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Original article

# The effect of some cosolvents and surfactants on viability of cancerous cell lines

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## Abstract

Improving the solubility of non-soluble herbal materials is an issue of interest in cell culture based experiments. Evaluating the biological activity of these materials could become possible with the aid of cosolvents/surfactants which obviously should have little or no cytotoxic activity. In the present study, the cytotoxic activity of six cosolvents/surfactants: ethanol, methanol, Tween 20 and 80, propylene glycol (PG) and poly ethylene glycol 400 (PEG) which are usually helpful in dissolving non-soluble herbal extracts, has been evaluated against HepG-2, MCF-7 and HT-29 cells by MTT assay. Among the investigated cosolvents/surfactants, Tween 20 and 80 demonstrated the highest and ethanol and methanol the lowest cytotoxicity to the evaluated cell lines, suggesting the two latter as proper aids for improving solubility in biological experiments.

**Keywords***:* cell culture, cosolvent, MTT assay, solubility, surfactant

## Introduction

Usually, the potency and pharmacological activity of the new synthesized compounds are over focused which often lead to poorly soluble compounds. Though cosolvents or solubilizing systems are employed, a large number of new drug candidates can't be evaluated in permeability models because of their poor aqueous solubility [1]. Similarly, assessing the biological activity of some materials would not be possible in cell-based experiments due to their insolubility in cell culture media or non-toxic solvents.

To increase aqueous solubility of an insoluble substance, methods such as pH adjustment and using cosolvents or surfactants can be employed. These methods can be used singly or in combination [2].

Evaluating the cytotoxic activity of materials is a beginning step in cancer research studies in cell culture laboratories; therefore, encountering the insolubility of a substance becomes a hindrance in cytotoxicity evaluations. Many of these researches are focused on herbal extracts which sometimes could not be evaluated due to their

 
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insolubility in the culture media. In the present study, the cytotoxicity of six usual cosolvents/surfactants has been evaluated in order to assess their induced cytotoxicity to find the cosolvents/surfactants with the least toxicity to the cultured cells. These cosolvents/surfactants have proved to be beneficial in dissolving non-soluble herbal extracts in our previous experiences; thus, we were encouraged to assess their toxicity on cultured cells. Subsequently, the least cytotoxic cosolvent/surfactant could be used as a solubility aid in the experiments.

## Experimental

*Chemicals*

The cosolvents/surfactants of analytical grade [ethanol, methanol, Tween 20 and 80, propylene glycol (PG), polyethylene glycol (PEG)] were provided from Merck (Germany).

Dulbecco's modified eagles medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco (New Zealand) and RPMI 1640 medium, penicillin-streptomycin and 3-(4,5-dimethylthiazol-2-yl)-2,4-diphenyltetrazolium bromide (MTT), were provided from Sigma (USA).

*Cancerous cell lines*

The cell lines MCF-7 (human breast adenocarcinoma), HepG-2 (human hepatocellular carcinoma) and HT-29 (human colorectal adenocarcinoma), were provided from the Pasteur Institute, Tehran, Iran.

*Preparation for MTT assay*

The cosolvents/surfactants were provided in culture media with the final concentrations (0.7-0.02 µL/mL), (50-1.5 µL/mL) and (200-6.25 µL/mL) for (Tween 20 and 80), (PG and PEG) and (methanol and ethanol), respectively.

*MTT assay*

The human cancerous cells were exposed to each cosolvent/surfactant for 72 h, thereafter the medium was replaced with medium containing MTT (final concentration of 0.5 mg/mL in each well) and the cells were incubated for another 4 h [3-5]. In MTT assay, the viable cells are capable of converting MTT to violet formazan crystals by their reductase enzymes. The absorbance of the dissolved formazan crystals in DMSO can be measured at 570 nm with an ELISA reader, which is correlated to the number of viable cells. The relative cell viability (%) was calculated by (A samples /A control) × 100, where A samples is the absorbance of test sample and A control is the absorbance of control wells (without cosolvent/surfactant). To calculate $IC_{50}$, viability (%) versus log concentrations was graphed by Microsoft Excel program [6,7]. Moreover, the cytotoxic activity of a sample which had been found to be insoluble in our previous experiments was evaluated by using an elective concentration of the most suitable cosolvents.

