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RESEARCH

Tattoo inks: evaluation of cellular responses and analysis of some trace metals

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Abstract After tattoo application, inks remain in the skin, mostly in the dermal layer, and manufacturers use inks that have not been adequately evaluated for safety in tattoo production. In this study, the metal contents (Cd, Hg, Pb, and Cr) of tattoo inks available in the Turkish market were determined and the relationship between cell viability and inflammatory response of the detected metal levels was investigated. Nine tattoo inks (3 colors) from 3 different brands abbreviated as E, I, and W were examined. ICP-MS was used for element analysis. The viability of human keratinocyte cells was determined by the WST-1 assay following ink exposures at various dilutions. IL-18 levels were measured in cell culture supernatant by ELISA method following ink or metal (Cd, Cr, Hg, and Pb) exposures. The concentrations of trace elements were found in inks as follows: Cd, 0.0641–1.3857; Hg, 0.0204–0.2675; Pb, 0.8527–6.5981; Cr, 0.1731–45.3962 μg mL^{-1}. It was observed that the levels of Pb and especially Cr in the samples exceeded the limit values. Tattoo inks reduced the cell viability in a dose- and color-dependent manner. IL-18 release was significantly increased in all groups except Cr and black ink of brand I treated cells ($p<0.05$). Our results show that the metal contents of tattoo inks exceed Council of Europe Resolution values in some samples and some inks induce immune system activation (IL-18 secretion) and cytotoxic effects. It is thought that these findings may contribute to the toxic/adverse effects of tattoo inks commonly used.

Keywords Tattoo ink · Metal toxicity · ICP-MS · Keratinocytes · Inflammation

Introduction

In recent years, tattoos have become very popular worldwide and also in Turkey. Many people

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especially young ones have tattoos in black and different colors. Clinical observations and case reports show that tattoos are frequently associated with adverse skin reactions (Cohen et al. 2020; Eghbali et al. 2014; Tighe et al. 2017). Numerous case reports are presented in the literature on dermatological diseases such as pseudolymphoma, allergic or granulomatous skin reactions resulting from tattoos (Bassi et al. 2014; Eghbali et al. 2014; Serup et al. 2016; Tammaro et al. 2021; Xue and Warshawsky 2005). The number of carcinogenic cases associated with inks after tattoo applications is also increasing but currently, there is no causal proof established that tattoos would increase the risk of cancer (Eghbali et al. 2014; Laux et al. 2016; Tighe et al. 2017). Tattoo inks are evaluated in the FDA's classification of permanent cosmetic products. However, the chemicals used in making both permanent and temporary tattoos are not FDA approved. The in vivo suitability of these chemicals is a matter of debate. (Giulbudagian et al. 2020; Prior 2015). Tattoo inks are injected into the dermis/epidermis, thus making direct contact with dermis cells, immune cells, blood, and the lymphatic system. Chemical compounds entering the cell cause disruption of cell metabolism. In this process, chemicals bind to a skin protein, form a hapten, and the hapten passes through the stratum corneum to the underlying epidermis (Bil et al. 2018; Weis et al. 2021). Activation of keratinocytes causes the secretion of cytokines such as interleukin (IL)-1α, IL-18 and tumor necrosis factor-α. IL-18 plays a crucial role in events such as skin sensitization and allergic contact dermatitis. This cytokine is considered a specific biomarker that is up-regulated by chemicals with the potential to be skin sensitizers (Bil et al. 2018; Karregat et al. 2021).

Inks containing hazardous chemicals have been detected in products sold in the European Union market. Metal-based compounds or pigments are used for the coloring of tattoo inks. When the tattoo labels are examined, it is seen that the contents are included as CAS no information and there is no information about the concentration of the metals it contains. It is seen that the solvents used are also indicated by the CAS no. The most well-known metals found in tattoos are mercury (Hg) and cadmium (Cd) in the red pigment, lead (Pb), and chromium (Cr) in the green pigment (Arl et al. 2018). However, nowadays mostly organic pigments are used to create colorful inks (Serup et al. 2020). Cadmium, lead and mercurybased pigments are not used for decades anymore. Black tattoo inks often contain carbon black, an inorganic pigment.

