cancer cells. The study attempted to help in developing a proper treatment for cancer. This was achieved through exploring the beneficial role of Quinoa seeds against the growth of cancer. The study was focused on the Evaluate the cytotoxicity effect of Quinoa alcoholic extract on different cancer cell line (Brest cancer, Liver cancer and skin cancer) using MTT reduction assay.

The result showed that the MCF-7 and HepG2 were the most sensitive to the C.quinoa ethanolic extract and A375 had the lower response. Further studies are needed to uncover the mechanism and chemical constituents of the obtained activity of C. quinoa.

Keyword: Cancer disease, Quinoa seed , Cytotoxicity.

1. INTRODUCTION

The cancer burden continues to grow globally, exerting tremendous physical, emotional and financial strain on individuals, families, communities and health systems (Pant *et al*., 2021). Many health systems in low- and middle-income countries are least prepared to manage this burden, and large numbers of cancer patients globally do not have access to timely quality diagnosis and treatment (Nageswaran, 2021).

Quinoa (*Chenopodium quinoa* Willd.) is a pseudo cereal that belongs to Amaranthaceous family. This crop is cultivated from ancient times in the Andean region of South America and it is capable of adapting and growing in different climate conditions, including the most detrimental abiotic factors like drought, hailstone, high altitude, heat, and salinity. Quinoa seeds are the main edible part of this grain, which is also the most known and valued portion (Angeli and Valanides, 2020). Recently, taking into account quinoa polyphenols content, some studies have been focused on its antioxidant properties and how this can be affected /modulated by different cultivation conditions (Fisher et al., 2017). Cancer treatment needs to be safe and efficient for the patients because cancer is still a major burden of disease worldwide. Each year, tens of millions of people are diagnosed with cancer around the world, and more than half of the patients eventually die from it (Atun et al., 2020). The study attempted to evaluate the cytotoxicity effect of Quinoa alcoholic extract on different cancer cell line (Brest cancer, Liver cancer and skin cancer) using MTT reduction assay.

2. MATERIAL AND METHODS

Samples collection on plant:

The Quinoa seeds were cultured in college of Agriculture / Basrah University Iraq. The seeds of the plants are properly washed in tap water and then rinsed in distilled water. The rinsed leaves are dried in an oven at a temperature of 35-40°C for 3 days. The dried leaves of each plant are pulverized, using a sterile electric blender, to obtain a powered form. The powdered form of these plants is stored in airtight glass containers, protected from sunlight until required for analysis.

Preparation on the extracts:

The extraction was performed by macerating 500 g in 1.5 L of ethanol (70% v/v) for one week with occasional stirring. The macerated mixture was filtered by filter paper and evaporated at 40°C up to one third of initial volume. Remaining solvent was completely evaporated at 40°C, using a hot air oven and kept in

desicator for two days. The yield (10% w/w) of the powdered plant material was collected dried and stored at 5°C in air tight container without light exposure.

Cell Lines

1. ***MCF-7 Cell Line:***

Michigan cancer foundation-7(MCF-7) was derived from the pleural effusion from a 69 year old female suffering from a breast adenocarcinoma (Soule et al., 1973).

2. ***WRL 68 Cell Line:***

The human hepatic cell line WRL 68 exhibits morphology similar to hepatocytes and hepatic primary cultures. Cells have been shown to secrete albumin and alpha-feto protein and express liver specific enzymes such as alanine amino transferase (Asita et al., 2013).

3. ***HepG2:***

HepG2 cells were the first to exhibit the key characteristic of hepatocytes. This line was isolated from a hepatocellular carcinoma of a 15-year-old, White, male youth with liver cancer in 1975.

4. ***A375:***

A375 is a cell line exhibiting epithelial morphology that was isolated from the skin of a 54-year-old, female patient with malignant melanoma.

The Cytotoxic Effect of Compounds Isolated from C.quinoa extract:

This *in vitro* method was performed to investigate the possible cytotoxic effect of different compounds isolated from C.quinoa extract on tumor cell lines (MCF-7 , HepG2 and A375) and normal cell line WRL 68.

Cell Line Maintenance (Freshney, 2010)

When the cells in the vessel formed confluent monolayer, the following protocol was performed:

A- The growth medium was aspirated and the cell sheet washed with PBS.

