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Cytotoxic Effects of Ethyl Acetate Extract of *Sambucus ebulus* Compared With Etoposide on Normal and Cancer Cell Lines

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ABSTRACT

Sambucus ebulus is a native botany and exists in large amount in Iran and consists of anti-cancer substances such as ebulin (RIP-II, ribosome inactivated protein-II), flavonoids, etc. Isolation and identification of some potent anti-tumor compounds from medicinal plants, has motivated researchers to screen different parts of plant species for anti-tumor effects. In previous studies, anti-inflammatory effects of n-hexane and methanolic extracts of *S. ebulus*, nephro- and hepato-toxic effects of ethyl acetate extract of this plant and evaluation of role of vitamins C and E on prevention of cellular and pathological disorders induced by the ethyl acetate extract was performed and reported. So, cytotoxic activity and IC₅₀ of specific concentrations of ethyl acetate extract of fruits of *S. ebulus* on 4 normal and cancer cell lines was studied.

Also, Etoposide, a chemotherapeutic drug, was administered to control positive group. The normal cell lines were CHO and rat fibroblast and cancer cell lines were HepG2 and CT26. The cytotoxic effects and IC₅₀ of the extract on the cell lines were studied followed by MTT assay.

The results showed that the ethyl acetate extract of *Sambucus ebulus* possesses lower IC₅₀ in the cancer cell lines in comparison with the normal cell lines. On the other hand, the extract possesses higher IC₅₀ in comparison with Etoposide on all 4 normal and cancer cell lines (P<0.05), but it manifested a good cytotoxic compound which can introduce as an anticancer compound.

KEYWORDS: *Sambucus ebulus*, Etoposide, Cytotoxicity, IC₅₀, MTT assay, cell line.

INTRODUCTION

At present time utilization of pharmaceutical plants (especially native plants) in treatment of diseases has been specific importance. Isolation and identification of some potent anti-tumor compounds, such as colchicine,

Vinca alkaloids, taxol and etc., as natural anticancer compounds, has encouraged scientists to screen different parts of plant species against cancer cell lines (1–4). While the plant *S. ebulus* (Caprifoliaceae L.) is a native botany, exists in large amount in Iran and consists of anticancer substances, such as ebulin (RIP-II, ribosome inactivated

protein-II), also flavonoids, steroid substances, cardiac glycosides, tannins, caffeic acid derivatives and other isolated substances (5–10).

According to our previous investigations, we observed the anti-inflammatory effects of n-hexane and methanol extracts of *S.ebulus*, and renal and hepato-toxic effects of ethyl acetate extract of fruits of this plant in mice; and the evaluation of role of vitamins C and E, as anti-oxidant compounds on prevention of cellular and pathological disorders which were produced by ethyl acetate extract of *S.ebulus* was performed (10, 11). Thus, we decided to study the IC₅₀ and cytotoxic effects of ethyl acetate extract of *Sambucus ebulus* on normal (CHO and rat fibroblast cell lines) and cancer cell lines (HepG2 and CT26 cell lines), and compare the parameters with the results concluded from cytotoxic effect of Etoposide on these cell lines.

MATERIALS AND METHODS

1. Plant materials

Fruits of *Sambucus ebulus* were collected from the Farah Abad region, Sari (Mazandaran province, Iran) in June 2007.

2. Isolation and Extraction

50 g of the fruits was chopped and soaked in 150 ml of ethyl acetate for 48 hours and then percolated (5 hours, 30 drops/min) (12). The extracts were concentrated by rotary evaporator, and the specific concentrations of the ethyl acetate extract (5, 25, 50, 100, 150, 25, 500 and 1000 µg/ml) were prepared using DMSO solvent and DMEM media (1:19).

3. Cell lines

CHO (Normal Chinese hamster ovarian cells), normal rat fibroblast, HepG2 (Human hepatocarcinoma) and CT26 (Human colon carcinoma) cell lines were purchased from Pasteur Institute of Iran in Tehran.

This completed media was sterilized by 0.22 µm microbiological filters and kept at 4°C before use.

