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Research Article

COMPOSITION ANALYSIS OF E-LIQUIDS AND THEIR EFFECTS ON HEALTHY LIVER AND PHARYNGEAL CARCINOMA CELL LINES

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Abstract: Electronic cigarettes have become popular worldwide in recent years although their effects on human health are still not properly known. The lack of regulations brings a problem of inconsistency between ingredients and the product label. We aimed to analyse the contents of widely used e-liquids and their effects on two different cell lines. Eleven e-liquid samples were selected according to their availability and popularity. Nicotine, propylene glycol (PG), glycerine (GLY), and volatile compounds in e-liquids were analysed by High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC). 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay was used to determine the effects of e-liquids on transformed human normal liver epithelial cell line (THLE-2) and human pharyngeal carcinoma cell line (Detroit 562). Nicotine amounts were found to be consistent with product labels. and GLY were not only different between brands but also for products within the same brand. THLE-2 cell viability was inversely correlated with e-liquid concentration. However, decreases in cell viability were not correlated with nicotine amount. Interestingly, effects of several samples on Detroit 562 cells were triphasic; decrease in viability at lower doses, cell survival in mid-concentrations and loss of viability in highest doses. The analytical composition of e-liquids differs greatly among products which corresponds to different cellular effects. Viability of cancer cells does not change in a dose-dependent manner, which suggest that cellular differences may play role in the outcome of these products.

Özet: Henüz sağlık üzerindeki etkileri tam olarak bilinmese de elektronik sigaralar, son yıllarda dünya çapında popüler hale gelmiştir. Yasal düzenlemelerin eksikliği, beraberinde ürün etiketinde yazan ve gerçekten içerikte bulunan maddeler arasında bir tutarsızlık olmasına sebep olmaktadır. Bu çalışmada, yaygın olarak kullanılan e-sıvıların içeriklerini ve iki farklı hücre hattı üzerindeki etkilerini analiz etmeyi amaçladık. Erişilebilirlik ve popülerliklerine göre on bir e-sıvı numunesi seçildi. E-sıvılardaki nikotin, propilen glikol (PG), gliserin (GLY) ve uçucu bileşikler, yüksek performanslı sıvı kromatografisi (HPLC) ve gaz kromatografisi (GC) kullanılarak analiz edildi. E-sıvıların, sağlıklı karaciğer hücre hattı THLE-2 ve faringeal karsinom hücre hattı Detroit 562 modelleri üzerinde hücre canlılığına etkisi, 3-(4,5-dimetiltiyazolil-2)-2,5-difeniltetrazolyum bromür (MTT) canlılık testi kullanılarak belirlendi. Nikotin miktarlarının ürün etiketleriyle uyumlu olduğu bulundu. PG ve GLY ise sadece markalar arasında değil aynı marka içindeki ürünler için de farklılık gösterdi. THLE-2 hücre canlılığı, e-sıvı konsantrasyonu ile ters orantılı olarak bulundu. Bununla beraber, hücre canlılığındaki azalmalar nikotin miktarı ile ilişkili değildi. İlginç bir şekilde, bazı numunelerin Detroit 562 hücreleri üzerindeki etkileri, düşük dozlarda canlılıkta azalma, orta konsantrasyonlarda hücre hayatta kalması ve en yüksek dozlarda canlılık kaybı olmak üzere üç fazlı olarak gözlemlendi. E-sıvıların analitik bileşimi, farklı hücresel etkilere karşılık gelen ürünler arasında büyük farklılıklar göstermektedir. Kanser hücrelerinin canlılığının doza bağlı bir şekilde değişmemesi, bu ürünlerin sonuçlarında hücresel farklılıkların rol oynadığını düşündürmektedir.

Introduction

Electronic cigarettes (e-cigarettes), which are designed to mimic smoking experience, emerged as a cessation strategy and become widely popular (Tarasenko *et al.* 2022). According to 2019 National Youth Tobacco Survey (NYTS) data, high school and middle school students reported current e-cigarette use as 27.5% and

10.5%, respectively, indicating the popularity among youth (Harrell *et al.* 2017, Cullen *et al.* 2019). There are still question marks on the safety and health consequences of e-cigarettes. The debate on whether e-cigarette is a smoking cessation method continues and contradicting results have been reported (Berry *et al.* 2019, Giovacchini *et al.* 2022). Marketing of e-cigarettes as "healthier alternative" compared to cigarettes arose concerns (Cobb *et al.* 2010, Collins *et al.* 2018).

