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Cytotoxic Effect of Hot Crude Extract of Fresh aloevera Plant on HepG2 Tumor Cell Line

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Abstract: This work Aimed to study cytotoxic study crude extract of fresh leaves aloe vera plant on HepG2 tumor cell line. To achieve this goal, hot crude extract of fresh leaves Aloe vera plant, the study utilized on *in vitro* evaluated of cytotoxic effect of hot crude extract on HepG2 tumor cell line at different concentrations and exposure at 72 hour treatment. The hot crude extract concentration ranging (0.3906-50) mg/ml in two fold serial dilution were used to treat HepG2 tumor cell line for 72 hour intervals. The results, appeared tumor cell line showed highest deity toward sensitivity toward the cytotoxic effect of the hot crude extra at concentration 3.125 mg/dl compare with normal cell line showed novel behavior effect, the lowest concentration of treatment give the most significant ($P<0.01$) inhibitor effect, however, lower inhibitor effect when exposure of 0.3906 mg/dl of hot crude extract. The conclusion is that there is enough evidence to support the claim that Aloevera plant, is chemotherapeutic agent against cancer.

Keyward : Aloevera plant, HepG2, cytotoxic assay.

التأثير السمي للمستخلص الحار لنبات الصبار في خط الخلايا السرطانية HepG2

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الخلاصة: هذه العمل يهدف لدراسة السمة الخلوية لمادة الخام المستخلصة من نبات الصبار على خط الخلايا السرطانية HepG2 للوصول الى الهدف تم الاستخلاص باستخدام الطريقة ودراسة سمة المراكب الخام في الزجاج على عالق الخلايا والمتضمنة. سرطان الخلايا السرطانية HepG2 في تراكيز مختلفة وزمن التعرض لمدة 72 ساعة من العلاج. وتركيز مستخلص الصبار يتراوح بين (50-) 0.396مايكروغرام / مليلتر سلسلة التخفيف النصفي للعلاج نوعين من الخلايا لفترات من 72 ساعة. واظهرت النتائج، ان تركيز المستخلص 3.125 مايكروغرام/مليلتر كان الافضل في تثبيط نمو الخلايا السرطانية HepG2 بالمقارنة مع خط الخلايا الطبيعية وذات فرق معنوي بمقدار ($P<0.010$) بالمقارنة مع التراكيز الاخرى. بينما كان اقل تأثير على خط الخلايا السرطانية للمستخلص بتركيز 0.3906 مايكروجرام / مليلتر والاستنتاج يدعم الادعاء بان مستخلص الصبار هو العلاج الكيميائي لمكافحة السرطان.

Introduction

Aloe barbadensis Miller is an important medicinal plant from Liliaceae family with African origin. Among 300 species, Aloe vera is considered as an important medicinal plant in many countries (1 and 2). The Aloe vera plant has been known and used for centuries for its health, beauty, medicinal and skin care properties. The name Aloe vera derives from the Arabic word "Alloeh" meaning "shining bitter substance," while "vera" in Latin means "true." 2000 years ago, the Greek scientists regarded Aloe vera as the universal panacea. The Egyptians called Aloe "the plant of immortality." The two major liquid sources of A. vera are yellow latex and clear gel, which is obtained from the large parenchymatic cells of the leaf (3). The main constituents of the latex are anthraquinones including the hydroxyathracene derivatives, aloin A and B, barbaloin, isobarbaloin and aloe amedin (4). A. vera possesses different biological and physiological activities, such as wound healing, anti-inflammatory, antibiotic, anti-bacterial, antiviral, anti-fungal, anti-diabetic and anti-neoplastic against some diseases (diabetes, cancer and allergy) (5 and 6). The Aloe vera plant have antiviral and antitumor activity, these actions may be due to indirect or direct effects. Indirect effect is due to stimulation of the immune system and direct effect is due to anthraquinones. The anthraquinone aloin inactivates various enveloped viruses such as herpes simplex, varicella zoster and influenza (7). It is well known that the use of plants as a therapeutic material due to their chemical substances of medicinal value is common all over the world (8). Solid material of A. vera leaves consists of a range of compounds including vitamins, minerals, enzymes, polysaccharides, phenolic compounds, and organic acids (9). Different studies indicated anti-tumor activity for A. vera gel in terms of reduced tumor burden, tumor shrinkage, tumor necrosis, and prolonged survival rates. In addition to these effects, A. vera gel was also shown to have chemo-preventative and anti-genotoxic effects on benzo (α) pyrene-DNA adducts (9). Stimulation of immune response is one mechanism of action that was proposed for these anti-cancer effects of Aloe polysaccharides (10). From the previous data we suggested our study to investigate the cytotoxic effects of crud extraction of Aloe vera on HepG2 tumor cell line by using different concentrations.

Materials and Methods

Sample collection of plant

The fresh of *Aloe vera* plant collected from the garden of plants in Baghdad University were included in this study. The plant parts were cleaned from the dust and other particles and stored in the freeze until use.

Hot crude extraction from plant tissues

After cleaning the plant tissues with distilled water, the plant tissues were homogenized and approximately 50 grams from sample were ground with 500 ml of hot distilled water, left it on magnetic stirrer for 15 minute, the extract was filtered and then solvent was evaporated using rotory evaporator.