4. MTT assay

In MTT assay, 50 µl of DMEM/F12 including 1×10⁵ cells were added to 3 wells for each concentration of the extract and Etoposide. Then, they incubated for 72 hours. After incubation, the cell lines were exposed to 50 µl of each 0, solvent (DMSO), 5, 25, 50, 100, 150, 25, 500 and 1000 µg/ml of ethyl acetate extract of *S.ebulus* and 0, 5, 25, 50, 100 µg/ml of Etoposide for 72 hours, and then washed using sterile normal saline 0.09%. After this period, the contents of wells were excluded; the cells were dyed by 30 µl of MTT (Methyl thiotetrazolium) and incubated for 4 hours. Then, MTT solution was excluded from wells, and 30 µl of DMSO was added to each well and the 96 well/plates were shaken for 15 minutes. At the end, the absorbance was determined by ELISA reader (λ_{max} =490 and 630 nm).

5. Statistical analysis

Prism ver.3 Software was used to perform statistical tests. Analysis of variance (one-way ANOVA) followed by Tukey test was used to determine the differences among the groups (p<0.05).

RESULTS

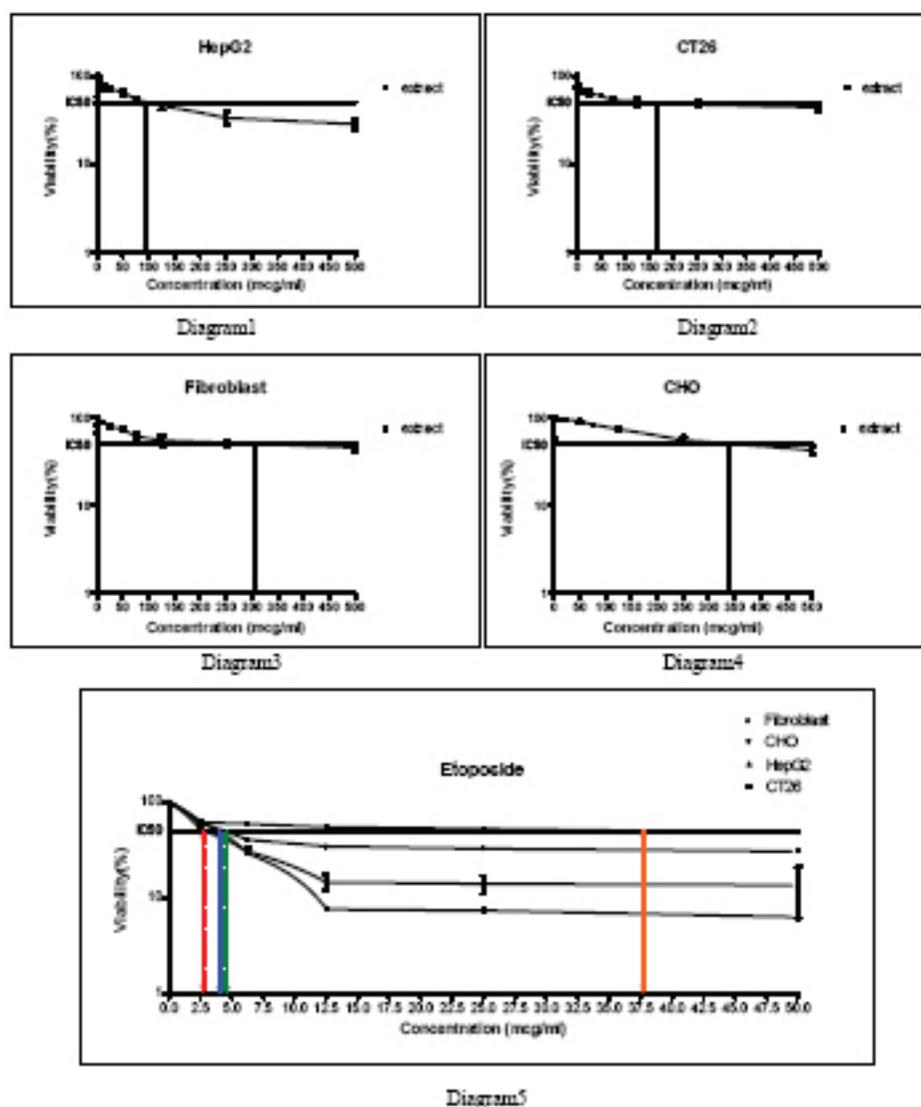
The results are prepared in Table 1. The results showed that the extract of *Sambucus ebulus* possesses higher IC₅₀ in comparison with Etoposide on all 4 normal and cancer cell lines (P<0.05); the lower IC₅₀ represent the higher potency of a compound to inhibit the growth of cells and cause toxicity and death of cells. But, the study manifested a good cytotoxic compound which can introduce as an anticancer compound which was toxic in the cell lines. The lowest and highest IC₅₀ of the extract was evaluated on HepG2 (97.03 µg/ml±1.52) and CHO (346.2 µg/ml±3.02) cell lines; also, IC₅₀ of the extract on normal cell lines was higher than that in cancer cell lines. This order was similar to the order of IC₅₀ of Etoposide on normal and cancer cell lines. Diagrams 1 to 5 shows the IC₅₀ of ethyl acetate extract of *S.ebulus* and Etoposide on the cell lines.

