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The effect of plant extracts on cancerous cells and the study of some Indicators

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Abstract: The method of treating many plants, including Rheum ribes, is known by many scholars and physicians. In contrast to what chemotherapy costs, the cost of plant treatment is very cheap. The aim of the study is to determine the Cytotoxicity Effect of Ethanolic Rheum ribes Leaf extract Against the HepG2 Cell line. The study used an in vitro assay at varying concentrations for the cytotoxic activity of the extract of Ethanolic Rheum ribes Leaf. The most notable decrease in viable cell count was 62.5, 125 and 250 mg/ml, which seemed to induce cell death induction. The findings showed that leaf ethanol extract at different concentrations affects the HepG2 parameters of cells (Effect of rheum ribes on viability cells, Effect of rheum ribes on Mitochondrial membrane permeability, and Effect of rheum ribes on Cytochrome c). Rheum ribes, the study states, may be treated as a dietary antioxidant and anticancer agent.

Keywords: *Cancer, Cytotoxicity, Ethanolic Rheum ribes, Extract, HepG2 cell line, Patient,*

1. Introduction

By using natural plants, cancer may be defeated, or at the very least, the severity of its spread within the body may be decreased. When cancer is spread all over the body, it becomes a terrible disease that makes the opportunity for cells to survival closer to zero than to one (Malvezzi et al., 2014) . The approach of treating several plants, including Rheum ribes, is widely acknowledged by many academics and clinicians. In trials involving cancer patients, a few medicinal herbs have been shown to lower the severity of this deadly illness, and employing plants as a type of treatment is far less expensive than using chemotherapy to treat malignant tumors. The use of traditional herbs, which have been preferred as a prescription medicine since ancient times, is generally known(Kuo et al., 2014 ; Ebrahimnezhad et al., 2013 ; Amirghofran et al., 2011) . The beneficial advantages of herbal plants include better accessibility and lower side effects. *Rheum ribes* (R. ribes) are the most common raw materials in Asian countries for obtaining crude drugs(Amirghofran et al, 2007; Amirghofran et al, 2005 ; Amirghofran et al, 2006). It can be consistent with environments of extremely dark, arid, and clayish acidic or alkaline soil and can remain alive as cold weather freezes(Shoeb , 2006). R. Ribes is a native, perennial vegetable plant that grows in North and Central Asian sub-regions (Nehate et al.,2014). It is primarily found in Iran and Anatolia between 1800 and 2800 altitudes of rocky countryside(Tafrihi et al, 2014). Ribes have been used in Turkey and Iran as a diuretic treatment(Wang et al, 2014). Humans can use the Rheum Ribes Root (EERR) as an anti-trichomonas agent(Machana et al, 2012). It has been used for the treatment of asthma, obesity, gastrointestinal disorders, and hemorrhoids. In prostate cancer cells and human umbilical vein endothelial cells, EERR demonstrated activity in the activation of oxidative cell injury(Elkady et al., 2013 ; Manosroi et al., 2012). The current research aims to identify the *Rheum Ribes* root activity on oxidative cell damage induction. There are a variety of adverse effects on healthy cells from medications used to treat cancer patients. Nature has become the provider of medicinal agents

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with a broad variety of illnesses to be remedied. In order to cure illnesses, medicinal plants are good sources of health-related therapeutic aids. *Rheum Ribes* has been shown to suppress cancer cell development and proliferation (Amirghofran et al, 2010). The aim of this study is the Cytotoxicity Effect of Ethanolic *Rheum ribes* Leaf extract Against the HepG2 Cell line.

2. Materials and Methods

In the laboratories of the College of Science, University of Baghdad - Baghdad. Dust and other contaminants were washed from the plant cuttings and placed in the fridge to be used (Haifa N.Muter and Ali S.Mohamed 2017). Under a 37 °C atmosphere containing 5% carbon dioxide, the cells were incubated. Through the use of high content assay (HCS) kits, a cytotoxic Multipara meter assay was performed. The high content screen method of array scanning (Cellomics, PA, USA) was used to examine the plaque with stained cells. Array Scan HCS is a digital, computerized fluorescence imaging microscope that tracks the quantity and distribution of light in each cell as well as automatically identifies stained cells. One thousand cells were analyzed in each well.

