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Research Article

Analyzing Lead Concentrations in Pregnant Women Following Topical Application of Eau de Goulard (2% lead subacetate) via ICP-MS

Davut Serdar Sayılgan[1], Tugrul Cagri Akman[2]*, Alptug Atila[3], Gamze Nur Cimilli Senocak[4]

[1]Erzurum Regional Criminal Police Laboratory Directorate, Erzurum, Turkey
[2]Department of Analytical Chemistry, Faculty of Pharmacy, Erzincan Binali Yildirim University, Erzincan, Turkey
[3]Department of Analytical Chemistry, Faculty of Pharmacy, Ataturk University, Erzurum, Turkey
[4]Department of Gynecology and Obstetrics, Faculty of Medicine, Ataturk University, Erzurum, Turkey,

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Abstract

Lead compounds having cytotoxic and genotoxic properties can enter the body via the skin. Although Eau de Goulard (EDG), which contains lead subacetate, is used to prevent edema and phlebitis, it raises concerns about lead exposure. In the study, lead levels in the whole blood of 10 pregnant women were measured by inductively coupled plasma mass spectrometry before and 120 minutes after topical application of the EDG. Upon analysis of the whole blood samples, the average lead concentration was found to be 12.763 µg/L at 0 minutes and 15.823 µg/L at 120 minutes. Statistical analysis using the Paired-Samples *t*-test indicated a significant correlation between lead levels in whole blood and time elapsed after the topical EDG application (R: 0.981, $p<0.05$). The study showed that even short-term application significantly increased the lead concentration in the whole blood of pregnant women ($p<0.05$). This application carries potential health risks due to lead exposure. The study revealed that the topical EDG application threatens both maternal and fetal health and that safer medical practices are needed to prevent phlebitis and edema.

Keywords: ICP-MS, Lead, Eau de Goulard, Trace elements, Validation

Eau de Goulard'ın (2%kurşun subasetat) Topikal Uygulanmasının Ardından Gebe Kadınlarda Kurşun Konsantrasyonlarının ICP-MS ile Analizi

Öz

Sitotoksik ve genotoksik özelliklere sahip kurşun bileşikleri deri yoluyla vücuda girebilir. Kurşun subasetat içeren Eau de Goulard (EDG), ödem ve flebiti önlemek için kullanılsa da kurşun maruziyeti konusunda endişeler yaratmaktadır. Çalışmada, 10 hamile kadının tam kanındaki kurşun seviyeleri, EDG'nin topikal uygulamasından önce ve uygulamadan 120 dakika sonra indüktif eşleşmiş plazma kütle spektrometresi ile ölçüldü. Tam kan örneklerinin analizinde ortalama kurşun konsantrasyonu 0. dakikada 12.763 µg/L, 120. dakikada ise 15.823 µg/L olarak belirlendi. Paired-Samples t-testi kullanılarak yapılan istatistiksel analiz, tam kandaki kurşun seviyeleri ile topikal EDG uygulamasından sonra geçen süre arasında anlamlı bir korelasyon olduğunu gösterdi (R:0,981, *p*:0,000). Çalışma, kısa süreli uygulamanın bile hamile kadınların tam kanındaki kurşun konsantrasyonunu önemli ölçüde artırdığını gösterdi ($p<0.05$). Bu uygulama kurşuna maruz kalma nedeniyle potansiyel sağlık riskleri taşır. Çalışma, topikal EDG uygulamasının hem anne hem de fetus sağlığını tehdit ettiğini, flebit ve ödemin önlenmesi için daha güvenli tıbbi uygulamalara ihtiyaç duyulduğunu ortaya koydu.

