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IN VITRO SUPPRESSION OF HEPATOCELLULAR CARCINOMA WITH ALEO VERA AND CALLIGONUM COMOSSUM

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ABSTRACT

This study is thought to evaluate the antitumor effect of Aleo vera (A. vera) and Calligonum comosum *(C. comosum)* extracts using human hepatocellular carcinoma cell line (HepG2). Cells were grown in the absence and presence of various concentrations of A. vera and *C. comosum* to study their effect in killing cancer cells of HepG2 cell line using Methylthiazol Tetrazolium (MTT) . Results show gradual increase in cell death of human hepatocellular carcinoma cell line in a dose and time dependant manner. These findings suggested that A. vera and *C. comosum* have antitumor effect and could be a kind of promising agent for further evaluations in the treatment of hepatocellular carcinoma.

INTRODUCTION

Hepatocellular carcinoma (HCC) is among the most lethal and common malignancies in the human population, with approximately 5, 50, 000 new cases and almost as many deaths per year (**Bruix *et al*., 2004& Raoul 2008).** For HCC, surgery (resection or transplantation) is curative but restricted to patients at an advanced stage, while non-surgical therapeutic methods prove to be unsatisfactory, with 1- and 3-year survival rates of 20 and 5%, respectively, and a median survival of only 8 months **(Song *et al*., 2004& Olsen *et al*., 2009).**

To meet this challenge, it is of a great significance to explore new nontoxic medicines with high efficacy. Natural products derived from medical plants have recently received much attention as potential chemopreventive and chemotherapeutic agents with low toxicity. Aleo vera (A. vera) and Calligonum comsum *(C. comosum)* "orta" are Egyptian desert plants that

are used as source of medicine by rural people. The aim of this study was to evaluate biochemically the antitumour effect of different doses of A. vera and C. comosum extracts on human hepatocellular carcinoma (HepG2).

MATERIALS AND METHODS

Drugs and reagents:

A. vera and C. comosum extracts was obtained from MBI company, United Kingdum. A. vera and *C. comosum* extracts were prepared to a concentration of 0.1 M in dimethyl sulfoxide (DMSO) as a stock solution and stored at -20°C. The working concentrations used in this study were from 50 µM to 2000 µM and were freshly diluted with medium before each experiment with a final DMSO concentration of less than 0.1% **(Samarakoon et al.2012).**

Cell culture

Human hepatocarcinoma (HepG2) cell lines were maintained at the Centre for Research and Development of Medical Experimental Research Center, Mansoura University. The cell culture medium was Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin and 100 µg/ml streptomycin. The cells were cultured at 37°C under a humidified atmosphere containing 5% CO2. Cells in a 75cm^2 tissue culture flask ***(Machana* et al.** *2011).*

HepG2 viability assays:

Cell culture

HepG2 (human hepatoma) cells were harvested by trypsinization, plated (5 x 10 ^{4} cells/ml) in 96-well cell culture plate and maintained in Dulbecco's Modified Eagle Medium (DMEM) for 48h at 37 0 C in 95% air / 5% CO 2 atmosphere, with 95% humidity. Cultures were exposed only to medium (1% DMSO, controls) or medium containing different concentrations of aqueous A. vera or C. comosum extracts dissolved in 1% DMSO. The crude extracts were dissolved as 20 mg/ml as stock solutions which were then diluted with DMEM to desired concentrations ranging from 10 to 20000 µg/ml (50, 100, 150, 200, 250, 500, 750, 1000, 1250, 1500, 1750 and 2000 µg/ml), and incubated for 48 h. At the end of this incubation period, cells were briefly washed with Phosphate-buffered saline (PBS). Fresh medium (100µl) was then placed in each well and MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays performed as **(Oka *et al*., 1992).**

Overall cell activity - MTT assay:

Effect on overall cell activity was determined by performing the MTT assay based on the method of **Oka *et al*., (1992)**. The MTT assay measures the metabolism of 3-(4, 5-dimethylthiazol-2yl) -2, 5 - biphenyl tetrazolium bromide to form an insoluble formazan

precipitate by mitochondrial dehydrogenases only present in viable cells. After exposure of cells to different concentrations of the aqueous extract for 48 h, one hundred microlitres of MTT (1mg/mL) solution was added to each well of the 96-well plate, and the plate was incubated at 37 ^{o}C for 2 hr.