Tattoo inks are currently covered by regulations based on Council of Europe Resolution (CoE ResAP) (2008)1 (EUR 27672. 2016). On 14 December 2020, an EU-wide legal requirement for ingredients found in tattoo inks or permanent makeup was published. These regulatory frameworks differ between countries. The European Chemical Agency (ECHA) has restricted a large number of hazardous chemicals found in tattoo inks and permanent make-up under The Regulation on the registration, evaluation, authorisation and restriction of chemicals (REACH) from January 2022. These rules require this use to be stated on the labels of mixtures intended for tattoo and permanent make-up.

In this study, we aimed to evaluate some metal contents as well as the cytotoxicity and inflammatory potential of widely used tattoo inks. Three colors (red, green, and black) of three different brands found in the market were selected for the analysis.

Materials and methods

Sampling

The viscous liquid form of nine tattoo samples in three colors (black, green, and red) of three brands (abbreviated as E, W, and I) were analyzed in this study. The inks were supplied from the market in Turkey. The brands were chosen for their usage popularity in Turkey.

Biochemical analysis

Cell culture and treatments

Cell culture experiments were conducted in an immortalized human keratinocyte cell line, (HaCaT) which was kindly gifted by Professor Çiğdem Yenisey PhD (Department of Biochemistry, School of Medicine, Aydın Adnan Menderes University, Aydın, Turkey). Cells were grown as monolayers at 37°C, in a 5% CO_2 atmosphere, in DMEM medium (Gibco Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich),1xamphotericin B (2.5 µg mL^{-1}), penicillin (100 U mL^{-1}) and streptomycin (100 U mL^{-1})

(Gibco-Invitrogen). Cells were seeded in poly-L-lysine coated polystyrene cell culture dishes, incubated at 5% CO_2 and 37 °C and passaged. In order to evaluate the effect of tattoo inks, 0.22 µm filtered tattoo ink was diluted in the medium at four different dilutions (1:10 – 1:10.000). For IL-18 analysis, a 1 mM stock solution of each metal was prepared using ultrapure water. Stock solutions of metals were stored at room temperature (25 °C) and fresh dilutions were made using a cell culture medium prior to each experiment. All experiments were run in triplicate.

Cytotoxicity test

Cytotoxicity was determined by using a tetrazolium salt, WST-1 (4-[3-(4-iodophenyl) 2-(4-nitrophenyl) 2 H-5-tetrazolio] 1,3-benzene disulfonate), which turns to a highly water-soluble, orange-colored formazan crystals by metabolic active cells. Following tattoo ink exposures, wells were washed with phosphate buffer solution (PBS). Then, medium containing WST-1 was added to cells and incubated for 4 h at 37 °C. The absorbance was measured at 450 nm test wavelength and 630 nm reference wavelength in a microplate reader (Multiscan FC, Thermo Scientific).

IL-18 ELISA

The secretion of IL-18 in the culture supernatant was measured with a commercially available specific ELISA kit according to the manufacturer's instructions (Bioassay Technology Laboratory Cat. No E0147Hu). Briefly, HaCaT cells were seeded into 6-well plates (4×10^5 cells/well/mL) and incubated for 24 h in a 37 °C CO_2 (5%) incubator. At the end of the incubation, 10 µL of Cd, Cr, Hg or Pb containing fresh medium) or 10 µL of each tattoo ink (final dilution: 1/100) were added to the wells and cells were incubated for 24 additional hours. Then, the cell culture supernatant was collected and centrifuged at 3000 rpm for 10 min. Supernatants were added to a 96-well plate coated with human IL-18 antibody according to the Human Interleukin 18 ELISA Kit protocol. Biotinylated IL-18 antibody and streptavidin-HRP were added to it, respectively. It was covered and incubated at 37 ° C for 1 h. The plate was washed, and substrate solutions A and B were added and incubated at 37 ° C for 10 min in the dark. At the end of the time, the reaction was stopped by adding a stop solution. The absorbance value was measured at 450 nm and IL-18 concentration was calculated using the calibration curve prepared with standard solutions (Park et al. 2007).

Analytical analysis

Sample preparation

Around 300 mg of each sample was carefully homogenized with a mixture of nitric acid, hydrochloric acid, and hydrofluoric acid in the ratio 6:3:0.8 and then digested in the microwave. For each color, three samples were prepared and each sample was analyzed 3 times.

