

# Time and Concentration Dependent Effects of Different Solvents on Proliferation of K562, HL60, HCT-116 and H929 Cell Lines

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Received: 22 October 2021 / Revised: 16 February 2022 / Accepted: 17 February 2022

**ABSTRACT:** Many organic solvents are used in cell culture models to dissolve polar and non-polar chemical compounds and drugs to test their activities. However, the solvents themselves can have cytotoxic effects on cells. The degree of cytotoxic effect may vary according to the solvent used. The aim of this study is to determine the time and concentration dependent effects of methanol, ethanol, dimethyl sulfoxide (DMSO), ethyl acetate and acetone on colorectal cancer (HCT-116), chronic myeloid leukemia (K562), multiple myeloma (H929) and acute promyelocytic leukemia (HL60) cell lines. According to our results, DMSO is the most toxic solvent and ethyl acetate and methanol are least toxic solvents in all cells. IC50 value of Acetone is greater than 10% in K562, HL60 and HCT-116 cells in all tested incubation times but in H929 cells IC50 value of acetone is decreased over time. In conclusion, since most of these solvents are currently being used for treatment of cells with different drugs and compounds in biochemical toxicology studies, it is essentially important to reveal their biochemical and toxic effects to determine the optimum concentration to be used.

**KEYWORDS:** DMSO; Methanol; Acetone; Ethanol; cytotoxicity; cell culture; MTT; cancer.

## 1. INTRODUCTION

*In vitro* cell culture models are frequently used to determine the underlying causes of toxicity mechanisms [1]. Before applying the bioactive compounds to the cell culture, it is necessary to determine the solvents that they are soluble and to choose the most suitable one among these solvents. Acetone, ethanol and DMSO are among the most commonly used solvents in cell culture studies. [2]. It is essential to select organic solvents with very little or no toxicity to the cells [3]. Several cell lines can be widely used in cell culture systems for research studies investigating the cytotoxic effects of different chemicals or natural products. It has been shown that different cells respond differently to different solvents [4].

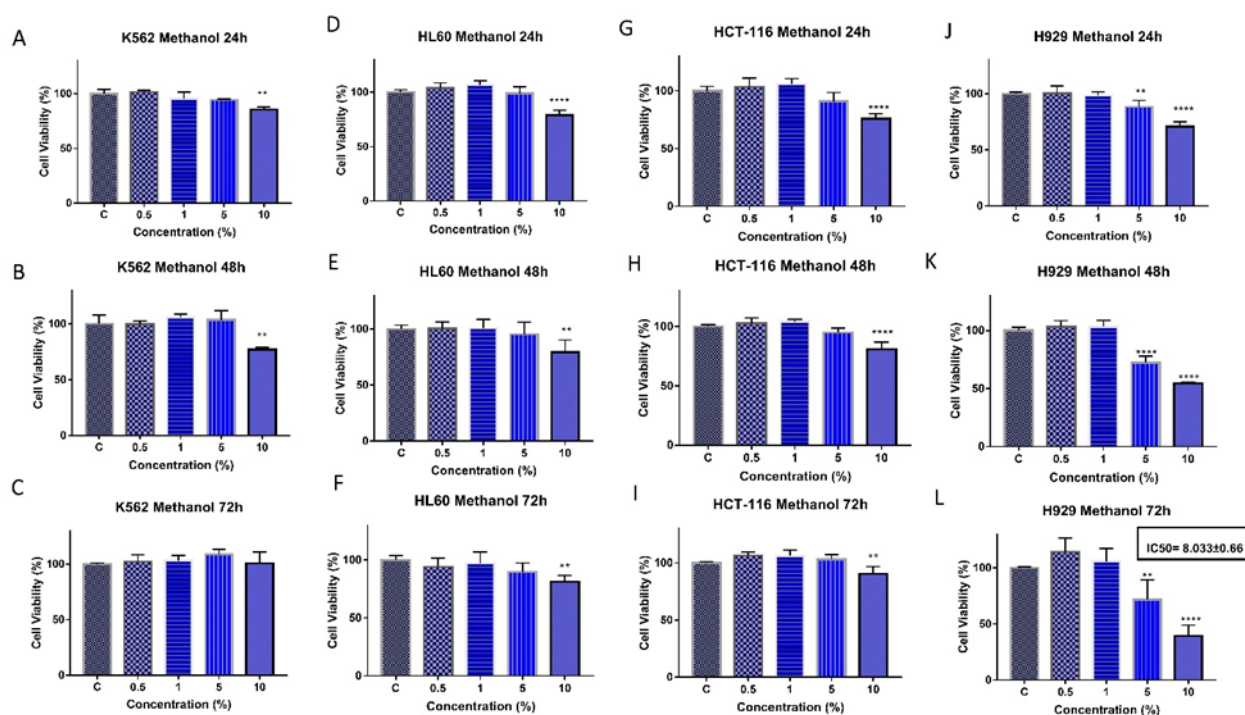
DMSO is frequently used in cryopreservation procedures, drug delivery studies, and screening. In addition to DMSO; ethanol and methanol are also used in drug screening research for the aim of dissolving plant extracts. In drug screening research, in addition to DMSO, it is used to dissolve plant extracts in methanol and ethanol [5]. It was shown that DMSO decreased number of choriocapillary endothelial cells [6]. Exposure to methanol and its toxic metabolites can pose a health risk [7]. It was reported that methanol (up to 100mM) didn't exhibit any effect on cell viability of rat thymocytes [8]. Ethyl acetate and its metabolites have several industrial applications and inhalation of ethyl acetate by rodents' effects significant respiratory parameters [9]. High ethanol concentrations induce apoptosis in both healthy and hepatoma tumor cells, while low ethanol concentrations induce apoptosis only in hepatoma tumor cells [10]. It has been reported that ethanol exposure is associated with reactive oxygen species (ROS) formation and stimulation of apoptosis [11]. It was reported that methanol and ethanol (10% and %5) decreased cell proliferation of HepG2, MDA-MB-231, MCF-7 and VNBRC1 cell lines [5].