B- Two to three ml trypsin/versine solution was added to the cell. The vessel was turned over to cover the monolayer completely with gentle rocking. The vessel allowed incubation at 37°C for 1 to 2 minutes, until the cells were detached from the vessel.

C- Fresh complete RPMI medium (15-20 mL) was added and cells were dispersed from the wedding surface into growth medium by pipetting.

D- Cells were redistributed at required concentration into culture vessels, flasks or plates whatever needed and incubated at 37°C in 5% CO_2 incubator.

Cell concentration was achieved by counting the cells using the haemocytometer and applying the formula:

Total Cell Count/ml: cell count x dilution factor (sample volume) x 10^4

MTT Protocol

The cytotoxic effect of different compounds isolated from C.quinoa extract was performed by using MTT ready to use kit:

A- Kit contents:

- MTT solution 1 ml x 10 vials.
- Solubilization solution 50 ml x 2 bottle.

B- Protocol:

- Tumor cells ($1x10^4$ – $1x10^6$ cells/mL) were grown in 96 flat well micro-titer plates, in a final volume of 200∟L complete culture medium per each well.

The microplate was covered by sterilized parafilm and shacked gently.

- The plates were incubated at 37∟C, 5% CO_2 for 24hrs.
- After incubation, the medium was removed and two fold serial dilutions of the desired compound (6.25, 12.5, 25, 50, 100, 200 ∟g/mL) were added to the wells.
- Triplicates were used per each concentration as well as the controls (cells treated with serum free medium). Plates were incubated at 37∟C, 5% CO_2 for selected exposure time (24 hours).
- After exposure, 10 ∟l of the MTT solution was added to each well. Plates were further incubated at 37∟C, 5% CO_2 for 4 hours.

- The media were carefully removed and 100∟L of solubilization solution was added per each well for 5 min.
- The absorbance was determined by using an ELISA reader at a wavelength of 575 nm. The data of optical density was subjected to statistical analysis in order to calculate the concentration of compounds required to cause 50% reduction in cell viability for each cell line.

Statistical Analysis

A one way analysis of variance ANOVA (Duncan) was performed to test whether group variance was significant or not, statistical significance was defined as $p \leq 0.05$. Data were expressed as mean± standard deviation and statistical significances were carried out using Graph Pad Prism version 6(Graph Pad Software Inc., La Jolla CA).

3. RESULTS AND DISCUSSION

Result

Cytotoxicity Results for MCF-7 Cell Line:

MCF-7 breast cancer cells were exposed to a different concentration of C.quinoa (6.25, 12.5, 25, 50, 100, 200 ∟g/mL).The results were obtained using ELISA reader and showed that the C.quinoa extract had an effect on the MCF-7 at high concentration and no effect on WRL68 normal cell line as shown in the Table (3-1) and Figure (3-1).

Table (3-1): Cytotoxicity Results for MCF-7 Cell Line Comparing to WRL68 Cell Line

	MCF-7			WRL68	
Concen.	**Mean**	**SD**		**Mean**	**SD**
200.00	57.99	7.58		77.28	1.62
100.00	61.00	1.48		87.96	1.51
50.00	74.92	3.24		92.13	1.56
25.00	89.82	2.34		95.41	0.37
12.50	94.41	1.99		95.79	0.71
6.25	95.02	1.45		94.91	2.20

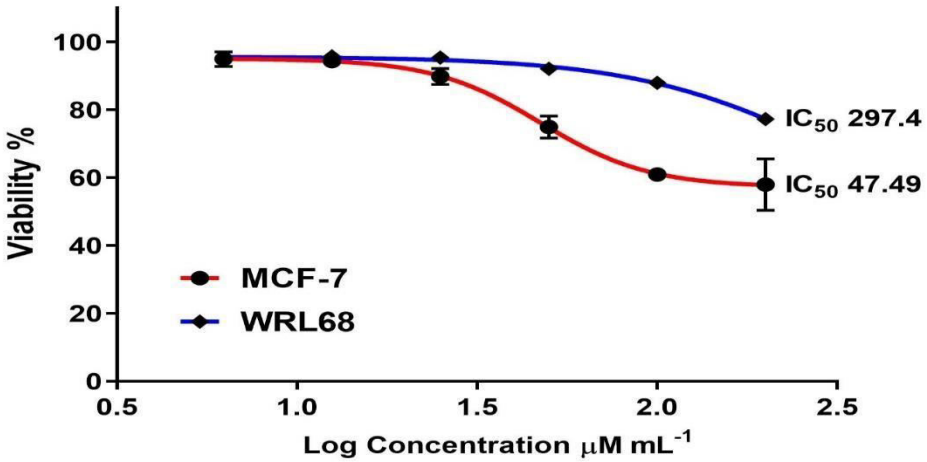

Figure (3-1): Cytotoxicity Results for MCF-7 Cell Line Comparing to WRL68 Cell Line