E-cigarettes vaporize the liquid present in their cartridges and inhalation of the vapour gives similar experience as in smoking. Consumers obtain refill solutions, known as electronic liquid (e-liquid), with various flavours and different nicotine contents to use with these devices. It is possible to encounter several brands and numerous products for each brand in this rapidly developing market. Two problems have been identified about these products since the early years of use: (i) inconsistency between label and contents (Cheng 2014, Bębenek *et al.* 2022) and (ii) batch-to-batch differences in formulas (Bahl *et al.* 2012). Main ingredients of e-liquids are nicotine, propylene glycol, glycerine, and volatile compounds. Especially, the composition of volatile compounds can substantially vary among e-liquids since they are responsible for the aromatic flavours. Phenolic compounds, polycyclic aromatic hydrocarbons, tobacco-specific nitrosamines (TSNAs) and free radicals were also reported in aerosols or cartridges (Zhao *et al.* 2018, Belushkin *et al.* 2019). TSNAs, carbonyl groups and volatile organic compounds found in e-cigarettes have been associated with cyto- and genotoxic effects (Huang *et al.* 2018). Considering these variables, studies are needed to investigate the contents of e-liquids and their biological effects together. Analysing them in parallel makes it possible to relate the seen biological effects and responsible molecules. While several studies carry this approach, there are no studies conducted in Türkiye with this design.

The first cell culture studies investigating the effects of e-liquids and/or aerosols biological effects started in early 2010s. These studies used various cell types such as human and mouse stem cells (Bahl *et al.* 2012, Behar *et al.* 2012, Zahedi *et al.* 2019), bronchial cells (Mathis *et al.* 2013, Scheffler *et al.* 2015), cardiac cells (Farsalinos *et al.* 2013), adipocytes (Zagoriti *et al.* 2020) and fibroblasts (Romagna *et al.* 2013). Liver cells, on the other hand, have generally been neglected in these studies. Recent *in vivo* studies showed that both direct exposure to e-liquids (El Golli *et al.* 2016) and exposure to vapours produced by e-cigarette (Espinoza-Derout *et al.* 2019) cause liver damage. When the role of the nicotine is considered, no correlation was found between nicotine amount and cytotoxicity (Bahl *et al.* 2012, Farsalinos *et al.* 2013). It is also worthy to mention that, e-cigarette vapor shows less cytotoxicity compared to tobacco smoke (Farsalinos & Polosa 2014). Understanding the current adverse effects of e-cigarettes is important to overcome these disadvantages.

The aim of study is to sample the frequently preferred e-liquids in Türkiye to represent different brands, various nicotine contents and several flavours to analyse and compare the ingredients. Chromatographic methods are golden standards when it comes to constituent analysis of e-liquids. Liquid and gas chromatography methods can be utilized to separate the analyse of interest from the mixture. Mass spectroscopy (MS), diode array detector (DAD), and flame ionization detector (FID) are examples of detector systems that can be used in combination with chromatographic separation. While DAD and FID are more suitable for targeted analyses such as nicotine, MS provides untargeted analysis of volatile components of e-liquids (Holt *et al.* 2021). In this study, validated liquid and gas chromatographic methods were used for nicotine, propylene glycol, glycerine and volatile compound detection. We also evaluated the amount of nicotine for the label consistency. Additionally, the effects of the sampled e-liquids on cell viability were shown *in vitro* by using transformed human normal liver epithelial cell line (THLE-2) and human pharyngeal carcinoma cell line (Detroit 562) as healthy and tumorigenic cell line models, respectively.