## Results and Discussion

The results of the MTT assay suggested the most cytotoxic activity for Tween 20 and 80 while PG and PEG 400 remained in the second place. The least cytotoxic activity was observed for methanol and ethanol (figures 1-6 and table 1).

Dimethyl sulfoxid (DMSO), has been considered as one of the major solvents in biological assays because it is water-miscible and also possesses high solubilizing capacity, as well as low viscosity which make it an acceptable solvent in biological experiments; however, in some cases some substances are even insoluble in DMSO, therefore, attempts to increase the solubility of materials have been the focus of many studies. Examples include the solubility of taxol in aqueous solutions, which has been increased by using cyclodextrins [8]. Also several cosolvents /surfactants have been examined for improving water solubility of benznidazole (a treatment for chagas disease), among which, solvent systems based on PEG 400, with addition of ethyl alcohol and/or potassium biphthalate buffer solution have seemed to be effective [9]. Using Tween 80 for improving the solubility of capsaicin has appeared to enhance the solubility while it had not prevented the breakdown of capsaicin during

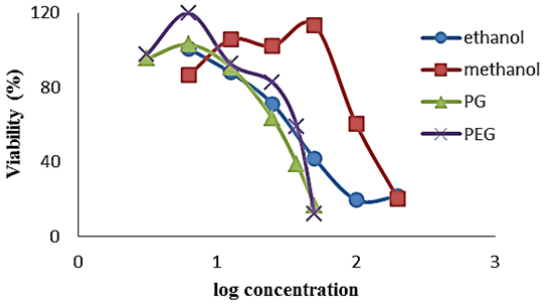


**Figure 1.** Viability of HepG-2 cells exposed to ethanol, methanol, PG and PEG 400

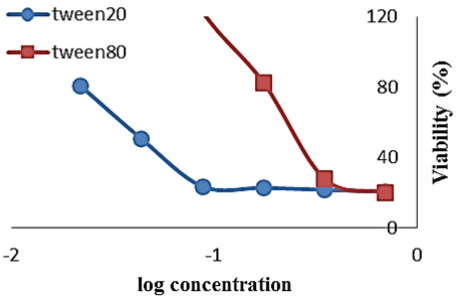


**Figure 4.** Viability of MCF-7 cells exposed to Tween 20 and 80

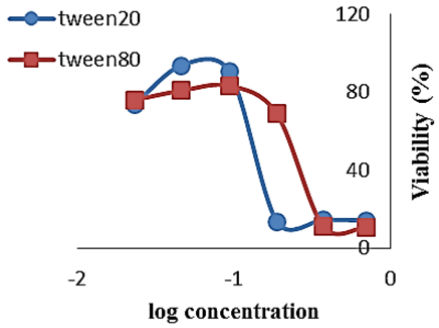


**Figure 2.** Viability of HepG-2 cells exposed to Tween 20 and 80

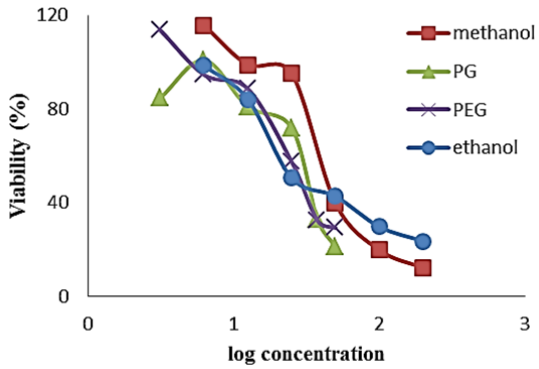


**Figure 5.** Viability of HT-29 cells exposed to ethanol, methanol, PG and PEG 400

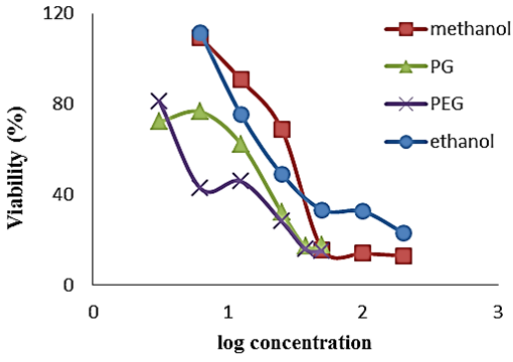


**Figure 3.** Viability of MCF-7 cells exposed to ethanol, methanol, PG and PEG 400

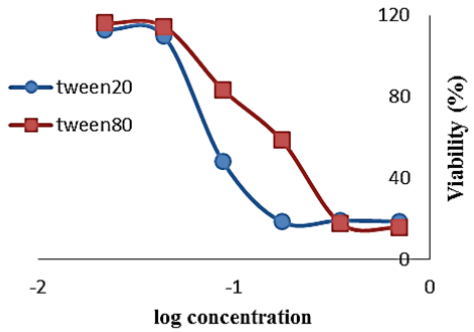


**Figure 6.** Viability of HT-29 cells exposed to Tween 20 and 80

**Table 1.** $IC_{50}$ of cosolvents /surfactants in HepG-2, MCF-7 and HT-29 cell lines

| Cosolvent/surfactant | $IC_{50}$ (µL/mL) | | |
|---|---|---|---|
| | HepG-2 | MCF-7 | HT-29 |
| **Tween 20** | 0.2 | 0.9 | 0.8 |
| **Tween 80** | 0.2 | 0.2 | 0.2 |
| **PG** | 28.9 | 15 | 24.6 |
| **PEG 400** | 36.5 | 10.8 | 25.6 |
| **Methanol** | 124.0 | 38.1 | 47.3 |