Cytotoxicity assay

It is also called a cell growth inhibition assay, the in vitro method was used to investigate the effect of crude extraction on tumor cell line at different concentrations (50; 25; 12.5; 6.25; 3.125; 1.5625; 0.78125; and 0.3906 µg/ml) and exposure times of 72 hours. HepG2 (Hepatocellular carcinoma, human) which was derived from the liver tissue of a 15 year old Caucasian American male with a well differentiated hepatocellular carcinoma. HepG2 are adherent, epithelial-like cells growing as monolayer and in small aggregates, have a model chromosome number of 55 (11). HepG2 cell line which used in this study supplied by Animal cell culture lab/ Biotechnology Research Centre / AL- Nahrain University. Cells were cultured in RPMI 1640 media supplemented with 10% fetal calf serum, 50 mg/ml streptomycin and 1000U/L penicillin. Cell line was grown as a monolayer in humidified atmosphere at 37°C with 5% CO2. The experiments were performed when cells were healthy and at logarithmic phase of growth. Single cell suspension was prepared by treating 25 cm3 tissue culture flask with 2 ml trypsin solution incubated for 5-7 min at 37°C in an incubator supplemented with (5%) CO2 after detachment of the cells from the flask surface single cell suspension was made by gently taping of the flask followed by the addition of 20ml of growth medium supplemented with 10% fetal calf serum then the viability test of the cells was made by using trypan blue dye which stains the dead cells. Cell suspension was well mixed followed by transferring 200µl/well of the 96 well flat bottom micro titer plate using automatic micropipette containing (1x105 cells/well). Plates were incubated at 37°C in an incubator supplemented with (5%) CO2 until 60-70% confluence of the internal surface area of the well, the cells were then exposed to different concentrations of hot crude extract of fresh leaves Aloevera plant, the extract added to the cells in triplicate form of each concentration, only cells incubated with culture media represented the negative control. The exposure times was 27hr, after elapsing the incubation period, 50 µl/well of neutral red dye freshly prepared were added to each well and incubated again for 2 hrs, viable cells will uptake the dye and the dead not, the plates washed by PBS to remove the excess dye , then 100µl/well of elute solution were added to each well to draw out the dye from the viable cells. Optical density of each well was measured by using ELISA reader at a

transmitting wave length on 492nm (12).

The inhibition rate was calculated according to following the formula:

% inhibition rate = (control – test / control) x 100

Statistical analysis

The statistical analysis is a very important final step in the research to analyses and evaluate the obtained results. Medical statistics of this study was conducted via computer based statistical program which was:

1. SPSS for Windows computer package (Programm 11.5).
2. Microsoft Excel 2003.

The statistical analysis tests which used in this were as follows: Duncan test is non-parametric test which used to determine whether there is a significant difference between the expected frequencies with respect to two variable. It is a well used test for the medical statistics. P value <0.05 is considered a significant correlation.

Results and Discussion

The cytotoxic effect on HepG2 tumor cell line: table (1) revealed that 3.125µg/ml concentrations gave a significantly (P<0.001) high inhibition rate of cells while being low gradually with low concentrations. The extracted hot crude extract was very sensitive and liable through many environmental changes even with suitable protection which led to get the best results at 72 hours of exposure. This might explain the more significant inhibition rate at different concentration for 72 hours of exposure.

Table (1): The cytotoxic effect as (IR%) of extract hot crude extract on HepG2 tumor cell line at (72) hours of exposure.

Concentration	72 hours
µg/ml 50	33.613±0.611 a
25µg/ml	30.710±1.014 a
12.5 µg/ml	20.546±0.358 b
6.25 µg/ml	19.879 ±0.381 b
3.125µg/ml	55.382±2.648 c
1.5625µg/ml	31.770±0.692 a
µg/ml 0.7812	25.260±1.113 d
µg/ml 0.3906	6.840±0.529 e

P < 0.001, the means within any column with different letters are of significant differences

Table (1) showed the results of the significant effect at (P<0.001) level, after 72 hours of exposure the highest concentration 50µg/ml of hot crude extract showed cytotoxic inhibitory effect (33.613±0.611%), the inhibitory effect decreased to reach to (6.840±0.529) % after 27 hours of exposure of concentration 0.3906µg/ml of hot crude extract and highest cytotoxic inhibitory effect (55.382±2.648 %) of exposure on HepG2 tumor cell line when using 3.125µg/ml from extract. The hot crude extract showed an antiproliferative effect a significant inhibitory effect (33.613±0.611; 30.710±1.014 and 31.770±0.692) % at concentrations (50µg/ml, 25µg/ml and 1.5625µg/ml) respectively after 72 hours of exposure HepG2 tumor cell line. The Aloe plant is a part of the lily family but its Aloe barbadensis is commonly called A. vera. Aloe was also mentioned as a laxative in the Egyptian Papyrus Ebers from 1552 BC (13). Juneby, 1999, (14) referred to use of A. vera is based on its historic and traditional use, and analysis of pharmacologic and toxicological research. The Allium L. test has important advantages (15) and has been used for many years in investigating physical and chemical mutagenesis, pollutant agents, plant extracts, and similar active material's cytogenetic effects in mitotic cell division. It is stated that the Allium test exhibits similar results with mammalian test systems (16 and 17). Aloe vera L. (Aloe barbadensi s Miller) has been used for its curative and therapeutic properties ranging from dermatitis to cancer for centuries (18), Kim et al., 1999, suggest a possible benefit of using aloe gel in cancer chemoprevention. El-shemy et al., (2010), shown the potential anticancer that extract from Aloe vera asignificant against acute myeloid leukemia and Acute lymphocyte leukemia cancerous cells (19). The conclusion is that there is enough evidence to support the claim that Aloe vera plant, is chemotherapeutic agent against cancer.

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