Koc A, Karabay AZ, Ozkan T, Buyukbingol Z, Aktan F. Time and concentration dependent effects of different solvents on proliferation of K562, HL60, HCT-116 and H929 cell lines. J Res Pharm. 2022; 26(3): 494-501.

In this study, it was aimed that to investigate the cytotoxic effects of different solvents (ethanol, methanol, acetone, ethylacetate, DMSO) in cancer cell lines including chronic myeloid leukemia (K562), colon cancer (HCT-116), multiple myeloma (H929), acute promyelocytic leukemia (HL60) cell lines. We also investigated whether these effects vary between cell lines in a dose- and time-dependent manner.

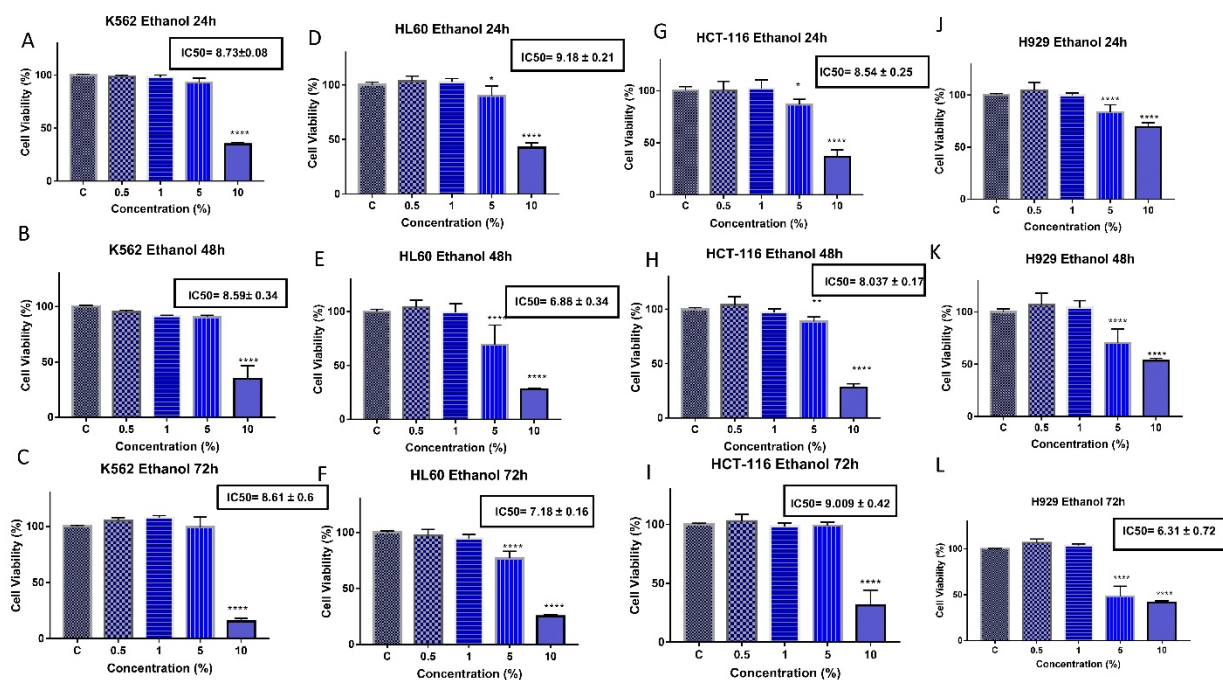