Reagents

Analytical grade reagents and bi-distilled ultra-pure water (Merck Millipore Milli 2 Integral 2) were used for all the steps of the analysis. Suprapure-graded nitric acid, hydrochloric acid, and hydrofluoric acids were used for the digestion. Stock solutions of certified multi-element standards of Cd, Cr, Hg, and Pb (1000 mg L^{-1}) (Merck, Darmstadt, Germany) were preferred for the calibration. ICP-MS tunning solution ^{7}Li, ^{89}Y, ^{205}Tl ve ^{6}Li, ^{45}Sc, ^{72}Ge, ^{103}Rh, ^{115}In, ^{159} Tb, ^{175}Lu, ^{209}Bi were used as internal standard for matrix effect.

Apparatus

Multi-element analysis was done by using an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS 7800, Agilent, USA). The working conditions of the ICP-MS are given in Table 1. Standards were given to the device in the form of a multi-element analysis in accordance with its optimum range.

Statistical analysis

All data were expressed as means RSD values and analyzed for statistical significance using Statistical Package for Social Sciences (SPSS version 23.0). Comparisons of means between two groups or multiple groups were performed by student *t*-tests or one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, respectively. Each sample was

Table 1 Working conditions for ICP-MS detection

ICP-MS parameters	Value
Plasma mode	General purpose
RF power	1550 W
RF matching	1.80 V
S/C temperature	2 °C
Sample depth	10 mm
Carrier gas flow rate	1.0 L/min
Nebulizer pump flow rate	0.1 rps
Internal standards	^{6}Li, ^{4}5Sc, ^{72}Ge, $_{103}$Rh, ^{115}In, ^{159} Tb, ^{175}Lu, ^{209}Bi
Tuning solvent	^{7}Li, ^{89}Y, ^{205}Tl

analyzed in triplicate. In all statistical tests, $p < 0.05$ was considered statistically significant.

Results

Biochemical results

Cellular viability was reduced by tattoo inks in a dose-dependent manner. All green and black tattoos significantly decreased cell viability at 1:10 dilution ($p < 0.05$) (Figs. 1 and 2). No viable cell was detected in either brand of green tattoos at higher concentrations (E and W). This suggested that the lowest viable dilution of tattoo ink in the evaluation of the WST-1 test was 1 in 100. There was a decrease in viability up to 1:1000 dilutions in black inks of two brands (E and I). All concentrations studied at brand E significantly reduced cell viability in green and red colors. In red tattoos, cell viability showed a significant decrease depending on the concentration only in the brand E (Fig. 3).

In addition to cell viability, tattoo inks at 1:100 dilutions were evaluated in terms of IL-18 release. As shown in Table 2, all three brands significantly increased the release of IL-18 from the cells ($p < 0.05$). However significant increases at the level of $p < 0.01$ were observed only in E and W-exposed cells. In addition to tattoo inks, four heavy metals (Cd, Cr, Hg, and Pb) were selected for IL-18 determination. As shown in Table 2, Cd, Hg, and Pb exposures led to an increase in IL-18 release in HaCaT cells ($p < 0.05$).

Analytical results

Method validation

The performance of the analytical method was evaluated in precision, accuracy, linearity, sensitivity, limit of detection (LOD), and limit of quantification (LOQ). The results of the evaluation of accuracy and the precision of the method are shown in Table 3. The correlation coefficient of the calibration of the

