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IN VITRO CYTOTOXIC ACTIVITY OF SOME WILD PLANTS EXTRACTS AGAINST RAW264.7 CELL LINE

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Abstract

This study investigated the role of ethanolic extracts of four plants are *Artemisia sieberi, Sonchus oleraceus, Savignya parviflora and Reomeria hybrida* as a cytotoxic agent against RAW264.7 cell line using different concentrations of this extracts 6.25, 12.5 , 25 and 50 µg / ml for exposure time 24 hour. The results revealed a clear cytotxoic activity of this extract on growth of RAW264.7 cell line, and the effect was concentration dependent.

The signification inhibition (P<0.05) was obtained at a concentration of 50 and 25 µg / ml as compared to control for all extracts. In addition, the growth of RAW264.7 cell line was more sensitive to *Sonchus oleraceus* and *Savignya parviflora* in comparison with *Artemisia sieberi and Reomeria hybrida* . In conclusion, the extracts showed inhibitory effect on RAW264.7 cell line and it is promising to use as anticancer drug.

Key word: *A. sieberi, S. oleraceus, S. parviflora,and R. hybrida* c*ytotoxicity,* RAW264.7 cell.

Introduction

The plants of Al Muthanna desert are a small herb that grows in dry and semi-dry regions. The greatest number of these species was attained in Asia (Gordanian *et al.*, 2014; Jose *et al.*, 2012). The most species have been used in traditional medicine as anti-infectious, anti-bacterial, gastric tonic, digestive and stomachic (Nezhadali and Parsa 2010). Recently, monoterpenes, sesquiterpenes, sesquiterpene lactones, flavonoids, coumarins, sterols, polyacetylenes have been isolated from Artemisia species (Hoffman *et al.*, 1999; Sengul *et al.*, 2009). Previous studies on some Artemisia species have shown that most species possess medicinal properties such as anti-bacterial and anti-cancer effects (Akrout *et al.*, 2011; Zahi *et al.*, 2010; Devmurari and Jivani 2010; Sarath *et al.*, 2007). Many in vitro and in vivo studies have been published on the anticancer activity of different species of Artemisia (Mcgovern *et al.*, 2010; He *et al.*, 2011; Perumal *et al.*, 2010).

Polyphenols constitute a distinct group of natural compounds of medicinal plants as *Savignya parviflora*; and ethanolic extracts exhibit a wide range of multi-hysiological activities such as antioxidant, antiinflammatory, and anti-ulcerogenic activities (Nermien *et al.*, 2017).

Roemeria hybrida, contains a rare class of isoquinoline alkaloids namely, proaporphine-tryptamine dimers and roehybridine â-N-oxide which considered as anticancer natural products (Samad *et al.*, 2016).

Sonchus oleraceus L. (Asteraceae) (SO) is a dietary and traditional medicinal plant that can be cooked and eaten to treat inflammatory disease (Qi Li *et al.*, 2017). Currently, studies have indicated that the extract of SO exert many bio-activities, including antioxidant (McDowell *et al.*, 2011).

Cytotoxicity screening models provide important preliminary data to help in selecting plant extract with potential antineoplatic properties (Cardellinall, *et al.*, 1999). Also, cytotoxicity test is a qualitative and quantitative test to determine how cell death (Rachmani, *et al.*, 2001). For this purpose, this study was designed to evaluate the cytotoxic activity of the different plant

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extracts on the growth of cell line *in vitro*.

Materials and methods

Plant collection

Young and adult leaves of plants were collected from several specimens located in the Al Muthanna desert, Iraq. The collected leaves were kept at room temperature and left to dry. The leaves were identified by a plant taxonomist at the Biology Department, College of Science, University of Baghdad.

Preparation of the plants extraction According to (Mostafa *et al.*, 2018; Freshney, 2012) as follows:

Ethanolic extract: Fifty grams of powdered leaves were extracted with 250 ml of absolute ethanol using soxhelt apparatus for four hours at 45ºC. The extraction was filtered and the filtrate was evaporated to dryness under reduced pressure at 45ºC and stored at 4ºC until use.