Materials and Methods

Reagents

Eagle's Minimum Essential Medium (EMEM), Bronchial Epithelial Cell Growth Medium (BEGM) Bullet Kit, penicillin/streptomycin (P/S, 10,000 UI/mL), L-Glutamine (200 mM), trypsin/EDTA (0.05% trypsin; 0.20 g/L EDTA), and phosphate buffered saline (PBS) used in cell culture experiments were purchased from Lonza. Foetal bovine serum (FBS) was obtained from Biowest. 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT), sodium dodecyl sulphate (SDS), fibronectin, collagen type I, bovine serum albumin, phosphoethanolamine, and epidermal growth factor (EGF) were purchased from Sigma. Transformed human normal liver epithelial cells (THLE-2) were purchased from American Type Culture Collection (ATCC). Human pharyngeal carcinoma cell line (Detroit 562) was kindly donated by Prof. Dr. Sarhan SAKARYA (Adnan Menderes University, Türkiye). Growth medium for THLE-2 was prepared with modification BEGM Bullet Kit as suggested by ATCC. Briefly, gentamycin/ amphotericin (GA) and epinephrine were discarded and EGF (5 ng/mL), phosphoethanolamine (70 ng/mL), 10% FBS, and 1% P/S (Final concentration: 100 units/mL of penicillin and 100 µg/mL) were added to BEGM. Growth medium for Detroit 562 was prepared by adding 10% FBS and 1% P/S (Final concentration: 100 units/mL of penicillin and 100 µg/mL) to EMEM.

Nicotine, propylene glycol, and glycerine were purchased from Sigma. Chromatographic grade solvents and reagents used in the content analyses were obtained from Merck and Sigma.

E-liquid samples

Eleven e-liquids were included in this study according to their availability and popularity (Table 1). Three

parameters, nicotine amount, brand and aroma, were taken into consideration regarding the contents of the e-liquids. In cell culture analyses, subsets of e-liquids were formed where two out of these three parameters were kept constant and one parameter (i.e., nicotine amount or aroma) varied.

Chromatographic analyses

E-cigarettes generally contain nicotine (NIC), propylene glycol (PG), glycerine (GLY), and aroma components. NIC in e-liquids was detected and quantified by using an Agilent 1100 RP-HPLC system consisting of a gradient pump, a DAD and an Agilent Eclipse XDB-C18 column (5.0 µm particle size, 4.6 × 150 mm). Detection and quantification were carried out at 260 nm wavelength. The column was injected with 20 µL and the column temperature was 35°C. The mobile phase consisted of (A) Water (1% phosphoric acid) (B) acetonitrile (90:10, v:v). The flow rate was 1.0 mL/min with a 20 µL of injection volume. Quantitative analysis was based on the peak areas by using ChemStation software. Standard solutions of NIC were dissolved in methanol. Specification and determination limit (LOD / LOQ) were determined for NIC. Validation parameters related to NIC analyses such as linearity and accuracy are given in the supplementary materials (Supplementary Material Tables S1, S2 and Figs S1, S2).

PG and GLY were analysed by gas chromatography (GC) according to CORESTA Recommended Method No. 60 "Determination of 1,2-Propylene Glycol and Glycerol in Tobacco and Tobacco Products by Gas Chromatography" (CORESTA 2014). The PG and GLY were analysed by gas chromatography-flame ionization detector (GC-FID). The Agilent 7890B GC-FID system consisted of a G4513A auto-liquid sampler and VF-35ms capillary column (0.10 µm particle size, 0.25 mm internal diameter, and 30 m length). The oven initial temperature was held at 110°C for 1 min and then increased to 150°C at a rate of 10°C/min., the secondary ramp rate was 30°C /min. until 220°C, where the temperature was held for 5 min., and helium gas was used as the carrier gas. The injector temperature was 280°C and injection was performed in the split mode (1:50). The injection volume was 1.0 µL. Hydrogen was used as the carrier gas at 30 cm/s. Standard solutions of PG and GLY were dissolved in methanol. The amounts of each substance, expressed in mg/mL, were calculated according to the CORESTA method. This method was validated in terms of linearity and accuracy (recovery) and given in the supplementary materials (Supplementary Material Tables S3-S5 and Figs S3, S4).