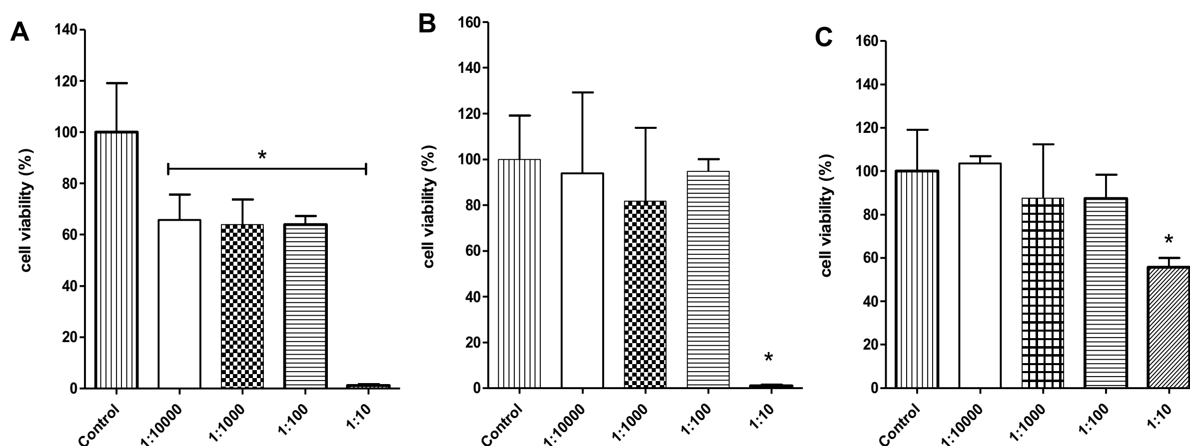

Fig. 1 Effects of green tattoo inks E (**A**), W (**B**) and I (**C**) on HaCaT cell viability. Cell viability was evaluated by the WST-1 assay. Cells were exposed to four different dilutions of inks for 24 h. Each bar represents mean + SD. *$p < 0.05$: significant differences than untreated cells. *SD* standard deviations

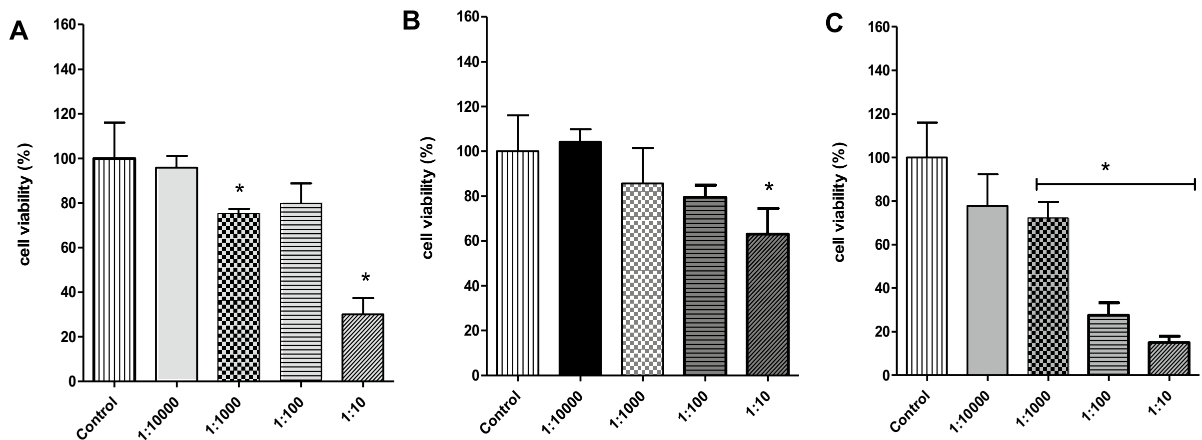

Fig. 2 Effects of black tattoo inks E (**A**), W (**B**) and I (**C**) on HaCaT cell viability. Cell viability was evaluated by the WST-1 assay. Cells were exposed to four dilutions of inks for 24 h. Each bar represents the mean + SD. *$p < 0.05$: significant differences than untreated cells. *SD* standard deviations

Table 2 Released IL-18 levels (ng mL^{-1}) in HaCaT cells following exposure to tattoo inks (1:100) and heavy metals

Groups (n = 3)		IL-18 ng mL^{-1})	
		Mean	RSD
Untreated cells		12,768	0.15
Cd-treated cells		20,710*	1.44
Cr-treated cells		14,411	0.96
Hg-treated cells		23,267*	1.37
Pb-treated cells		23,414*	0.41
Green ink	E-treated cells	28,888*	0.24
	I-treated cells	17,793*	0.55
	W-treated cells	18,912*	0.51
Black ink	E-treated cells	22,185*	1.01
	I-treated cells	15,153	1.32
	W-treated cells	20,859*	0.17
Red ink	E-treated cells	20,482*	0.01
	I-treated cells	16,309*	1.16
	W-treated cells	18,876*	0.42

RSD relative standard deviation

*$p < 0.05$: significant differences than untreated cells. Cd (0.0112 μg mL^{-1}), Cr (0.519 μg mL^{-1}), Hg (0.0200 μg mL^{-1}), Pb (0.0207 μg mL^{-1})

elements and the other validation parameters are given in Table 4. Validation studies are based on ICH guideline (EMEA, Note for Guidance on Validation of Analytical Procedures: Text and Methodology, CPMP/ICH/381/95, 1995, 1–15).