Cell culture and Cytotoxicity

This study was conducted at Tissue Culture Unit of the Biotechnology Research Center, Al-Nahrin University. The RAW 264.7 cell line (macrophage cell line) was used. The colorimetric cell viability MTT assay was used (Zahi *et al.*, 2010). Cells were grown in RPMI-1640 medium containing 10% fetal calf serum (FCS and 1% penicillin-streptomycin antibiotic. The cytotoxicity of the ethanolic extracts from different plants was tested using the method of (Gaom *et al.*, 2003). In brief, the extracts were diluted with complete RPMI-1640 medium to give concentrations ranging from 6.25-50 µg/ml. The cells were grown in tissue culture flasks containing growth medium at 37ºC in an atmosphere of 5% CO_2 and 95% relative humidity in a CO_2 incubator. The cells at subconfluent stage were harvested from the flask by treatment with trypsin-versine solution (20 ml trypsin in 370 ml PBS containing 10 ml versine) and suspended in the medium. Cells with more than 97% viability (trypan blue exclusion) were used for determination of cytotoxicity. Cells were plated in 96-multiwell plate for 24 hours in a CO_2 incubator at 37ºC. Different concentration of the tested substance (6.25, 12.5, 25 and 50 µg/ml) were added to the cells (four replicate wells were prepared for each individual concentration) and re incubated for further 24 hours. Control cultures containing RPMI-1640 alone were tested for back ground cytotoxicity. After that, 10 µL of MTT solution (5 mg/mL) was added to each well and incubated at 37°C for 4 hours. Finally, 50 µL of DMSO (dimethyl sulfoxide) was added to each well and incubated for 10 min. The absorbance was measured for each well at 620 nm using an ELISA reader. Inhibitory rate of cell growth was calculated as following formula (Marinova *et al.*, 2005):

Inhibition (%) = [(optical density of control wells – optical density of test wells)/optical density of control wells]*100

Statistical analysis:

The experiments data were analyzed using statistical software SPSS (SPSS 16.0 for windows, SPSS Ins. III., USA). Significant difference between control and sample means was assessed using student's t-test and p values < 0.05 were considered significant.

Results and Discussion

The effect of treating RAW 264.7 cells of *A. sieberi, S. oleraceus, S. parviflora, and R. hybrida* leaves extracts are shown in Fig. 1 and table 1. Growth inhibition in a dose – dependent manner was observed in RAW 264.7 cell line. The extracts have significant effect ($p < 0.05$) on RAW 264.7 cell line at the concentrations (25 and 50 µg / ml) at 24 hrs. Also, there is significant effect ($p < 0.05$) for the concentration (12.5 µg/ ml) for the *S. oleraceus and S. parviflora* extracts, and 6.25 µg/ml for the *S. oleraceus* extract. This means that all concentrations of *Sonchus oleraceus* extract have significant effect ($p < 0.05$) on RAW 264.7 cell line. Where the other concentrations showed nonsignificant effect during the period of exposure, Fig. 1.

Based on the results of the viability, the growth inhibiting (G1) effect was calculated, the results in table 1- revealed that the RAW264.7 cell line was sensitive to the *A. sieberi, S. oleraceus, S. parviflora,and R. hybrida* leaves ethanolic extracts after period of exposure. Also, the results in table 1- showed that effects of extracts were a dose – dependent manner and Sonchus

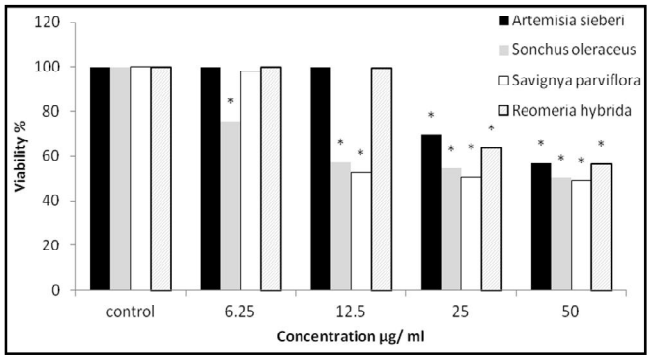

Fig. 1. Effect of *Artemisia sieberi, Sonchus oleraceus, Savignya parviflora and Reomeria hybrida* leaves ethanolic extracts at different concentrations on viability of RAW 264.7 cell line during 24 hours of exposure.

*Significant at $p < 0.05$.

Table 1: Percentages of inhibition of RAW264.7 cells by the ethanolic extracts of *A. sieberi, S. oleraceus, S. parviflora,and R. hybrida* during 24 hours of exposure.

R. *hybrida*	S. *parviflora*	S. *oleraceus*	A. *sieberi*	Plant extract CON. µg/ml
0	2.1	24.5	0	6.25
0.6	47.4	41.7	0	12.5
36.2	49.5	45.6	29.5	25
53.4	50.9	49.7	43.1	50

oleraceus extract was more sensitive to RAW264.7 cell line.

The results of the present study showed potent cytotoxic effects against RAW264.7 cell line in concentration 50 mg/ml it gave 53.4% inhibition fo *R. hybrida* and extract and at 25 mg/ml was 49.5% inhibition of *S. parviflor*. also, other extracts have the effect but a dose-dependent manner. The effect which may be due to existing phytochemicals such as flavonoids and alkaloids of this plant (Hoffman *et al.*, 1999; McDowell *et al.*, 2011). Cytotoxic drugs as anticancer are known to kill cells by inhibiting cell cycle. Many phytochemicals of plant damaging DNA during the S-phase of the cell cycle or by blocking the formation of the mitotic spindle in M phase (Marinova *et al.*, 2005).

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