Anahtar Kelimeler: ICP-MS, Kurşun, Eau de Goulard, Eser elementler, Validasyon

*Corresponding Author: eczcagri87@gmail.com
Davut Serdar SAYILGAN, https://orcid.org/0000-0001-7385-9244
Tugrul Cagri AKMAN, https://orcid.org/0000-0002-5439-950X
Alptug ATILA, https://orcid.org/0000-0001-7027-809X
Gamze Nur Cimilli SENOCAK, https://orcid.org/0000-0002-6750-9210

1. Introduction

In hospitals, intravenous injection (IV) is the most preferred method of medication administration. Peripheral intravenous catheters are used for 83% of hospitalized patients receiving intravenous bolus/push medication [1]. IVs are multipurpose devices that may be used to provide nutrients, fluids, drugs, and imaging-related contrast chemicals. However, despite these varied IV applications, complications such as edema, phlebitis, infiltration, burning sensation, and excessive fluid volume make it impossible to continue therapy [2].

Especially, phlebitis is characterized by inflammation of the vessel wall and symptoms such as erythema, pain, and edema, along the affected vessel or surrounding the catheter insertion site. Thus, patients with phlebitis endure greater pain, need to stay in the hospital longer, and spend more money on their medical care [3]. To prevent edema and phlebitis among hospitalized patients, a standard practice involves the regular administration of Eau de Goulard (EDG) solution, which contains 2% lead subacetate. This therapeutic approach is used together with antibiotic and anti-inflammatory creams. The reason for including EDG is that the lead subacetate component in the solution reduces the symptoms of edema with its vasoconstrictor effect in vascular complications [4–11].

Lead (Pb) compounds have toxic effects at the cellular level, negatively affecting many organs and systems. Their genotoxicity can cause DNA damage, disrupt cellular replication and pave the way for serious health problems such as cancer [12]. The use of EDG raises concerns due to the toxic nature of Pb. Pb exposure can potentially cause severe complications during pregnancy and also adversely affect the health of fetus [13,14]. The topical application of EDG may release Pb into the bloodstream of pregnant women by absorption from the skin. So, this situation may threaten the health of the mother and the fetus. To eliminate this risk, minimizing Pb exposure of pregnant will be an effective method to safeguard the health of the fetus [15,16]. Therefore, monitoring Pb concentrations in the blood following topical application of EDG is crucial to determine the safety of this therapeutic application for pregnant.

Blood is responsible for transporting trace elements between tissues. Therefore, it is an important source of information about the amount and metabolism of trace metals in the human body [17,18]. Whole blood, serum and plasma have been used in recent biological research for the analysis of trace metals [19–22]. In the literature, some studies for trace metal analysis in pregnant women have been reported. In these studies, whole blood [22–28], plasma [20,22,29], serum [19,30], and urine [21,31] samples were used. There have been no published investigations on the reliability of topical EDG use and its relationship to Pb concentrations.

The purpose of this study was to examine whether Pb from the topical application of Eua De Goulard (2% lead subacetate solution) reaches the bloodstream after being applied to the catheter area. To accomplish this, Pb concentration in the whole blood of pregnant women was measured before and after the first EDG was applied, using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Pb levels before and after the application were then analyzed statistically.

2. Materials And Methods

2.1. Study Population

The study adheres to the principles of the World Medical Association Declaration of Helsinki (WMADH). Ten pregnant women, aged between 18 to 40 years, seeking medical attention at Atatürk University Faculty of Medicine, Department of Obstetrics and Gynecology, between March 2020 and September 2020, were enrolled as participants. The study population consisted of pregnant patients at risk of premature birth with a mean age of 30.32 ± 5.3 years and a BMI > 30 kg/m^2.

Written informed consent was obtained from each patient. The study was carried out in accordance with the Declaration of Helsinki and approved by the Atatürk University Clinical Research Ethics Committee on 07.11.2019 (Permission Number: B.30.2.ATA.0.01.00, Decision Number: 65/482). The study included pregnant women who were at risk of premature birth and were administered IV medication (Ritodrine) via the catheter. To prevent phlebitis and edema around the catheter on the left arm, nurses soaked sterile hydrophilic gauze with EDG solution and pressed the gauze on the catheter area for fifteen minutes every hour. A qualified nurse collected a 3-mL blood sample from the right arm of each participant both prior to and two hour following the first administration of EDG. These samples were collected in evacuated tubes (BD Vacutainer®), which were free of trace metals and contained heparin as an anticoagulant. 2 mL of blood was transferred to an Eppendorf tube which was previously cleaned in a 100-clean room. Before analysis, the blood sample was immediately frozen at -80 °C.