The precision value of the method is given by the RSD value as a result of three consecutive measurements. Intra-day precision was determined by preparing three of the same standard samples and calculating the RSD values of the results obtained from these three analyses, and inter-day precision was determined by measuring the same standard solutions again on different days and calculating the RSD values of the results obtained. 0.1 μg mL^{-1} standard solution of Cd, Cr, Pb, and 0.02 μg mL^{-1} standard solution of Hg were used for the precision analysis.

Linearity was determined by a mixture of standards containing 5 different concentrations of each metal given at optimum operating conditions of the device. Standard solutions were prepared from 1000 μg mL^{-1} stock solution of each element by dilution in different ranges. The slope of this calibration curve also shows the sensitivity of the method. The limit of detection measurements was made with the measurement of blank samples. LODs and LOQs were experimentally calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response of ten blanks and S is the slope of the calibration curve (EURACHEM 2000).

Accuracy of the method was determined with LGC7162-CRM (LGC, Germany). The systematic error was calculated by the formula of A = ((CRM value-Sample value)/CRM value)*100.

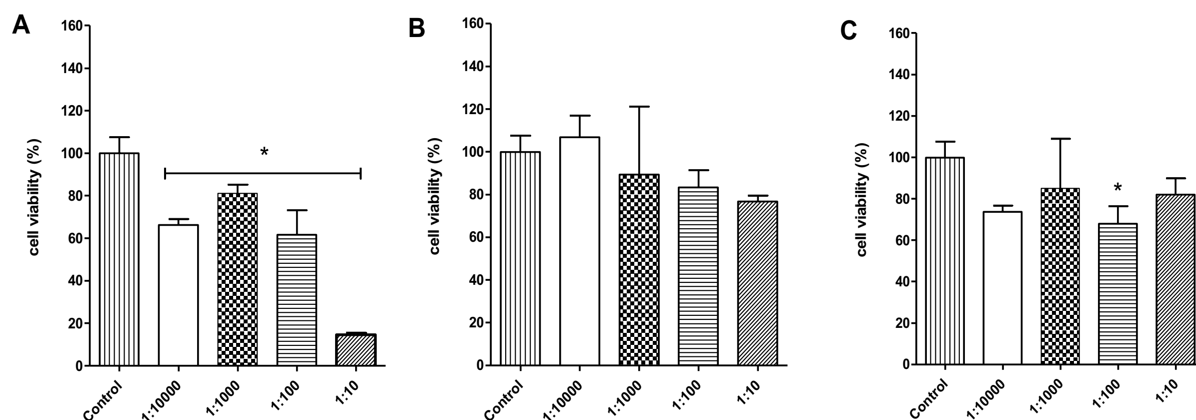

Fig. 3 Effects of red tattoo inks E (**A**), W (**B**), and I (**C**) on HaCaT cell viability. Cell viability was evaluated by the WST-1 assay. Cells were exposed to four dilutions of inks for 24 h. Each bar represents the mean + SD. *$p < 0.05$: significant differences than untreated cells. *SD* standard deviations

Table 3 Precision and accuracy of the method for the tattoo ink determination

Element	Precision (RSD)	Intra-day precision (RSD)	Inter-day precision (RSD)	Accuracy (%)
Cd	0.37	0.63	1.97	13
Cr	0.63	2.77	4.36	17
Hg	2.56	8.35	17.35	*
Pb	2.34	1.69	2.37	9

CRM certified reference material

*Hg values could not be given due to the absence of Hg in the CRM

Table 4 Some validation parameters of the ICP-MS method for the determination in tattoos inks

Elements	Calibration curve equation	Calibration curve Range (μg mL^{-1})	r^2	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
^{111}Cd	y = 0.0012 * x + 1.4858E-005	0–100	0.9999	0.0117	0.0353
^{52}Cr	y = 0.1527 * x + 1.0201	0–100	1.0000	0.0059	0.0182
^{201}Hg	y = 0.0029 * x + 5.6137E-005	0–20	0.9971	0.0998	0.0302
^{208}Pb	y = 0.02747 * x + 0.001364	1–100	1.0000	0.0319	0.0966

ICP-MS results

The results of laboratory analyses showed large variations in heavy metal concentrations in the samples of tattoo inks tested in Table 5.