Volatile components were analysed by HP-INNOWAX capillary column (0.25 µm film, 0.320 mm diameter and 60 m length) by using a gas chromatograph 7890 B coupled to a mass spectrometer series MSD 5977 A (Agilent Technologies) and Head Space sampler (PAL Sampler). The 85°C incubation temperature in the PAL cycle was applied for 10 minutes and then 500 µL was injected. The flow rate of the carrier helium gas is 0.7 mL/min. The temperature program for the GC was as follows; 60°C initial temperature, 210°C held for 3 min with 4°C/min, and then programmed rise the temperature 280°C with 20°C/min held for 5 min. The injection was performed in the split mode (1:50). The injection volume was 1.0 µL. The GC–MS interface was heated to 250°C with the actual temperature reaching 180°C in MS source and 150°C in MS-quadrupole. The electron impact energy was set at 70 eV, and data were collected in the range of 40–300 atomic mass units (amu). Compound identifications were based on mass spectra by comparison with Wiley MS spectra database. The integrations were performed with Agilent MassHunter software. E-liquid samples were dissolved in methanol in all chromatographic analyses.

Statistical analysis

Characterization and classification of volatile compounds in e-liquids samples were carried out by using principal component analysis method (PCA) (Ward's algorithmic method). The multivariate analyses were performed by using MINITAB 15 Statistical Software. All data and auto scaled variables were normalized prior to the chemometric analysis. Results of PCA are visualized by scores and loading plots.

IC_{50} values were calculated by nonlinear "[Inhibitor] vs. response -- Variable slope (four parameters)" regression analysis (GraphPad Prism 7) (Sebaugh 2011).

Cell culture

THLE-2 and Detroit 562 cells were maintained in their corresponding media (BEGM and EMEM, respectively) at 37°C in a humidified incubator with 5.0% CO_2. Cells were subcultured at 80% confluency by trypsinization. Flasks were precoated with 0.01 mg/mL fibronectin, 0.03 mg/mL collagen type I and 0.01 mg/mL bovine serum albumin for THLE-2 subculturing.

Table 1. Nomenclature used for e-liquid samples.

Sample ID	Aroma	Nicotine Amount (mg/mL) [a]	Brand [b]
A-Car-0	Caramel	0	A
A-Car-9	Caramel	9	A
A-Car-18	Caramel	18	A
B-Cap-0	Cappuccino	0	B
B-Car-0	Caramel	0	B
B-Str-0	Strawberry	0	B
B*-Str-9	Strawberry	9	B*
B*-Str-18	Strawberry	18	B*
C-Mix-0	Mixed	0	C
C-Mix-6	Mixed	6	C
C-Mix-24	Mixed	24	C

[a] As written on product label. [b] Brand names were denoted as capital letters. Brand B have changed label and formulation during the study. Renewed products of brand B are denoted as B*.

Cell viability assay

Colorimetric MTT assay was used to assess relative cell viability. Briefly, 10,000 cells/well were incubated in 96-well cell culture plate for 24 h at standard culture conditions. THLE-2 and Detroit 562 cells were treated with e-liquids for 24 h. Samples were diluted with BEGM or EMEM with final concentrations of 1% - 20% (v/v). After desired incubation, MTT solution (0.5 mg/mL in growth medium) was added to each well and incubated for 4 h. Intracellular formazan crystals produced by the enzymatic activity of living cells were dissolved in 10% SDS (in 0.01 M HCl) and quantified by reading the absorbance at 570 nm. Absorbance at 620 nm was used as reference wavelength (BioTek™ ELx800™).

Either BEGM or EMEM without any sample was used as control and considered as 100% viable. Relative cell viability was plotted as the percent absorbance of sample treated cells.

Results

Nicotine amount in e-liquids is in concordance with product labels

The method was validated at a concentration range between 1.00 µg/mL and 50.00 µg/mL for nicotine. The retention time was determined by using nicotine standard and found as 1.253 min. (Supplementary Material Fig. S2). Linearity criterion was measured by standard test solution at 6 different concentrations. Linearity calculations were performed over the peak area obtained for each concentration. The equation of calibration curve was y=31.831x+16.661. The results showed a good correlation between the peak areas and concentrations with R^2=0.9999. The limit of detection (LOD) and the limit of quantification (LOQ) for nicotine were analysed to evaluate the sensitivity of the methods. LOD and LOQ of the nicotine were estimated from the signal-to-noise ratio of about 3:1 and 10:1, respectively. LOD and LOQ values of nicotine were 0.157 µg/mL and 0.525 µg/mL, respectively.

Sample analyses revealed that nicotine amount in e-liquids were in concordance with the labels on the products (Table 2).