During the study period, all patients were closely monitored and their treatment continued in the Gynecology and Obstetrics Service. All participants were provided with comprehensive high-risk pregnancy care, including regular uterine ultrasonography and fetal heart monitoring.

2.2. Reagents and standard solutions

All aqueous solutions utilized in the study were prepared using purified water obtained through the Milli-Q Advanced A 10 purification system (Millipore, USA). To prevent contamination during sampling, storage, and analysis, all equipment, including tubes, glass bottles, and micropipette tips, used in the study were cleaned. This ensured the elimination of potential contaminants that could affect the accuracy and reliability of the results. Whole blood samples, the solutions of internal standard and standard were prepared using a mixture solution consisting of 65% HNO_3 (Merck, USA) and 33% H_2O_2 (Sigma Aldrich, Germany) (>99%). This solution served as the medium for dissolving and diluting the respective samples, maintaining consistency in the analytical process. To create the calibration curve, a standard stock Pb solution was prepared at a concentration of 100 ppm by diluting the Agilent® Trace Elements (USA) solution. By utilizing increasing concentrations of 2% nitric acid solution, both standard Pb samples in the range of 1-500 µg/L and quality control samples (25, 200 and 400 µg/L) were prepared. The internal standard stock solution containing 10 ppm Bismuth was prepared. Each sample contained the internal standard at a concentration of 1 µg/L to adjust for calibration curve deviations during analysis.

2.3. Instrumentation

The ICP-MS method was used for quantification of Pb in the whole blood samples [32]. The drying process was carried out using an oven (Milestone connect ETHOS UP microwave), and ultra-pure water was obtained with Direct-Q 8 UV Ultrapure Water systems. To analyze the samples, and process the data, the Agilent 7800 Quadrupole ICP-MS device, equipped with an Integrated Sample Introduction System (ISIS 3) and SPS 4 Autosampler, along with Mass Hunter 4.2 Workstation Software 7800 ICP-MS Top C.01.02, was used. The device was calibrated using Agilent tune solution (1 ppm Ce, Co, Li, Mg, Tl, Y). For Pb analysis, the helium (He) collision mode was employed, with argon (Ar) gas used as the carrier gas. The ICP-MS system was run at 1550 W of radio frequency power. The detailed settings and operating parameters of the ICP-MS system applied in the method are provided in Table 1. Values for the measured concentrations (μg/L) were recorded.

Table 1. Agilent 7800 Quadropole ICP-MS operating parameters

Parameter	Value
Plasma conditions	Forward power 1200W
Plasma gas flow	15.0 L/min
Carrier gas flow	0.99 L/min
Carrier gas pressure	1.45 kPa
Dilution gas flow	1 L/min
He gas flow	4.5 mL/min
QP bias	-15 V
Oct bias	-18 V
Cell entrance	-40 V
Cell exit	60 V
Deflect	-0.8 V
Plate bias	-60 V
Nebulizer pump speed	0.30 rps
Sample uptake rate	1.5 mL/min

2.4. Sample Preparations

In the sample preparation process, measures were taken to prevent atmospheric particle contamination. In accordance with the publication by Meyer et al.[32], the sample processing methodology was optimized. To prevent contamination from air particles, the whole blood samples were prepared for ICP-MS analysis using the Milestone Connect ETHOS UP microwave oven and Direct-Q 8 UV Ultrapure Water equipment. Initially, the the whole blood samples were homogenized by vortexing for one minute. A 15 ml polypropylene tube with a screw cap (VWR labs) was filled with 0.5 mL of whole blood. After adding 0.5 mL of hydrogen peroxide (H_2O_2) and 1 mL of 65% nitric acid (HNO_3), the liquid was quickly vortexed. Tubes were then placed in a heating system and heated to 130°C for 60 minutes in order to dissolve.