Cd concentration in all brands and colors was in the range of 0.0641–1.3857 μg mL^{-1}. In general, all black tattoo ink brands had similar Cd concentrations. The highest amounts of Cd were found in the green ink of brands E and I respectively. If we sorted by the Cd results according to brand name, in red ink of W in black ink I, and in green ink E had the highest concentration of the element. On the other hand, the red inks of I and E brands and the green ink of W had the lowest amount of Cd (Table 4).

Cr concentrations dramatically varied according to both the brand and color of tattoo inks. The concentration range changed between 0.1731 and 45.3962 μg mL^{-1}. The highest Cr concentration was observed in the red ink of brand W, which was found to be well above the CoE ResAP (2008)1 guideline value (0.2 μg mL^{-1}) the lowest one is the green ink of brand E.

Hg concentrations of the tattoo inks were in the range of 0.0204–0.2675 μg mL^{-1} in three brands.

Table 5 The amount of some trace elements in (μg mL^{-1}) in different colors and different brand tattoo permanent ink samples

Color	Brand	Cd		Cr		Hg		Pb	
		Mean	RSD	Mean	RSD	Mean	RSD	Mean	RSD
Green	E	1.39	3.53	0.17	3.36	0.04	1.32	2.13	0.50
	I	0.51	1.68	1.90	0.93	0.05	2.83	3.37	0.84
	W	0.10	0.83	6.34	0.72	0.17	1.69	4.01	0.97
Black	E	0.13	5.64	6.93	2.18	0.27	4.30	6.60	2.83
	I	0.15	1.17	8.21	0.65	0.22	2.53	3.50	2.29
	W	0.14	1.69	4.79	0.33	0.09	2.06	4.69	0.05
Red	E	0.10	0.67	11.87	2.09	0.04	2.31	0.85	0.74
	I	0.06	0.81	4.27	0.55	0.02	1.48	0.70	0.66
	W	0.11	1.45	45.40	0.67	0.05	1.00	1.23	0.02
Limit of ResAP (2008)1		0.2		0.2		0.2		2	
Commission Regulation 2020/2081 MAC Concentration limit (by weight)		0.5 0.00005%		0.5 0.00005%		0.5 0.00005%		0.7 0.00005%	

ResAp Council of Europe (CoE) Resolutions, *MAC* maximum allowed concentrations (maximum concentration in ready for use preparation)

Among the heavy metals tested, only Hg values were found to be close to each other and within the guideline limits in all analyzed samples. The black tattoo inks had a higher amount of Hg than the red and green ones. The highest Hg concentrations were observed in the black inks of brands E and I, and the green ink of brand W, respectively. The highest concentration of Pb was determined in the black inks of brands E. The range or the analysis for Pb determination was 0.8527–6.5981 μg mL^{-1}. As shown in Table 4, the Pb concentration was found lower in red tattoo inks than the other colour inks. The highest Pb concentrations of the brands were found in black inks of brand E and I, green ink of brand W, respectively.

Discussion

In this study, commercially available tattoo inks were evaluated in terms of metal contents and cellular responses. These brands are among the most consumed products in European countries. It has been hypothesized that subcutaneous application of various chemical compounds, including toxic metals used in tattoos, in certain amounts may be an important risk factor for health. It is considered that the longevity of tattoos, especially at young ages, may threaten health.

In this study, tattoo inks were added to culture medium to mimic intradermal exposure. We observed that tattoo inks induce inflammatory IL-18 release from HaCaT cells. These findings suggest that inks may trigger skin sensitization, inflammatory and allergic reaction. They may cause undesirable and lifelong effects such as prolonged infection. These reactions have been found to be associated with inorganic metals such as Cd and Hg, and it has been argued in recent studies that the reactions may be related to organic pigments (Karregat et al. 2021). In our study, IL-18 release appears to be increased for all brands in green and red colors and also for Cd, Hg and Pb. Literature reviews report that Cd accumulates in the skin, causes inflammation and tissue damage (Tucovic et al. 2018), and induces apoptosis in skin epidermal cells via a caspase-independent mitochondria-mediated pathway (Son et al. 2010). Mercury has also been reported to stimulate inflammatory activation leading to the secretion of IL-1β and IL-18 cytokines via the caspase-1-mediated pathway (Alphonse et al. 2023). These results support that the increased IL-18 levels in our study may be metal-mediated. Skin reactions mostly appear in inks with organic red pigments (Serup et al. 2020). On the other hand, chromium treatment did not induce any significant changes in IL-18 release.