Table 2. Comparison of NIC, PG, and GLY compositions between e-liquids.

	NIC (mg/mL)	PG (mg/mL)	GLY (mg/mL)
A-Car-0	0.021	250	1791
A-Car-9	8.850	271	2126
A-Car-18	17.578	153	59
B-Cap-0	<LOD	492	446
B-Car-0	<LOD	545	540
B-Str-0	<LOD	415	339
B*-Str-9	7.380	205	1254
B*-Str-18	16.240	257	1582
C-Mix-0	<LOD	<LOD	1733
C-Mix-6	5.572	253	1910
C-Mix-24	26.259	174	1555

LOD: Limit of detection

PG and GLY amounts in e- liquids show differences among products

Since the CORESTA standard method was used for the analyses of PG and GLY, only method transfer was performed to the instrument. For this, linearity and recovery parameters are examined from the method validation parameters. The concentration ranges, linear regression equations, correlation coefficient and retention times of PG and GLY were given in Table 3.

The GC-FID method has good correlation coefficient values in terms of linearity. The difference between the retention times shows that the resolution was also quite good. The recovery study was carried out at two different concentrations of PG and GLY (0.3 mg/mL and 0.6 mg/mL) for the accuracy of method. The RSDs were less than 4.0%, thus representing the good reliability and accuracy of the method (Supplementary Material Table S5).

PG values differ between 205 - 545 mg/mL, except for C-Mix-0, which was below LOD, among samples (Table 2). The differences between GLY amounts were found to be higher compared to PG (59 - 2,126 mg/mL) (Table 2). Formulations were similar for the same brand but differed between the brands. Interestingly, new products of Brand B (B*) were found to include more GLY and less PG than its previous formulation.

Volatile components don't correlate with the aromas used

The classification and characterization of the aroma components found in e-cigarette liquids is important to determine the chemical properties of the aromas. Thus, when determining chemical changes and differences resulting from aroma, it can be used to determine whether there is a relationship between aroma components and cell culture studies. Unsupervised PCA was performed to determine the differences between e-liquid samples to generate an overview of the volatile components (Fig. 1). According to the analysis, A-Car-0 was found to be an outlier and new products of the Brand B (B*) were found to be in far cluster compared to other samples (Fig. 1). Indeed, the composition of A-Car-0 differs greatly from other samples (Supplementary Material Table S6). It is interesting that the A-Car-0 comprise several additional molecules which is not the case with the other caramel aromas of the same brand.

Table 3. Linear regression equation, calibration curve and retention times of the analytical method for PG and GLY.

GC-FID method	PG	GLY
Linear Ranges (mg/mL)	0.2 -1.2	0.2 - 1.2
Linear Regression Equation	y=2E+06x-65818	y=1E+06x-113497
Correlation Coefficient	0.999	0.998
Retention Time (min)	3.409	6.989

Results also show that, composition changed in new products of the Brand B (B*). Methyl isobutyl ketone (4-Methylpentan-2-one) was found to be in all samples but not in new formulation of Brand B (B*). On the contrary, ethyl n-valerate, ethyl n-butanoate, and ethyl 2-methylbutanoate were found only in B* (Supplementary Material Table S6).

Nicotine was also screened with other volatile compounds. In concordance with liquid chromatography results, no nicotine was found in e-liquid samples labelled as 'without nicotine'.

E-liquids lower the viability of THLE-2 cells in a dose dependent manner

The effects of e-liquids were tested on two distinct cell lines, transformed human normal liver epithelial cell line (THLE-2) and human pharyngeal carcinoma cell line (Detroit 562). THLE-2 was used as a model of healthy liver. They express phenotypic characteristics of normal adult liver epithelial cells and constitute an *in vitro* model for pharmacotoxicological studies.

Increasing doses in all samples chosen in this study were negatively correlated with cell viability in THLE-2 cells (Fig. 2).

IC_{50} values were calculated by nonlinear regression analysis by using dose-dependent inhibition curves (Table 4). IC_{50} values were found to be similar for samples without nicotine (except samples with mixed aroma). No correlation between nicotine amount and cell viability was found. Higher nicotine amount resulted in lower IC_{50} in mixed samples, whereas opposite relation was seen in caramel samples.