3.1.2 Cytotoxicity Results for HepG2 Cell Line:

HepG2 liver cancer cells were exposed to a different concentration of C.quinoa (6.25, 12.5, 25, 50, 100, 200 ㄴg/mL).The results were obtained using ELISA reader and showed that the C.quinoa extract had an remarkable effect on HepG2 cell line even at lower concentration and no effect on WRL68 normal cell line as shown in the Table (3-2) and Figure (3-2).

Table (3-2): Cytotoxicity Results for HepG2 Cell Line Comparing to WRL68 Cell Line

	HepG2			WRL68	
Concen.	**Mean**	**SD**		**Mean**	**SD**
200.00	40.90	2.43		77.28	1.62
100.00	47.65	2.04		87.96	1.51
50.00	66.09	2.71		92.13	1.56
25.00	75.27	4.26		95.41	0.37
12.50	82.86	2.07		95.79	0.71
6.25	94.41	0.55		94.91	2.20

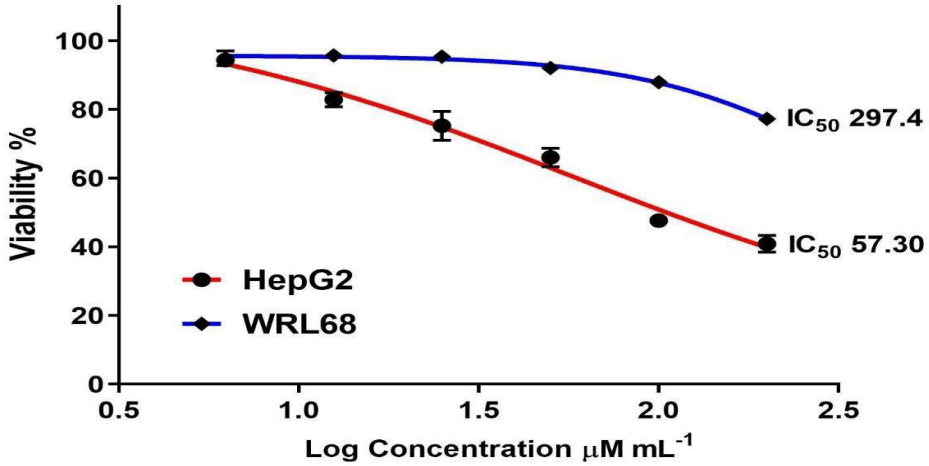

Figure (3-2): Cytotoxicity Results for HepG2 Cell Line Comparing to WRL68 Cell Line

3.1.3 Cytotoxicity Results for A375 Cell Line:

A375 skin cancer cells were exposed to a different concentration of C.quinoa (6.25, 12.5, 25, 50, 100, 200 ㄴg/mL).The results were obtained using ELISA reader and showed that the C.quinoa extract had no effect on A375 cell line and it is almost the same effect on WRL68 normal cell line as shown in the Table (3-3) and Figure (3-3).

Table (3-3): Cytotoxicity Results for A375 Cell Line Comparing to WRL68 Cell Line

	A375			WRL68	
Concen.	**Mean**	**SD**		**Mean**	**SD**
200.00	62.12	1.53		77.28	1.62
100.00	81.70	1.25		87.96	1.51
50.00	92.09	5.92		92.13	1.56
25.00	92.71	3.30		95.41	0.37
12.50	94.41	2.87		95.79	0.71