The medium was then removed by aspiration. Finally, 100 µl DMSO was added per well, the plate was shaken for a further 30 min and the absorbance at 520 nm with a 650 nm reference wavelength was measured using a microplate reader EL x 800 Universal Microplate Reader, BIO-TEK INSTRUMENTS, USA) and The percentage of cytotoxicity compared to the untreated cells was determined with the equation given below. A plot of % cytotoxicity versus sample concentrations was used to calculate the concentration which showed 50% cytotoxicity (IC50).

IC50 (%) = [100×(Absorbance of untreated group−Absorbance of treated group]/Absorbance of untreated group. **(Oka *et al*., 1992).**

DISCUSSION

Since current cancer therapies are minimally effective and exhibit intolerable toxicities in most cases, natural products and their derivatives are increasingly considered as a new and an ideal source for anticancer drugs discovery (**Butler, 2005; Aggarwal et al., 2006).** Antitumor activity of 50% ethanol extract (100 mg/kg) of A. vera was evaluated by **Bharath (2011)** against Ehrlich ascites carcinoma tumor in mice. He found that the 50% ethanol extract of A. vera exhibited antitumor effect by modulating lipid peroxidation and augmenting antioxidant defense system in Ehrlich ascites carcinoma bearing mice. Some studies have found that Aloe-emodin (a natural active compound present in the leaves of Aloe vera (**Reynolds, 1985**) exhibit anticancer activity on neuroectodermal tumors, lung squamous cell carcinoma and hepatoma cells (**Pecere et al., 2000; Lee et al., 2001; Kuo et al., 2002**). Indeed, anti-inflammatory, anti-ulcer and anti-cancer activities of C. comosum have been reported in rat and shrimp animal models (**Liu et al., 2001; *Badria et al., 2007).***

Abdel-Sattar et al., (2012) showed that C. comosum methanolic and aqueous extracts ameliorated haloperidol induced neuro- and hepatotoxicities in male albino rat. In Fig. 1. Effects of A. vera (50–2000 µg) on viability of HepG2 cells after 48 h. HepG2 viability were measured by MTT assay showed that A. vera causes cell death in a dose and time dependant manner . Also Fig. 2. Effects of C. comosum (50-2000 µg) on viability of HepG2 cells after 48 h showed that C.comosum causes cell death in a dose and time dependant manner.

RESULTS

A. vera concentration	O.D
0	100
50	98
100	90
150	86
200	80
250	77
500	70
750	68
1000	55
1250	45
1500	40
1750	31.5
2000	30

C. comosum concentration	O.D
0	100
50	96
100	89
150	85
200	80
250	78
500	68
750	58
1000	48
1250	45
1500	40
1750	36
2000	33

(5)

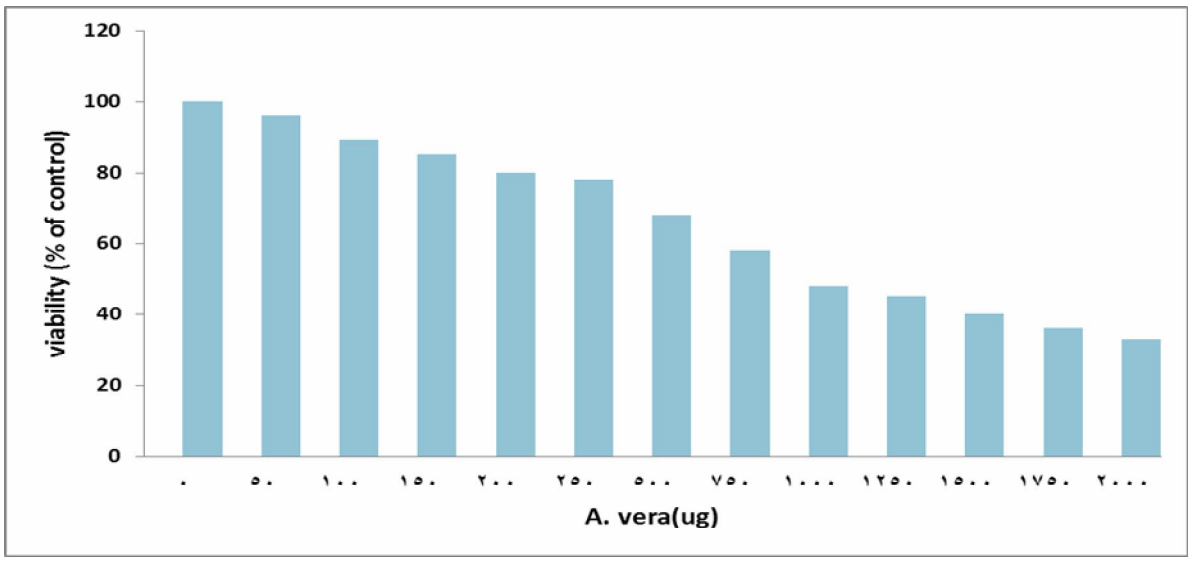

Fig. 1. Effects of A. vera (50–2000 µg) on viability of HepG2 ells after 48 hr. HepG2 viability were measured by MTT assay. Results are shown as mean ± SEM, derived from at least n = 4 replicates IC50 = 1045.