Viability of Detroit 562 cells either decreased in a dose dependent manner or showed triphasic response

Effects of e-liquids on cell viability were also tested in Detroit 562, human pharyngeal carcinoma cell line (Fig. 3). Samples containing mixed aroma (Brand C) and new formulation of Brand B (B*) showed similar trend as in THLE-2 cells, i.e., dose-dependent decrease in cell viability (Figs 3c, d). However, there were no direct relations between dose increase and cell viability in other samples tested. Generally, cell viabilities of Detroit 562 cells decreased at lower doses. However, increasing doses resulted in similar viability compared to non-treated controls. Interestingly, highest doses, 10% and 20%, were found to be toxic for most of the samples.

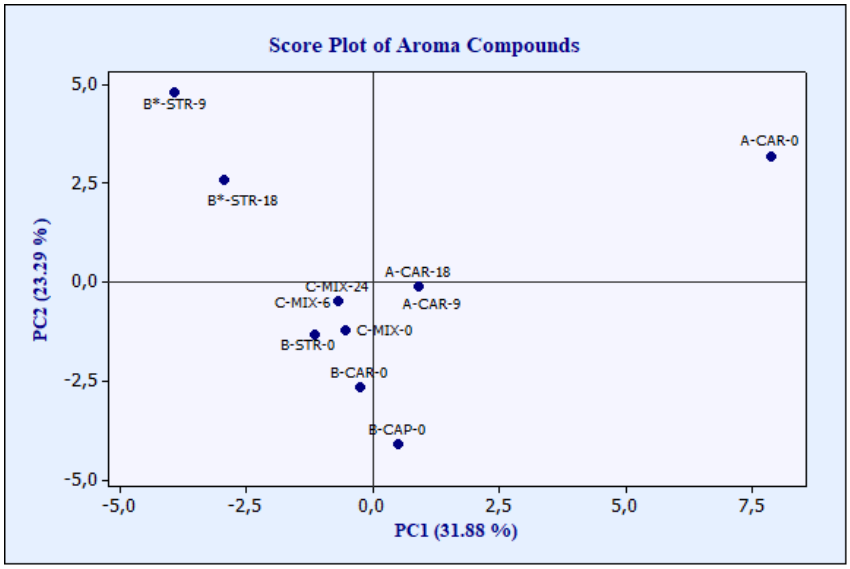

Fig. 1. PCA graph of e-liquid constituents.

Table 4. IC_{50} values (v/v) of e-liquid samples for THLE-2 cell line.

A-Car-0	**A-Car-9**	**A-Car-18**
1.467	1.719	2.415
B-Cap-0	**B-Car-0**	**B-Str-0**
1.539	1.211	1.404
B*-Str-9	**B*-Str-18**	
2.963	2.544	
C-Mix-0	**C-Mix-6**	**C-Mix-24**
5.101	4.661	1.739