Cr(VI) is known to induce inflammatory responses in cells through TNF-α. In addition, the reduction in cell viability and the induction of apoptosis were reported to be observed following Cr (VI) treatments at higher concentrations (> 30 μg mL^{-1}) (Lee et al. 2014). Skin reactions mostly appear in inks with organic red pigments (Serup et al. 2020). Nowadays, most red tattoos consist of organic pigments. More rarely, iron oxides are used. However, even these pigments and inks may have heavy metal impurities as seen in our and other studies. In our study, considering the ICP result (Cr concentrations in red ink of brand I; 45.39 μg mL^{-1}) and based on cell viability assay, highest concentration (0.519 μg mL^{-1} = 10 μM) was chosen for Cr to be performed in cell culture studies. Probably, the reason behind not observing significant alterations in IL-18 levels following Cr treatments might be the concentration used. The recommended 0.5 ppm (parts per million, μg mL^{-1}) limit for tattoo inks applies to Cr (VI) form, not the total Cr content. In this study, Cr is not speciated, the data obtained belong to the total Cr. The CoE ResAP(2008) and 2020/2081 report state, "The presence of traces of Cr (VI) in products for tattoos and PMU should be mentioned on the package together with a warning (for example, "Contains chromium Can cause allergic reactions)" rule. Schreiver and Luch (2020) emphasize that high amounts of Cr metal residues can enter the skin in tattoo needles used during application. This is also a matter of toxicological concern.

In their study, Bil et al. (2018) evaluated whether it would Pb to an increase in the release of IL-18, a biomarker of inflammation. Evaluating the IL-18 results, it was noted that 4 of the 5 inks tested were cytotoxic (and thus have irritating properties) and 2 inks may have sensitizing potential. The results are similar to our study. It was determined that glycerol and isopropanol, which are tattoo ink carriers, *Hamamelis virginiana* extract, and lactic acid, did not increase IL-18 release (Bil et al. 2018). According to the CoE ResAP latest regulation, the Pb limit value has been reduced from 2 to 0.7 μg mL^{-1}.

We showed that 7 of 9 tested inks are cytotoxic at high concentrations (except red inks of brand W and I). Falconi et al. (2009) reported that red ink caused loss of viability in fibroblasts and determined the black ink as the safest. In this study, cell viability was not significantly decreased in 2 of 3 different brands of red inks. Cell viability was very low at the highest ink concentration in all 3 brands of green and black inks.

The result of laboratory analyses showed large variations in heavy metal concentrations in the samples of tattoo inks tested in Table 4. Of the four metals we analyzed, except Cr, the other 3 metals (Cd, Hg, Pb) are xenobiotic. Although Cr is necessary for the body, it is a highly toxic metal in high doses.

Cd concentration in all brands and colors was in the range of 0.0641–1.3857 μg mL^{-1}. Cd affects genome stability by causing the formation of free radicals. It is a carcinogenic compound that causes DNA damage by inhibiting DNA repair. Cd toxicity may trigger the induction of inflammatory processes, attenuation of apoptosis, changes in gene expression, cell proliferation, and abnormal DNA methylation (Genchi et al. 2020).

Cr concentrations dramatically varied according to both the brand and color of tattoo inks. The concentration range changed between 0.1731 and 45.3962 μg mL^{-1}. It is seen that chromium oxide is used for green inks. The highest Cr concentration was observed in the red ink of brand W, which were found to be well above the guideline value (0.5 μg mL^{-1}) the lowest one is the green ink of brand E.

Hg concentrations of the tattoo inks were in the range of 0.0204–0.2675 μg mL^{-1} in three brands. Among the heavy metals tested, only Hg values were found to be close to each other and within the guideline limits in all analyzed samples. The black tattoo inks had a higher amount of Hg than the red and green ones. In the literature, cytotoxic properties of organic and inorganic Hg such as inducing apoptosis, disruption of the antioxidant system, and increasing cytokine release are seen, but there are differences in toxicity mechanisms. It is not clear how organic and inorganic Hg cause differences in the mechanism of action (Yang et al. 2020).