## 2. RESULTS

According to our results, methanol at 10% concentration has significant cytotoxic effects on K562 cells at 24h and 48h (Figure 1A and 1B) and IC50 value of methanol is greater than 10% at all tested incubation times. In H929 cells, methanol (%5 and %10) has significant cytotoxic effects at 24, 48 and 72h (Figure 1J, 1K, 1L). IC50 value is greater than 10% at 24 and 48h but IC50 value of methanol is % 8.033±0.66 at 72h. In HL60 and HCT-116 cells, methanol exhibits cytotoxic effects only at %10 concentration (Figure 1D, 1E, 1F, 1G, 1H, 1I) and IC50 value of methanol is greater than 10% in HCT-116 and HL60 cells at all tested incubation times.



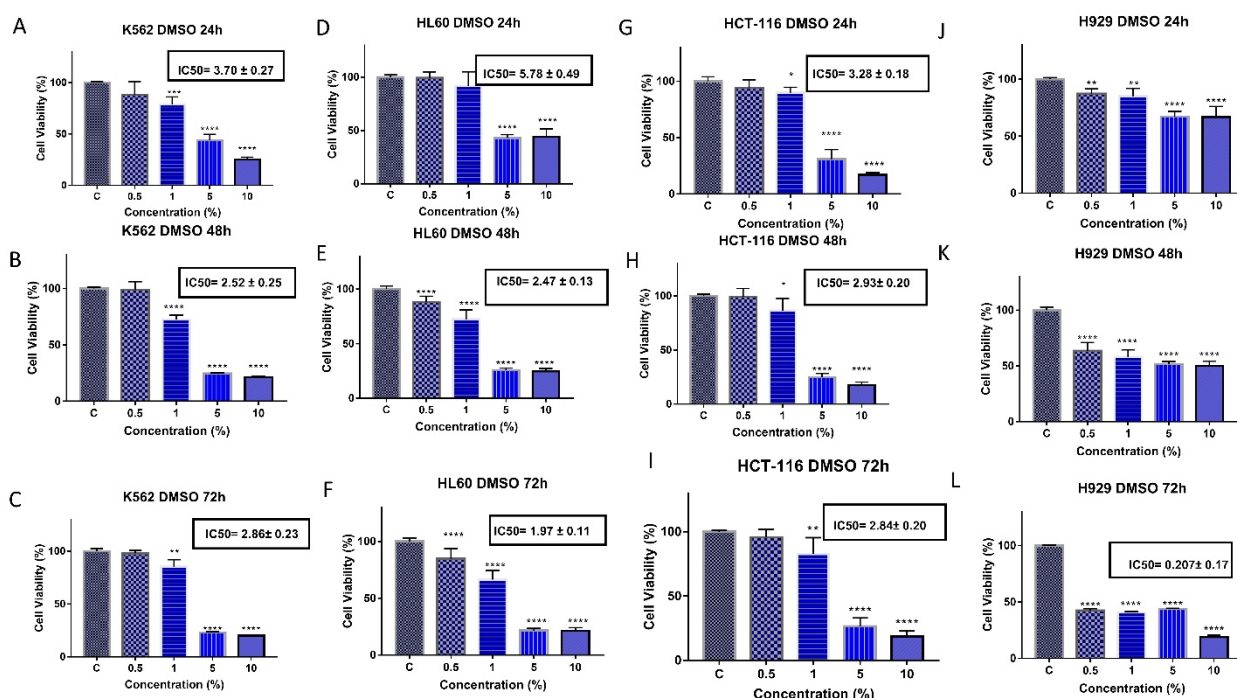
**Figure 1.** Cytotoxic effects of methanol (0.5-10%, v/v) on K562, HL60, HCT-116 and H929 cells at 24, 48 and 72h. (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ )

Ethanol decreased K562 cell proliferation at 10% concentration significantly at 24, 48 and 72h (Figure 2A, 2B, 2C) and IC50 values of ethanol are %8.73± 0.08, %8.59± 0.34, %8.61± 0.60 at 24, 48 and 72h respectively. It decreased HL60 and H929 cell proliferation at %5 and %10 concentration significantly at 24, 48 and 72h (Figure 2D, 2E, 2F, 2J, 2K, 2L). In HL60 cells, IC50 values of ethanol are %9.18± 0.21, %6.88± 0.34, %7.18 ± 0.16 at 24, 48 and 72h respectively and in H929 cells, IC50 values of ethanol are greater than 10% at 24 and 48h and %6.31 ± 0.72% at 72h. It decreased HCT-116 cell proliferation significantly at %5 and %10 concentration at 24 and 48h and at %10 concentration at 72 h (Figure 2G, 2H, 2I) and in HCT-116 cells, IC50 values of ethanol are %8.54± 0.25, %8.037± 0.17, %9.009 ± 0.42 at 24, 48 and 72h respectively.



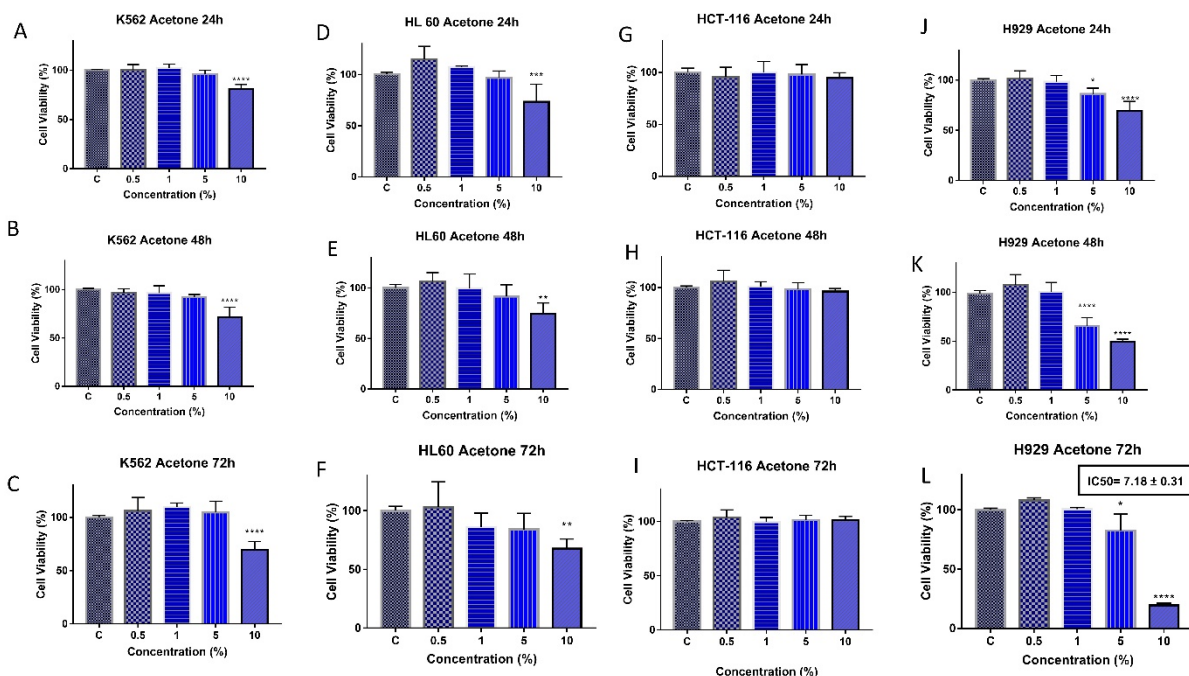
**Figure 2.** Cytotoxic effects of ethanol (0.5-10%, v/v) on K562, HL60, HCT-116 and H929 cells at 24, 48 and 72h). (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ )

DMSO decreased cell viability at 1-10% the concentrations of in K562 and HCT-116 cells, at 0.5-10 % the concentrations of in H929 cells at 24, 48 and 72 significantly (Figure 3A, 3B, 3C, 3G, 3H, 3I, 3J, 3K, 3L). In HL60 cells, it decreased cell viability significantly at 5-10% (24h) and at 0.5-10% (48 and 72h). IC50 values of DMSO are %3.70 ± 0.27, %2.52 ± 0.25, %2.86 ± 0.23 for K562 cells; %5.78 ± 0.49, %2.47 ± 0.13, %1.97 ± 0.11 for HL-60 cells; %3.28 ± 0.18, %2.93 ± 0.204, %2.84 ± 0.208 for HCT-116 cells at 24, 48 and 72h respectively. For H929 cells, IC50 values of DMSO are greater than 10% for 24 and 48h and %0.207 ± 0.17 at 72h.



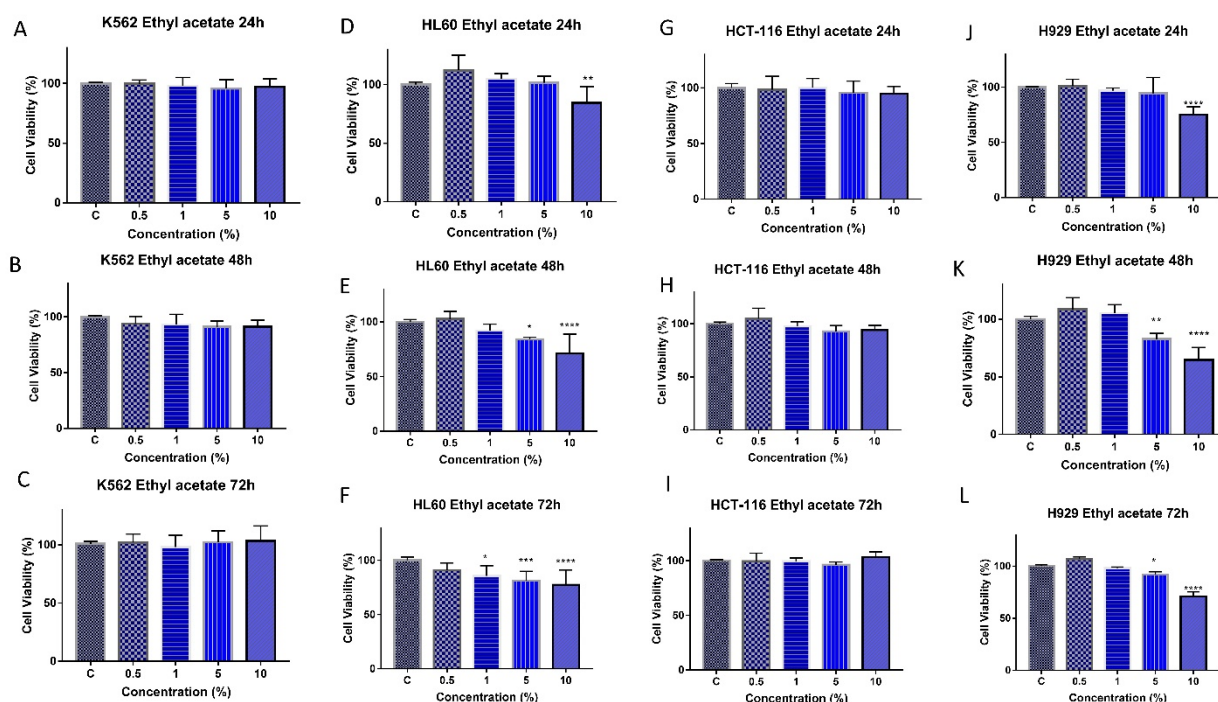
**Figure 3.** Cytotoxic effects of DMSO (0.5-10%, v/v) on K562, HL60, HCT-116 and H929 cells at 24, 48 and 72h). (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ )

Acetone decreased cell viability at the concentrations of 10% in K562 and HL60 cells, at the concentrations of 5-10% in H929 cells at 24, 48 and 72 significantly and it didn't exhibit any significant effect in HCT-116 cells (Figure 4). In H929 cells, IC50 value of acetone is  $7.18 \pm 0.31$  at 72h.



**Figure 4.** Cytotoxic effects of acetone (0.5-10%, v/v) on K562, HL60, HCT-116 and H929 cells at 24, 48 and 72h). (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ )

Ethyl acetate didn't affect the proliferation of K562 and HCT-116 cells. In HL60 cells it decreased cell viability at 10% at 24h, at 5-10% at 48h and at 1-10% at 72h. In H929 cells, it decreased cell viability at 10% at 24h and 5 and 10% at 48h and 72h (Figure 5).



**Figure 5.** Cytotoxic effects of ethyl acetate (0.5-10%, v/v) on K562, HL60, HCT-116 and H929 cells at 24, 48 and 72h). IC50 values are expressed as average  $\pm$  SEM (\* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001, \*\*\*\* $p$ <0.0001)

### 3. DISCUSSION

In our study, IC50 values of methanol are greater than 10% in all cell lines and all-time intervals except in H929 cell line and at the end of 72h incubation (IC50 value is  $8.033 \pm 0.66\%$ ) period and we determined that methanol and ethanol significantly reduced cell viability at 24 and 48 hours. However, cell viability at 72 hours was not significantly changed with these solvents (K562 cells for methanol and HCT-116 cells for ethanol) [12, 13]. This may be because of the evaporation rates of these solvents over time. In addition, surviving cells after 48 hours may have continued to divide and increase their proliferation in the presence of lower solvent concentration. These can be the causes of determination of less toxicity at 72 hours of incubation with ethanol and methanol. In previous studies, methanol (30-100mM) didn't exhibit any cytotoxic effects on rat thymocytes [14]. It has been shown that ethanol and methanol exhibit low toxic effects on HepG2, MDA-MB-231, MCF-7 and VNBRC1 cell lines [5].

In HL60 and HCT-116 cells, IC50 values of DMSO decrease with time. In addition, in H929 cells, IC50 value was above 10% at 24 and 48 hours, and  $0.207 \pm 0.17\%$  at 72 hours. At 72 hours, the sensitivity of the cells increases markedly. DMSO is an important solvent that dissolves poorly soluble polar and nonpolar molecules [15]. Galvao et al demonstrated that low concentrations of DMSO stimulates retinal apoptosis *in vivo* [15]. DMSO has not been shown to exert significant effects on cell proliferation in mouse peritoneal macrophages [16]. It has been shown that DMSO exhibited cytotoxic effects on human leukemic THP1, U937, Jurkat and Molt-4 cells and mouse fibrosarcoma Wehi 164 cells after 24 hours of incubation at concentrations higher than 2% with the following time dependency: 72h>48h>24 h [17]. DMSO has been shown to inhibit HepG2, MDA-MD-231, MCF-7 and VNBRC1 cell proliferations between 1.25-10% concentration and ethanol decreased HepG2 cell proliferations between 2.5%-10% concentration whereas methanol was the least effective solvent on the inhibition of HepG2, MDA-MD-231, MCF-7 and VNBRC1 cell proliferations [5].

In all cells except H929 cell line, the cytotoxicity of ethanol did not increase with time. In HeLa cells, it was shown that ethanol decreased cell viability above 5% concentrations [18]. It was found that the viability of RAW264.7 cells wasn't affected by ethanol up to 600 mM [19] and up to 5% concentrations of ethanol did not show any effect on Mono Mac 6 cells [4].