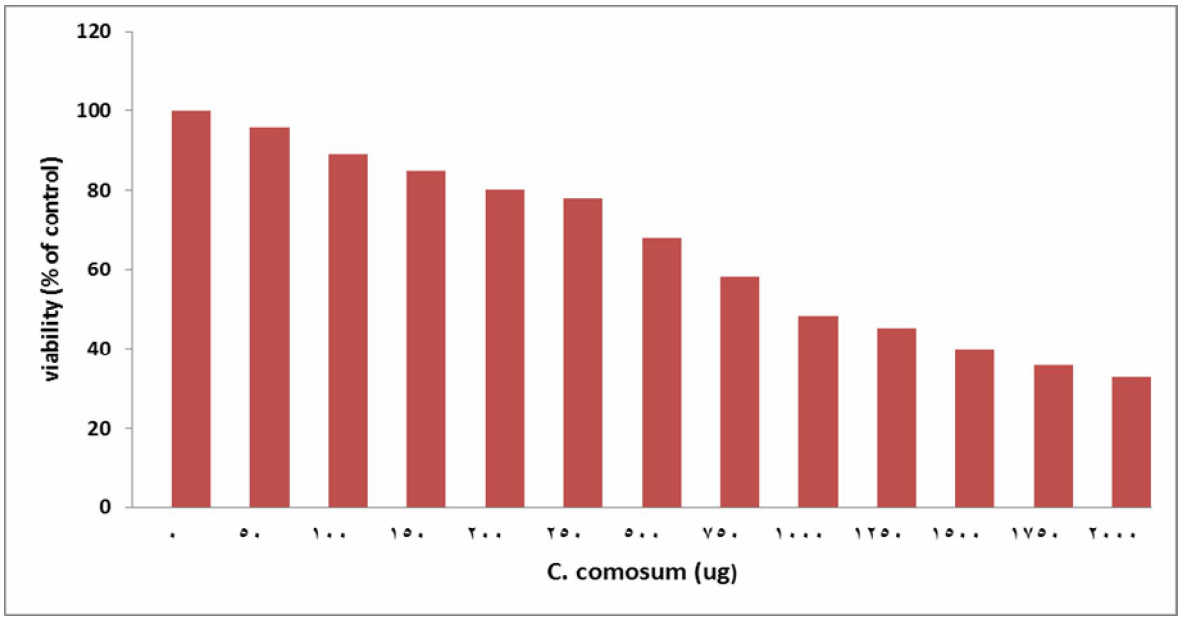

Fig. 2. Effects of *C. comosum* (50–2000 µg) on viability of HepG2 ells after 48 hr.

HepG2 viability were measured by MTT assay. Results are shown as mean ± SEM, derived from at least n = 4 replicates IC50 = 960.

(6)

CONCLUSION

We could be concluded that, the present study demonstrates that the herbal extracts of A. vera and C. comosum can induce cell death in human hepatocellular carcinoma HepG2 cell, in a dose and time dependent manner. These findings suggested that A. vera and C. comosum have antitumor effect andcould be a kind of promising agent for further evaluations in the treatment of hepatocellular carcinoma.

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الملخص العربى

تثبيط سرطان الخلايا الكبديه بواسطة خلاصه نبات الألوفيرا و نبات الأرطة في المعمل

يعتقد من هذه الدراسة ان خلاصه نبات الألوفيرا وخلاصه نبات الأرطة لهم دور في مكافحه السرطان بواسطة استخدام نسيج خلايا سرطان الكبد البشرية. تم إنماء الخلايا في وجود او عدم وجود تركيزات مختلفة من خلاصه الألوفيرا و خلاصه الأرطة لدراسة قدرتهم علي قتل خلايا السرطان عن طريق تجربه الميثيل ثايوزول تيترازوليم . أوضحت النتائج زيادة تدريجية في نسبه موت خلايا سرطان الكبد. وتشير النتائج الي أن الألوفيرا و الأرطة لهما تأثير علي مكافحه الورم ومن المحتمل أن يكونا عنصر واعد لمزيد من التقييمات في علاج سرطان الك