Following incubation, ultrapure water was added to the digested sample volume until it reached 15 milliliters. For sample preparation and analysis, around 18 MΩ cm^{-1} ultrapure water was utilized. Samples were prepared for measurement after centrifugation (3 min/ 2500 rpm) and vortexing. To eliminate particulate matter, the prepared samples were filtered using a 0.45 µm syringe tip. The filtered samples were subjected to ICP-MS analysis six times, and the results were averaged to ensure accuracy and precision in the measurements. The standard and whole blood samples were diluted at the same ratio using ultrapure water. Parameters such as final volume, sample volume, and dilution coefficient were accurately entered into the calculation section of the total dilution sub-tab in the software. The dilution factor, calculated using the formula: "Dilution factor = (final weight or volume / sample quantity or volume) * dilution coefficient," was used to verify the analytical process's accuracy. Table 2 shows the ramping conditions for the microwave program in the degradation process.

Table 2. The ramping conditions of the microwave program.

Step	Time	T1	T2	Pressure	Power
1	00:10:00	200 °C	100 °C	45 bar	Max power*
2	00:15:00	200 °C	100 °C	45 bar	Max power*

*Max power: 1500W for Ethos and 1200W for Start units.

2.5. Method Validation

The validation of the ICP-MS method involved a series of assessments to determine the accuracy, precision, and sensitivity of the method.

Standard samples (1-500 µg/L) were analyzed, and a calibration curve was established by performing linear regression analysis on the obtained results. The curve equation and correlation coefficient (R) were determined, providing an indication of the method's linearity. The sensitivity of the method was evaluated through the determination of the limit of detection (LOD) and the limit of quantification (LOQ) values. LOD and LOQ values were calculated as three and ten times the standard error of the curve slope, respectively, signifying the minimum concentration of Pb that could be reliably detected and quantified.

Intra-day and inter-day precision and accuracy of the ICP-MS method were assessed using three replicates of the quality control (25, 200 and 400 µg/L) samples. The accuracy and precision of the method were examined through the calculation of percent relative error (RE%) and percent relative standard deviation (RSD%) values, respectively, for both intra-day and inter-day analysis results of the control samples. Acceptance criteria required values below 15% to indicate satisfactory precision and accuracy.

The range of 80–120% is the acceptable range for the percent recovery value. This criteria further validates the method's applicability and reliability for the analysis by verifying that the amount of Pb recovered from the samples was within an acceptable range.

2.6. Statistical Analysis

The statistical analysis was performed using the SPSS 15.0 package program to evaluate the impact of topical EDG application on whole blood Pb levels. The mean and standard deviation values of whole blood Pb levels before and after the application were computed using the Paired-Samples *t*-test. This analysis aimed to evaluate any significant changes in Pb concentrations resulting from the application. $p < 0.05$ was considered significant. Any observed *p*-value below this threshold indicated a statistically significant difference in the Pb levels between before and after the application.

3. Results and Discussion

Before the analyses, the method was validated by evaluating in terms of accuracy, precision, linearity, sensitivity and percentage recovery parameters in the study. Finally, it was successfully applied to the whole blood samples of the pregnant women for Pb analysis.

Standard Pb samples prepared in the range of 0.1-1000 µg/L were analyzed by the method. The results were plotted against concentrations. In the linear regression analysis, the equation of the calibration curve was determined as $y = 33242.1153x + 6031.6467$. This showed that there was a strong linear relationship between the measured values and the Pb concentration in the samples. The correlation coefficient was calculated as 0.9999, confirming the excellent fit of the data to the calibration curve (Figure 1). Determining the linear working range of the developed ICP-MS method between 1-500 µg/L (1, 10, 50, 100, 250, 500 µg/L) reveals the concentration range in which accurate and precise quantitative analysis of Pb levels can be made reliably.