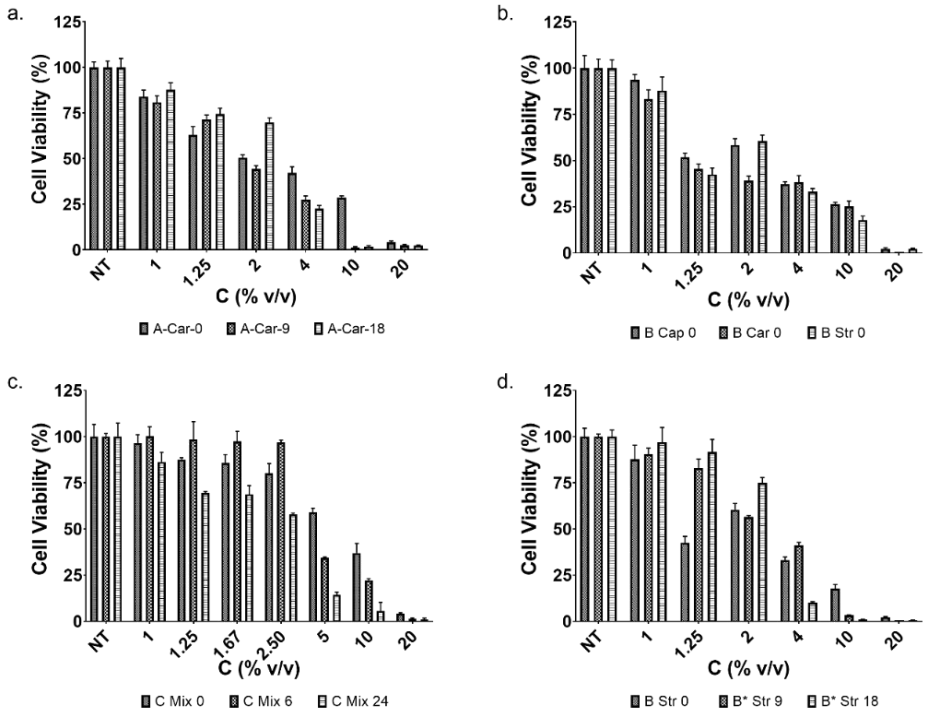

Fig. 2. Dose dependent effects of e-liquids on THLE-2 cell viability. **a.** Brand A, caramel aroma, nicotine amounts are 0, 9, and 18 mg/mL, **b.** Brand B, without nicotine, cappuccino, caramel, and strawberry aromas, **c.** Brand C, mixed aroma, nicotine amounts are 0, 6, and 24 mg/mL, **d.** Brand B*, strawberry aroma, nicotine amounts are 9 and 18 mg/mL (Brand B, strawberry aroma without nicotine is same as Fig. 1b for comparison), (Bars represent ±SD).

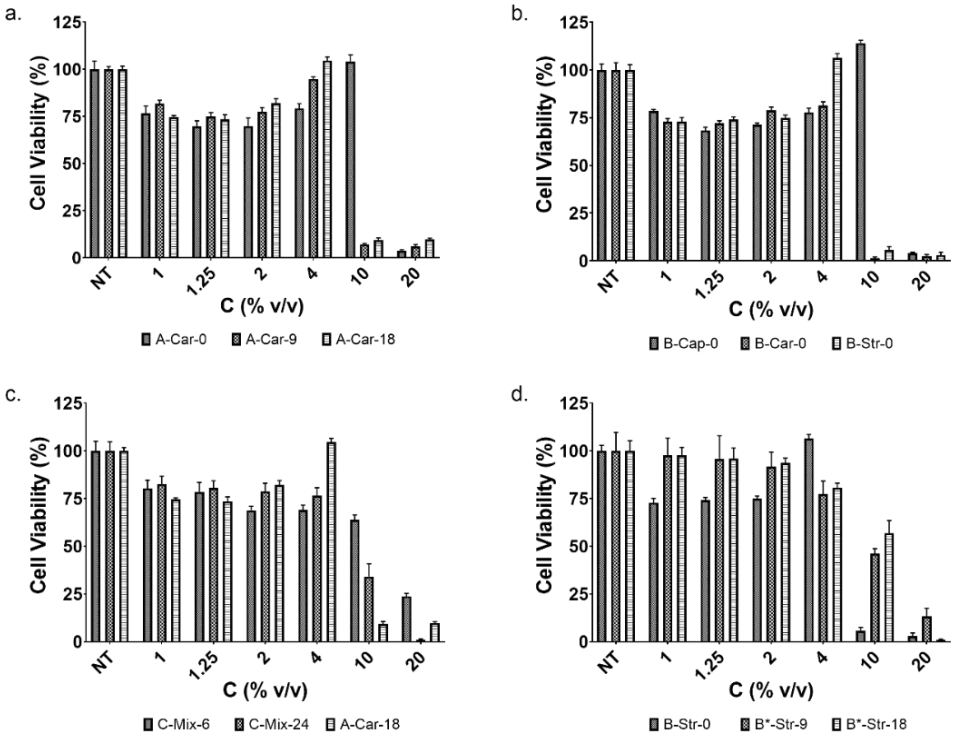

Fig. 3. Dose dependent effects of e-liquids on Detroit 562 cell viability. **a.** Brand A, caramel aroma, nicotine amounts are 0, 9, and 18 mg/mL, **b.** Brand B, without nicotine, cappuccino, caramel, and strawberry aromas, **c.** Brand C, mixed aroma, nicotine amounts are 0, 6, and 24 mg/mL, **d.** Brand B*, strawberry aroma, nicotine amounts are 9 and 18 mg/mL (Brand B, strawberry aroma without nicotine is same as Fig. 2b for comparison), (Bars represent ±SD).

