Cytotoxicity of Alcoholic Extract of Xanthium Strumarium Against Different Cancer Cell Lines

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Abstract
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. Xanthium strumarium is a well known herb which has been used since ages as a cure for various ailments like leucoderma biliousness, epilepsy, salivation, fever and poisonous bites of insects. The most important chemical constituents of Xanthium strumarium include phenolic compounds. The study attempted to help in developing a proper treatment for cancer. The study attempted to help in developing a proper treatment for cancer. This study aimed to investigate the possible in vitro cytotoxicity of Xanthium strumarium 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 X. strumarium ethanolic extract and A375 had the lower response. Further studies are needed to uncover the mechanism and chemical constituents of the obtained activity of X. strumarium.
Keyword: Cancer disease, X. strumarium L, Cytotoxicity

1. Introduction

Cancers as a group account for approximately13% of all deaths each year [1]. Herbs derived products have been used by about80% of populations particularly in developing countries [2].Although chemotherapeutic drugs and radiation are more powerful maneuvers for treatment of malignancy, but they are associated with serious adverse effects[3
Another challenge in the treatment of cancer is the development of resistance against therapy [4]. Hence, the major goals of herbs derived products in treatment of malignancy are; prevention of malignancy through induction of unsuitable environment for cancer cells growth, prevention of recurrence of malignancy, and stimulating immunity [5]. Herbs and medicinal plants have played an important role in pharmaceutical, cosmetics, and food industries.
Cocklebur (Xanthium strumarium L.) is an annual plant species belonging to the Asteraceae family. This plant possesses antitussive, cytotoxic, hypoglycemic, antibacterial, antifungal, antimalarial, sinusitis, urticaria, constipation, diarrhoea, lumbago, leprosy, stomachic, tonic, diuretic, sedative, allergic rhinitis, antirheumatic, antispasmodic, pruritis and antidiabetic properties [6]. The study attempted to evaluate the cytotoxicity effect of X. strumarium 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

This research was conducted from November 2021 until April 2022. Fresh leaves of Xanthium strumarium that used in this study were collected in November /2021 from farms (Iraq – Sulaimany**).** The plants were left at room temperature (20-25°C) until use

Figure 1: Xanthium strumarium L.

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

doi.org/10.31838/hiv23.03.292 Received: 26.11.22, Revised: 21.12.22, Accepted: 14.01.23.

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 (7).

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 **(8).**

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 Xanthium strumarium extract

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

Cell Line Maintenance (9)

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

1. The growth medium was aspirated and the cell sheet washed with PBS.
2. 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.
3. Fresh complete RPMI medium (15-20 mL) was added and cells were dispersed from the wedding surface into growth medium by pipetting
4. 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 104

MTT Protocol

The cytotoxic effect of different compounds isolated from X. strumarium extract was performed by using MTT ready to use kit

A- Kit contents

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

B- Protocol

1. Tumor cells ($1x10^4$– $1x10^6$ cells/mL) were grown in 96 flat well micro-titer plates, in a final volume of 200L complete culture medium per each well.
2. The microplate was covered by sterilized parafilm and shacked gently
3. The plates were incubated at 37C, 5% CO_2 for 24hrs.
4. After incubation, the medium was removed and two fold serial dilutions of the desired compound (6.25, 12.5, 25, 50, 100, 200
5. µ g/mL) were added to the wells
6. Triplicates were used per each concentration as well as the controls (cells treated with serum free medium). Plates were incubated at 37C, 5% CO_2 for selected exposure time (24 hours)
7. After exposure, 10 l of the MTT solution was added to each well. Plates were further incubated at 37C, 5% CO_2 for 4 hours
8. The media were carefully removed and 100L of solubilization solution was added per each well for 5 min.
9. 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 µg/mL).The results were obtained using ELISA reader and showed that the X. strumarium extract had an effect on the MCF-7 at high concentration and no effect on WRL68 normal cell line as shown in the Table (1) and Figure (2).

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

	MCF-7		WRL68	
Concen.	Mean	SD	Mean	SD
200.00	52.90	7.58	76.28	1.62
100.00	58.00	1.48	82.96	1.51
50.00	69.92	3.24	90.10	1.56
25.00	78.82	2.34	92.40	0.37
12.50	84.41	1.99	94.79	0.71
6.25	92.02	1.45	95.91	2.20

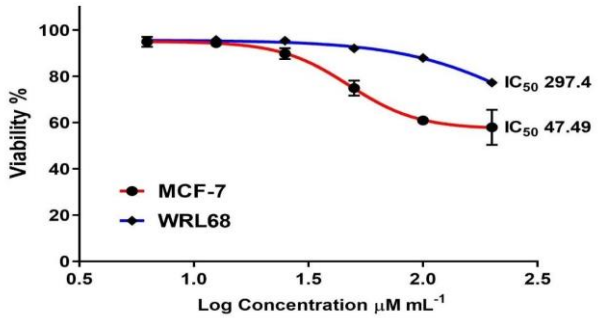

Figure (2): Cytotoxicity Results for MCF-7 Cell Line Comparing to WRL68 Cell Line

Cytotoxicity Results for HepG2 Cell Line

HepG2 liver cancer cells were exposed to a different concentration of X. strumarium (6.25, 12.5, 25, 50, 100, 200 µg /mL).The results were obtained using ELISA reader and showed that the X. strumarium 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 (2) and Figure (3).