Plant parts were separated from dust and other contaminants and kept for use in the freezer. The plant tissues were homogenized subsequent to a two-week period in which the mixture of plant tissues was washed with 70% ethanol rather than purified through a filter unit (Millipore, pore size: 0.22 mm). The impact of various concentrations of ethanolic Rheum ribes leaf extract (1000, 500, 250, 125, 62.5 µg/Ml (B.M.J. Alwash.,2017) on a HepG2 cell line maintained in RPMI (Gbico, Carlsbad, CA), supplemented with 10% FBS (Sigma), 1% 5000 units/ml penicillin, and 5000 lg/ml streptomycin (Sigma), was examined. The cytotoxic assay was performed for the multi-granule scale using high material assay kits (HCS), and these kits measure parameters including cell death, cytochrome c release, and improvement of cell membrane permeability(Fu et al., 2004 ; Hazra et al., 2002) .

Briefly, MTT dye and cell permeability dye were applied to live cells after 28 h of incubation and incubated at 37 for 30 min. Until assay with primary and secondary cytochrome c antibodies made from light-stained mice for 1 hour each, cells were modified, permeability and inactivated with 1x buffer solution Hoechst was applied to inverting the plating in stain solution(Susanti et al., 2012; Abdulla, WK and Maha, FA. 2022). A high-content assay method array assay (Cellomics, PA, USA) was used to examine plaque with stained cells. The HCS array assay is an automated computerized imaging microscope that tracks the quantity and distribution of fluorescence in each cell as well as automatically identifies stained cells. One thousand cells were analyzed in each well (Awale et al,2006).

2.1. Cell Lines and Cell Cultures

Human leukemia, T cell leukemia and Burkitt's lymphoma cell lines were used in the study, as well as solid tumor cell lines. Cells with 5 percent CO2 and 95 percent humidity were held at 37 ° (Amirghofran et al, 2011; Chan et al, 2011).

2.2. Statistical Analysis

Using GraphPad Prism 5.01 program, statistical analysis was performed. ANOVA Newman-Keuls assessed the details.

3. Result and Discussion

Different concentrations of leaf ethanol extract influence the HepG2 parameters of cells. An ethanolic extract of Rheum ribes was obtained from the leaves of the plant and subjected to cytotoxic activity analysis using HepG2. Various parameters, including cell loss rate, membrane potential intensity, and cytochrome C realizing intensity, were assessed. The results are presented in the following tables: (1) The impact of Rheum ribes on viability cells; (2) The influence of Rheum ribes on mitochondrial membrane permeability; and (3) The Effect of Rheum ribes on Cytochrome C.

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The objective of this study was to investigate the cytotoxic effects of an ethanolic Rheum ribes leaf extract derived from a locally cultivated Rheum ribes plant on a living Hep G2 cell line that was resistant to apoptosis, as shown in Table 1. The objective of this study was to investigate the cytotoxic effects of an ethanolic Rheum ribes leaf extract derived from a locally cultivated Rheum ribes plant on a living Hep G2 cell line that was resistant to apoptosis. The study employed an in vitro method to evaluate the cytotoxic effects of different concentrations (1000, 500, 250, 125, 62.5 µg/ml) of the ethanolic Rheum Ribes leaf extract via serial dilutions on the HepG2 cell line for 28 hours. The largest significant decline in viable counting cells occurs at 62.5, 125, and 250 µg/ml concentrations. All concentrations of ethanolic rheum ribes extract had no significant effect on the strong cell (P<0.001).

Table 1.
Effect of *rheum ribes* on viability cells.

	Mean ± SE
Treatment	**Valid cell counts**
Positive ctrl.	419.5 ± 63.5 d
Untreated	1179.0 ±4.0 a
1000 µ/ml	1030.0±80.0 ab
500 µ/ml	899.5 ±73.5 abc
250 µ/ml	830.5 ±21.0 bc
125 µ/ml	741.0 ±59.5 c
62.5 µ/ml	830.0 ±99.5 bc

Note: *(P<0.001): Means of different letters within the same column represented a significant difference.