Discussion

Chromatographic methods used in this study were validated for measurement parameters and reliable results were obtained. The results show noteworthy differences between the ingredients of e-liquids confirming previous studies (Cheng 2014). Especially, the concentration of main components, PG and GLY, differ tremendously. These results suggest that there is no standardisation in e-liquid formulations for neither intra- nor inter-brand products. This information may be of importance for regulatory bodies, such as International Organization for Standardization (ISO) or manufacturer organizations dedicated for safer production, such as American E-liquid Manufacturing Standards Association (AEMSA). Another important finding was the differences in volatile component composition. Normally, the same aromas (e.g., caramel) are to be expected to have similar compounds. However, this was not the case with our samples. On the other hand, nicotine amounts on the product labels were consistent with the analytical results.

The i*n vitro* results showed that nicotine amount solely cannot be responsible for inhibition of cell viability. Previous studies also showed that nicotine amount is not associated with cell survival or disrupted metabolic events (Bahl *et al*. 2012, Farsalinos *et al*. 2013, Madison *et al*. 2019). The whole composition of e-liquids affected normal and cancer cells in different manners. It is evident that Detroit 562 cells, but not THLE-2 cells, have adaptive intracellular mechanisms activated as a response to changing e-liquid concentrations. It is known that cancer cells have altered autophagy-apoptosis mechanisms compared to healthy cells. Especially, autophagy mechanisms could result either cell survival or cell death under environmental stress (Hippert *et al*. 2006, Esteve & Knecht 2011). While the exact mechanisms of action for e-liquids are not known, it is possible that they interfere with autophagy-apoptosis balance in cells in a dose-dependent manner. A previous study showed that vapour form can impair autophagy (Shivalingappa *et al*. 2016). Additionally, similar viability trend was reported for a cytostatic drug, paclitaxel (Liebmann *et al*. 1993). It is later found that lower doses resulted in apoptosis whereas, higher doses resulted in G2/M arrest in cell cycle (Torres & Horwitz 1998). Further studies aiming to find the difference between the responses of these two cell lines may enlighten main intracellular mechanisms causing alteration in cell viability after e-liquid insult.

It is of great interest to understand the potentials and risks of e-cigarettes. In this study, we initially validated a liquid chromatographic method to detect nicotine in e-liquid samples in a range of 1.00 μg/mL and 50.00 μg/mL concentration. Additionally, propylene glycol, glycerine, and volatile compounds were analysed in e-liquids. Nicotine amounts were found to be consistent with the product labels chosen in this study. It has been reported that estimated fatal dose of nicotine is 30-60 mg for adults and 10 mg for children (Etter *et al*. 2011). Thus, correct labelling of nicotine amount is important for not only users, but also in cases of accidently ingestion of e-liquids by children. Content analysis showed that not only different brands but also same brand with new products differ in PG and GLY ratios in formulation. In the case of use of these products in a therapeutic approach, their standardization will be needed. We also investigated the biological effects of e-liquids in terms of cell viability. Previous studies showed that inflammation (Wu *et al*. 2014, Muthumalage *et al*. 2017), oxidative stress (Lerner *et al*. 2015, Muthumalage *et al*. 2017), and DNA damage (Lee *et al*. 2018) seem to be the major pathways that play role in cytotoxicity. However, a recent review reported that there are inconsistencies regarding biologic effects of e-liquids in terms of cytotoxicity (Sood *et al*. 2018). In our study, while cell viability of THLE-2, normal liver cells, were found to be lower with increasing concentration of e-liquids, which was not the case for carcinoma cell line Detroit 562. When considering cellular effects of e-liquids, origin of cell and disease status should be considered. Further studies are needed to enlighten the exact molecular mechanisms responsible for different viability responses of cancer cells.

Strengths and limitations

This study reveals the ingredients in e-liquids and corresponding cellular health effects for popular brands. The experimental groups were designed to give various combinations of nicotine amount and flavours. Thus, validated analytical methods are complemented by biological response measurement. On the other hand, this study can give information on e-liquid exposure as a household item not the vaping experience since e-liquids were directly used in the experiments.

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