| **Ethanol** | 46.4 | 40.3 | 43.8 |

storage [10].In another study, using tetrahydrofuran and DMSO as cosolvents was found to be unsuitable for solubilizing lycopene in prostate cell cultures due to their cytotoxicity [11]. Investigations for a suitable solvent for triclosan (a broad spectrum antibiotic) have demonstrated that aceton-solublized triclosan could be assessed in MCF-7 cell culture for cytotoxic activity evaluations [12]. Cosolvents and surfactants such as glycofurol 75, propylene glycol, polyethylene glycol 400, polysorbate 20, Cremophor RH40 and 2-hydroxypropyl-β-cyclodextrin have been examined to improve the solubility of (+)-usnic acid (a dibenzofuran derivative), to find the last agent capable of solubilizing usnic acid without cytotoxicity on the cultured cells [2].

In the present study Tween 20 and 80, PG and PEG 400, ethanol and methanol have been evaluated for their cytotoxic effects in three tumor cell lines. The results indicated that since Tween 20 and 80 were toxic to the cells even at very low concentrations (<1 µL/mL), their usage as solubility aids would not be beneficial due to their high toxicity to the cultured cells. While PG and PEG exhibited moderate cytotoxic effects, methanol and ethanol showed more promising results. They presented higher $IC_{50}$ in the evaluated tumor cells which suggested their probable use as cosolvents in *in vitro* biological systems. To assess our assumption for their advantage in being used in cell culture based experiments, we evaluated the cytotoxicity of a *n*-hexane fraction of a herbal extract which was not soluble in the culture media in our previous experiments, by adding 12.5 µL/mL of ethanol/ methanol prior to the MTT assay in MCF-7 cells. The fraction dissolved in the culture media in both cases (12.5 µL/mL of ethanol or methanol) and no precipitate or immiscibility was observed. It was concluded that in confronting non-soluble materials, methanol and ethanol could be used in concentration of 12.5 µL/mL or lower as cosolvents for improving the solubility, whenever the substances are insoluble in the culture media.

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**Declaration of interest**

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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