Table (2): Cytotoxicity Results for HepG2 Cell Line Comparing to WRL68 Cell Line				
	HepG2		WRL68	
Concen.	Mean	SD	Mean	SD
200.00	43.90	2.43	79.28	1.62
100.00	49.65	2.04	88.96	1.51
50.00	68.09	2.71	92.13	1.56
25.00	77.27	4.26	95.98	0.37
12.50	80.86	2.07	95.90	0.71
6.25	95.41	0.55	95.91	2.20

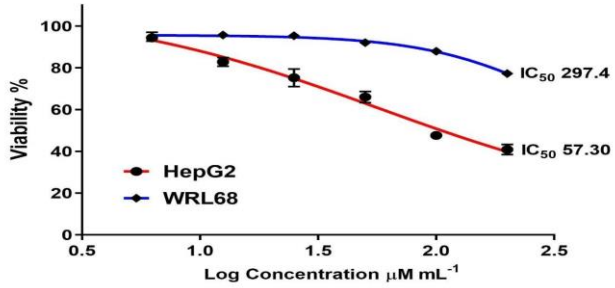

Figure (3): Cytotoxicity Results for HepG2 Cell Line Comparing to WRL68 Cell Line

Cytotoxicity Results for A375 Cell Line

A375 skin cancer cells were exposed to a different concentration of X. strumarium (6.25, 12.5, 25, 50, 100, 200 µg /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) and Figure (4).

Table (3): Cytotoxicity Results for A375 Cell Line Comparing to WRL68 Cell Line				
	A375			WRL68
Concen.	Mean	SD	Mean	SD
200.00	69.12	1.53	78.28	1.62
100.00	82.70	1.25	86.96	1.51
50.00	93.09	5.92	94.13	1.56
25.00	93.71	3.30	95.41	0.37
12.50	95.41	2.87	95.79	0.71

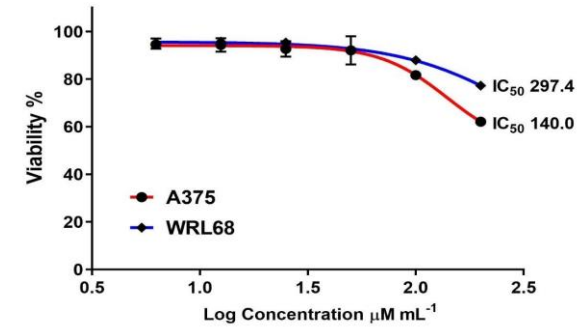

Figure (4): Cytotoxicity Results for A375 Cell Line Comparing to WRL68 Cell Line

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 **(10).**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 **(11).**

Several factors influencing the amount of extract; such as shaking speed, maceration period, solvents types and concentrations, water temperature in the water bath that contain leaves powder of Xanthium strumarium; the previous factors explain the variability in the percentage of extract amount**(12).**

These studies had explored the beneficial role of X. strumarium extract against the growth of different cancer types (Brest cancer, Liver cancer and skin cancer) by preparation of ethanolic extracts from X. strumarium 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 X. strumarium leaves. Evaluation of anticancer activity was carried out using X. strumarium 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 X. strumarium have a potential for use as anticancer, antioxidant and immune regulating agents **(13).** A study by Atheer S. Alsabah et al..,2018 showed that ., Chloroform extract has potent cytotoxicity in a dose dependent manner, against AMN3, AMJ13, MCF7 cell lines and less toxic effect on normal REF cell line;; where IC50 (2.93µg/ml), (2.99µg/ml), (2.67µg/ml), and (15.84µg/ml) respectively. The cytotoxicity of extract combined with Doxorubicin showed a synergistic activity on AMN3, AMJ13, MCF7 cell lines, and dose dependent effect on normal REF cell line (synergism at 3.125-6.25µg/ml, addition at 12.5µg/ml, and antagonism at 25-100µg/ml) **(14).**

5. Conclusions

1. The X. strumarium 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 X. strumarium ethanolic extract and A375 had the lower response.
2. The anticancer properties of the X. strumarium extracts, it is important to continue examine the X. strumarium 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 X. strumarium ethanolic extract on specific cancer cell lines, further research could be performed on examining the anticancer properties of X. strumarium to fully understand the mechanism of the cytotoxicity effects.

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