Table 1. The evaluated IC₅₀ (µg/ml) of ethyl acetate extract of *Sambucus ebulus* and Etoposide on normal and cancer cell lines

Cell lines Substance	Cancer Cell Line		Normal Cell Line	
	HepG2 (IC ₅₀ ±SD)**	CT26 (IC ₅₀ ±SD)**	CHO (IC ₅₀ ±SD)**	Fibroblast (IC ₅₀ ±SD)**
<i>Sambucus ebulus</i> (Ethyl acetate extract of fruits)	97.03±1.52	152.70±3.36	346.2±3.02	312.29±2.01
Etoposide	2.80±2.64	3.89±4.46	4.375±3.76	37.5±6.58

*µg/ml;

**P< 0.05



Diagrams 1 to 5: The evaluated IC₅₀ (µg/ml) and viability percent of the normal (Fibroblast and CHO) and cancer (HepG2 and CT26) cells exposed with the of ethyl acetate extract of *Sambucus ebulus* and Etoposide.
 Diagram1 Diagram2
 Diagram3 Diagram4
 Diagram5

The order of IC₅₀ of Etoposide in the 4 cell lines was: Fibroblast > CHO > CT26 > HepG2.

On the other hand, the order of IC₅₀ of the ethyl acetate extract of *Sambucus ebulus* in the 4 cell lines was: CHO > Fibroblast > CT26 > HepG2.

Otherwise, the viability percent (50%) of HepG2 and CT26 cells treated with the extract (orderly 97.03 and 152.70 µg/ml) was similarly closed to 2.80 and 3.89 µg/ml of Etoposide. Also, the viability percent (50%) of CHO and Fibroblast cells treated with the extract (orderly 346.2 and 312.29 µg/ml) was similarly closed to 4.375 and 37.5 µg/ml of Etoposide.

DISCUSSION

According to the results, IC₅₀ of the extract and Etoposide, as positive control compound, on normal cell lines were higher than that on cancer cell lines. This difference can be resulted from dysfunction of cellular organisms following cancer incidence which cause higher rate of proliferation and increased cellular intake. Also, defend disorders and effusion insufficiency to escape toxic substances can lead to lower amounts of cytotoxic compounds to inhibit the growth of cancer cells in comparison with normal cells.

On the other hand, the extract possesses higher IC_{50} in comparison with Etoposide on all 4 normal and cancer cell lines ($P < 0.05$); but, comparison of IC_{50} and viability percent of the cells exposed with the extract of *Sambucus ebulus* with Etoposide, as a common chemotherapeutic drug, allows us to conclude that extract of fruits of *Sambucus ebulus* is a good candidate for further studies of activity-monitored fractionation to identify their active components.

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