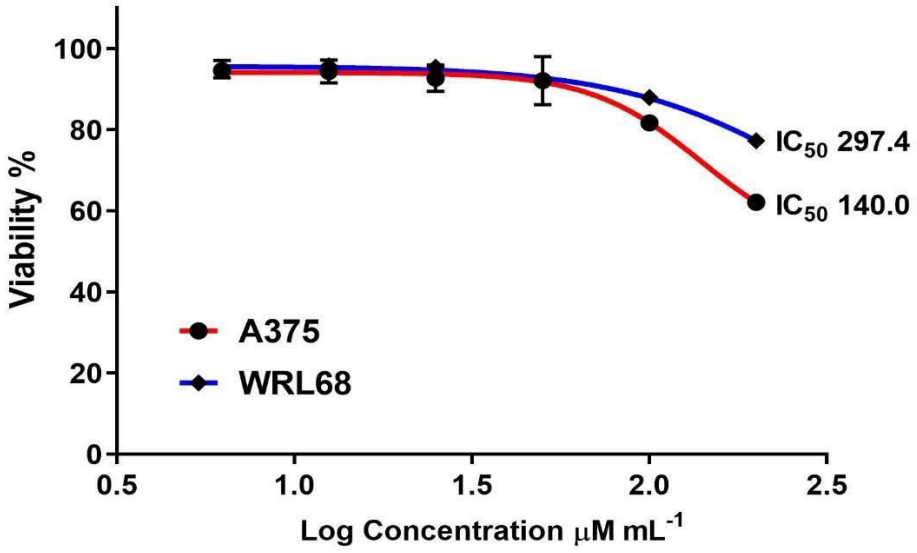

Figure (3-3): Cytotoxicity Results for A375 Cell Line Comparing to WRL68 Cell Line

4. DISCUSSION

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body (Pant et al., 2021).There are many types of cancer treatment. The types of treatment that you receive will depend on the type of cancer you have and how advanced it is. Some people with cancer will have only one treatment. But most people have a combination of treatments, such as surgery with chemotherapy and radiation therapy. However, all these treatment approaches have limitations such as: Cost, Side effects, and High Risk (Miller et al., 2019).

These studies had explored the beneficial role of Quinoa seeds against the growth of different cancer types (Brest cancer, Liver cancer and skin cancer) by preparation of ethanolic extracts from Quinoa seeds then test it on cancer cell lines. A study by Hu et al. (2017) evaluated the chemical characterization, antioxidant anticancer activity, and immune regulating activities, of bioactive polysaccharide components isolated from Chenopodium quinoa seeds. Evaluation of anticancer activity was carried out using C. Quinoa Polysaccharide (CQP) on MCF-7 breast cancer cell lines and human liver cancer SMMC 7721. The result has shown that CQP possesses cytotoxic activity against breast cancer cells and liver cancer cells, with no effects on normal cells. Moreover, the result indicated that the bioactive components present in Chenopodium quinoa have a potential for use as anticancer, antioxidant and immune regulating agents.

A study by ALbadri (2020) evaluated the effect of Chenopodium Quinoa Saponins on the proliferation of MCF-7 and MDA-MB-231 breast cancers. The study's main objectives were to evaluate the cytotoxic effects of bioactive compounds, present in Chenopodium quinoa seed. this study was examined the cytotoxic effect of saponins extracted from the Chenopodium Quinoa seeds on MCF-7 and MDA-MB 231 breast cancer cell lines. And according to their investigations, the best concentration that significantly affected the MCF-7 cells proliferation was 25μM at 48 hours, while the best concentration found to significantly affect the MDA-MB231 cells proliferation was 50μM at 24 hours.

This study was focused on the cytotoxicity assay of ethanolic extract from C.quinoa. The results were obtained from the cytotoxicity activity carried out on MCF-7 cancer cell lines, HepG2 cancer cell lines and A375 cancer cell lines then were expressed as IC50 values, The lower the IC50 values the greater the anticancer activity, whereas the higher the IC50 values the lower the anticancer activity (Purnamasari et al., 2019). Measurement of cell viability was done with MTT assay. This assay is quantitative, sensitive, and very reliable. It is based on the ability of the mitochondrial dehydrogenase enzyme resident in living cells to turn the yellow water-soluble MTT into an insoluble dark purple formazan which is produced in direct proportion to the number of cells in a range of cell lines (Charles-Okhe et al., 2022).

This study had evaluated the effect of ethanolic extract from C.quinoa seeds on MCF-7 cell line in vitro. As seen from the results of the MTT assay [shown in Table (3-1) and Figure (3- 1)]. The C.quinoa extract induced varying levels of cytotoxicity. Each of different concentrations were added in three replicates then the mean for

each concentration was found. IC50 for MCF-7 after adding different concentrations was (47,49) of the cells. The C.quinoa seeds ethanolic extract showed an effect on MCF-7.