The highest concentration of Pb was determined in the black ink of the brand E. The range of the analysis for Pb determination was 0.8527–6.5981 μg mL^{-1}. As shown in Table 4, the Pb concentration was found lower in red tattoo inks than in the other color inks. The highest Pb concentrations of the brands were found in black inks of brand E and I, and green ink of brand W, respectively. Exposure to Pb can induce neurological and cardiovascular disorders due to immune, oxidative, and inflammatory mechanisms.

Pb can disrupt the balance of the oxidant-antioxidant system and has hepatotoxic effects (Kianoush et al. 2012).

There are limited studies on the elemental content of tattoo inks. In previous studies, Eghbali et al. (2014) found Pb concentrations in the range of 57.0978–6.3191 μg mL^{-1} and Cd concentrations in the range of 0.8333 – 0.5544 μg mL^{-1} in their measurements of black, red and green inks. In the comprehensive analysis of 18 elements published by Battistini et al. in 2020, metal concentrations were found to be high in some samples.

In this study, Hg concentrations in each color and brand were found to be acceptable according to the guidelines (ResAP). However, Cr concentrations were exceeded in all samples except E brand green ink. Cd concentrations were within limits in all samples except E and I brand green inks. Pb concentration was determined above limits in all samples. [0.7 μg mL^{-1} for Pb, 0.5 μg mL^{-1} for Cr, Cd and Hg (Commission Regulation 2020/2081)].

Similar to our observations, previous studies suggest that different results on metal contents in tattoo inks may depend on the manufacturer (Battistini et al. 2020; Eghbali et al. 2014; Forte et al. 2009a, b; Tighe et al. 2017). The danger of using ink seems to depend on the color of the ink used (black/color), the different ingredients in brands, and the size and location of the image on the body.

When the cell study results and the elemental analysis results in the ink were evaluated together, some inferences were made from the results In green colored inks, cell viability decreased significantly in brand E and the highest level of IL-18 release was seen in brand E. When the metal content was examined, it was seen that Cd was high in E, Cr was high in W, and Pb was high in all green brands.

When black colored inks are evaluated; It was observed that cell viability decreased dose-dependently in all brands, and there were significant decreases at high doses of brands E and I. It was determined that Il-18 release showed a significant increase in E and W brands. Looking at the ICP-MS results, it was seen that Cr and Pb values were high in all brands. It was evaluated that the increase in IL-18 secretion may be related to Pb.

When red-colored inks are evaluated, the highest loss of cell viability appeared with brand E, while the W brand is not decreasing cell viability. It was determined that IL-18 release showed a significant increase in all three brands. In ICP-MS results, the highest Cr values were seen in W. Therefore, the element composition cannot explain the reduction of cell viability. The lack of an increase in Cr on an elemental basis in the release of IL-18 suggests that the release is related to different mechanisms or may have a common effect with other metals.

As a result, we think that it can be evaluated that Cd and Pb, which are elements that are not involved in metabolism, have an effect on cell damage. However, it seems that mechanistic studies that will explain the effect of elements on cell damage are necessary.

Conclusion

In this study, it was observed that the concentrations of Pb and especially Cr in the samples exceeded the CoE ResAP limit values. It was determined that tattoo inks reduced cell viability and triggered inflammation. From the results, it can be concluded that some metals contain high concentrations of harmful elements. Consequently, epidemiological data on the long-term health effects of tattoo inks in humans require the development of better risk assessment and safety testing strategies.

To protect people from chronic exposure, the Commission decided that the restriction would in the future limit the harmonized classified chemicals which are carcinogenic, mutagenic, or toxic to reproduction; skin sensitizer; skin corrosive skin irritant; eye irritant; or eye-damaging. There is no specific legislation regarding tattoo safety in Turkey. In this direction, we think that the regulations to be made in this regard are important.

Author contributions SSK, OS substantial contribution to conception and design, SSK, OS and GA acquisition of data, analysis, and interpretation of data; drafting the article and revising it critically for important intellectual content; SSK final approval of the version to be published. GK and IC analyzing of samples. All authors reviewed the manuscript.

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Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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