According to our results, IC<sub>50</sub> values of methanol are greater than 10% in all cell lines and incubation times except values obtained in H929 cells at 72h. It has been shown that ethanol and methanol (1.25%-0.15%) exhibit toxic effects on HepG2, MDA-MB-231, MCF-7 and VNBRC1 cell lines and 0.6%-0.15% concentration range can be used for DMSO [5].

We found that ethyl acetate had an IC<sub>50</sub> value of >10% at all incubation times and in all cell lines. Similarly, ethyl acetate, acetone showed a similar cytotoxic effect except the IC<sub>50</sub> value found in H929 cells (7.189±0.31%) at 72 hours. Incubation time appears to be important in H929 cells. While IC<sub>50</sub> values for, methanol, DMSO, ethanol and acetone were >10% at 24 and 48 hours, it decreased below 10% at 72 hours. The most remarkable decrease at 72 hours was observed in DMSO. Among all these solvents, in the cell lines tested, the least toxic solvent was ethyl acetate. Methanol and acetone follow ethyl acetate. It was shown that ethanol and methanol exhibited (1 and 2% v/v) are better tolerated in normal (CCL-1, HaCaT) or tumor (A-375, A-431) cells [20]. Jamalzadeh et al. also showed that DMSO has higher cytotoxic effects than ethanol and acetone in RAW 264.7, HUVEC and MCF-7 cell lines and it was found that acetone has the least cytotoxic activity on these cells [2].

#### 4. CONCLUSION

In our study, the effects of different solvents, which are frequently used to dissolve drugs or compounds used in different cell culture models were examined and it was concluded that the cytotoxic effects of the solvents used may differ depending on the cell line. In addition, it was determined that some solvents, such as ethyl acetate, exhibited less toxic effects than others in all cells used in this study. Therefore, where any of these solvents may be preferred, we recommend that the solvent exhibiting less toxic effects be used.

#### 5. MATERIALS AND METHODS

##### 5.1. Cell Culture

Human chronic myelogenous leukemia cell line (K562), colorectal cancer cell line (HCT-116), acute promyelocytic leukemia cell line (HL-60), multiple myeloma (H929) cell line were grown in RPMI 1640 medium (Sigma-Aldrich, St. Louis MO, U.S.A.) supplemented with 1% L-glutamine (Capricorn Scientific, Germany), Penicilin/Streptomycin (1%) (Capricorn Scientific, Germany) and 10% heat inactivated fetal calf serum (Capricorn Scientific, Germany).

##### 5.2. Cell Viability

Cell viability was tested by MTT assay [21]. For this purpose, human chronic myelogenous leukemia cell line (K562), colorectal cancer cell line (HCT-116), acute promyelocytic leukemia cell line (HL-60), multiple myeloma (H929) cell line was seeded to 96 well plates and incubated with the solvents (ethanol, methanol, acetone, chloroform, hexane, ethylacetate, DMSO) between %0.5-%10 concentrations for 24, 48 and 72 hours. After incubation time, MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (5mg/ml) was added and incubated for 4h at 37°C. After incubation, SDS-HCl solubilizing agent was used to solubilize insoluble formazan crystals and absorbance at 550nm was measured. The cells not treated with any solvent were used as a control group. IC<sub>50</sub> values were calculated by GraphPad Prism 7 program.

##### 5.3. Statistical Analysis

Statistical analysis was performed with One-way Anova variance analysis by Graphpad prism program. P<0.05 was accepted statistically significant (\*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001). IC<sub>50</sub> values are expressed as average ± SEM

**Author contributions:** Concept – A.K., A.Z.K.; Design – A.K., A.Z.K., T.O.; Supervision – A.K., A.Z.K., T.O.; Resources – A.K., A.Z.K.T.O.; Materials – A.K., A.Z.K., T.O.; Data Collection and/or Processing A.K., A.Z.K., T.O.; Analysis and/or Interpretation A.K., A.Z.K., Z.B., F.A.; Literature Search – A.K., A.Z.K.T.O, Z.B., F.A.; Writing –A.K., A.Z.K., T.O., F.A., Z.B.; Critical Reviews – A.K., A.Z.K. T.O, Z.B., F.A.

**Conflict of interest statement:** “The authors declared no conflict of interest” in the manuscript.

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