The purpose of this study was to investigate the cytotoxic effect of an ethanolic Rheum ribes leaf extract derived from local Rheum ribes plants on the live Hep G2 cell line, as demonstrated in Table (2). To achieve this purpose, the analysis employed an in vitro assessment of the cytotoxic activity of the extract of Ethanolic *Rheum ribes* Leaf at various doses (1000, 500, 250, 125, and 62.5 µg / ml) in serial dilutions on the HepG2 cell line and exposure length (28 hours). The largest substantial decreases in mitochondrial membrane permeability were at 62.5, 125, and 250 µg/ml. All concentrations of Ethanolic Rheum ribes checked *permeability of the mitochondrial membrane* extract, but without impact (no important value P<0.001).

Table 2.
Effect of *rheum ribes* on mitochondrial membrane permeability.

	Mean ± SE
Treatment	**Mitochondrial membrane permeability**
Positive ctrl.	99.00 ± 4.0 b
Untreated	384.0 ±29.0 a
1000 µ/ml	501.0 ±0.00 a
500 µ/ml	419.0 ±7.5 a
250 µ/ml	422.0 ±13.0 a
125 µ/ml	412.5 ±3.0 b
62.5 µ/ml	422.0 ±1.50 b

Note: (P<0.001): Means of different letters within the same column represented a significant difference.

The purpose of this study was to investigate the cytotoxic effect of an ethanolic Rheum ribes leaf extract derived from local Rheum ribes plants on the live Hep G2 cell line, as displayed in Table (3). The study employed an in vitro method to evaluate the cytotoxic effects of different concentrations

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(1000, 500, 250, 125, 62.5 µg/ml) of the ethanolic Rheum Ribes leaf extract via serial dilutions on the HepG2 cell line for 28 hours. At 62.5, 125, and 250 g/ml, the most significant reductions in cytochrome c were observed. Various concentrations of ethanolic Rheum ribes extract, including cytochrome c, were examined; however, no significant results were obtained (P<0.001).

Table 3.
Effect of *rheum ribes* on cytochrome c.

Treatment	**Mean ± SE**
	Cytochrome c
Positive ctrl.	650.0 ± 50.0 a
Untreated	212.5 ±22.5 b
1000 µ/ml	240.5 ±40.5 b
500 µ/ml	190.0 ±14.0 b
250 µ/ml	180.0 ±36.0 b
125 µ/ml	75.0 ±16.0 b
62.5 µ/ml	90.00 ±10.5 b

Note: *(P<0.001): Significant disparities were expressed by the means of several letters in the same column.

The intrinsic pathway and the exogenous pathway associated with death receptors such as Fas (CD 95) leading to activation of caspase-8 are two main pathways leading to apoptosis(Asl et al., 2008; Duaa, A. and Reema; MA,2022). The activation of these proteins contributes to the permeability of the mitochondrial membrane, resulting in the reapplication of cytochrome C(Deslnaker et al., 2011). The oleuropein isolated from Ethanolic *Rheum ribes* Leaf was to investigate the toxicity of tamoxifen and the role of oleuropein in protecting against TAM-induced oxidative liver damage in Balb / c mice, and the ability to directly scavenge free radicals is at least partially linked to preventive action. Ethanolic *Rheum Ribes* Leaf has been shown to cause large We investigated the levels of apoptosis, viability, cell proliferation, and apoptosis in MCF-7 human breast cancer cells. Bovine brain capillary microspheres, human bladder cancer (T24), human breast cancer (MCF-7), rheum ribes in ethanol, and (BBCE), leaf rudimentary extracts have been shown to inhibit cell proliferation(Lumaret et al,2001). The Effect of the Ethanolic *Rheum ribes* oleuropein and leaf extract on chronic UV-induced skin damage, carcinogenicity, and tumor development was shown in several research by lowering the synthesis of VEGF, MMP-2, MMP-9, and MMP-13 by a two-level COX reduction(Thomas et al.,2008).

4. Conclusion

Natural plants will prevent or at least lessen the amount of cancer, the dreadful illness that spreads throughout the body and brings its cells closer to death than life expectancy. A lot of scientists and doctors know how to treat certain plants, like Rheum ribes. Studies on cancer have shown that using some medical herbs can help make this dangerous disease less severe, and it does not cost much to take care of plants. Based on the comprehensive results obtained from this study, Rheum ribes can be identified as a potential food antioxidant and anticancer agent. The results obtained from our research do not support the notion that this approach can be utilized as a comprehensive cancer treatment.

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