A study by Bata et al. (2020) investigated the lipoidal matter of Chenopodium quinoa seeds and its cytotoxicity potential against three human cancer cell lines. examination the lipoid constituents of C. quinoa seeds and assessment of cytotoxic activity of C. quinoa seeds lipoidal components and different fractions (methanol, chloroform, ethylacetate, and butanol) against three different types of human cancer cell lines, including lung cancer (A549), liver cancer (HepG2), and colon cancer (Caco-2). The result has shown that the tested extracts and fractions revealed that human lung cancer cells A549 were the most sensitive to treatment with single dose of 100 μg/ml. Lower response was observed against cell viability of HepG2 and Caco-2 cells after treatment with extracts and fractions

Another study by Doha A. Mohamed et al in 2019 made research on In vitro Anticancer Activity of Quinoa and Safflower Seeds and Their Preventive Effects on Non-alcoholic Fatty Liver, Quinoa seeds powder was promising in cytotoxicity against hepatocarcinoma cell line HEPG2 (IC50 was 14.6 μg). Feeding rats on HFD produced dyslipidemia and significant increase in liver functions and lipid peroxidation with significant elevation in liver triglycerides and total cholesterol. Quinoa and safflower seeds powder produced improvement in the biochemical parameters with different degrees. The result has shown that Quinoa and safflower seeds powder possessed cytotoxicity against hepatocarcinoma cell line HEPG2 and afford hepato-protection against NAFLD.

Another study by Khaled G. Abdel-Wahhab in 2021, cancer is a disease that is associated with abnormal proliferation and growth of living cells, and cyclophosphamide® therapy results in hepatotoxicity. So his study aimed to investigate ameliorative effect of Chenopodium quinoa ethanolic extract (QEE) against cyclophosphamide®-induced hepatotoxicity. Four groups (10 male Wistar rats each) were used: (1) healthy control group; (2) rats treated orally with QEE (400 mg/kg/day) for 4 weeks; (3) rats injected intraperitoneally with cyclophosphamide® (150 mg/kg/week) for 4 weeks; (4) rats received QEE after cyclophosphamide® intoxication another 4 weeks. The results revealed that QEE succeeded to decrease the hepatotoxicity-induced by cyclophosphamide®. QEE succeeded also in improving the histopathological picture of the liver. It could be concluded that QEE succeeded, to a great extent, to counteract the oxidative stress and regenerated the liver against cyclophosphamide®-resultant hepatotoxicity. QEE could be considered a promising candidate as a food supplement for the protection against the side effects of cyclophosphamide®.

This study had evaluated the effect of ethanolic extract from C.quinoa seeds on HepG2 cell line in vitro. As seen from the results of the MTT assay [shown in Table (3-2) and Figure (3-2)]. The C.quinoa extract induced varying levels of cytotoxicity. Each of different concentrations were added in three replicates then the mean for each concentration was found. IC50 for HepG2 after adding different concentrations was ~57 which is the concentration that killed 50% of the cells. The C.quinoa seeds ethanolic extract showed a very effective response on HepG2 even at lower concentrations which was the highest response.

This study had evaluated the effect of ethanolic extract from C.quinoa seeds on A375 cell line in vitro. As seen from the results of the MTT assay [shown in Table (3-3) and Figure (3-3)]. The C.quinoa extract induced varying levels of cytotoxicity. Each of different concentrations were added in three replicates then the mean for each concentration was found. IC50 for A375 after adding different concentrations was 140 which is the concentration that killed 50% of the cells. There was almost no effect nor change on A375 after being treated with C.quinoa seeds ethanolic extract.

5. CONCLUSIONS

- The C.quinoa ethanolic extract resulted to be modestly cytotoxic against the cancer cell lines (MCF-7, HepG2, and A375) which showed that the MCF-7 and HepG2 were the most sensitive to the C.quinoa ethanolic extract and A375 had the lower response.
- The anticancer properties of the Chenopodium quinoa extracts, it is important to continue examine the Chenopodium quinoa against specific cancer cell lines in an order to help finding a way to be able to use these properties in cancer therapeutics and by identifying how effective Chenopodium quinoa ethanolic extract on specific cancer cell lines, further research could be performed on examining the anticancer properties of Chenopodium quinoa to fully understand the mechanism of the cytotoxicity effects.

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