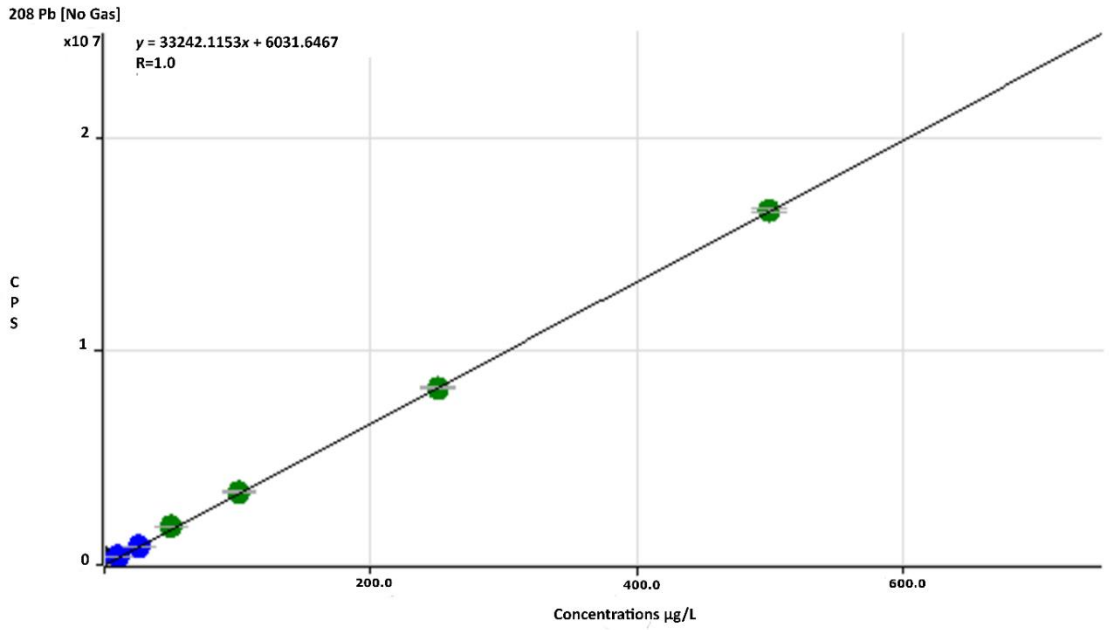

Figure 1. Calibration curve of standard Pb solutions at 1-500 µg/L (Agilent® Trace Elements)

Additionally, the sensitivity of the method was evaluated by determining the LOD and LOQ values. LOD and LOQ values were found to be 0.0418 and 0.1395 µg/L, respectively. These values indicate the minimum concentration of Pb that the method can detect and reliably quantify, further affirming the method's high sensitivity for trace element analysis.
To assess the accuracy and precision (intraday and inter-day) of the ICP-MS method, quality control samples were analyzed three times. According to the analysis results, RSD% and RE% values were found to be 0.662% and 1.540%, respectively. The method has high accuracy and precision for the quantitative analysis of Pb in whole blood samples.
Recovery experiments were carried out at two different concentrations (25, 200 and 400 µg/L). Calculated recovery values (100.2%) were within the acceptable range of 98-102%; This result proves the reliability of the method in accurately recovering and quantifying trace elements in samples. Additionally, the repeatability of the method was evaluated by analyzing 25, 200 and 400 µg/L samples in ten replicates. The results were found to be the same over 95%. (24.96±0.161 µg/L, 200.35±2.425 µg/L and 403.94±1.295 µg/L)

To eliminate differences in analyte sensitivity caused by matrix components in the sample, internal standards must be used in trace element analysis. In this study, a 1 µg/L concentration of bismuth was added to each sample. Consequently, the normalizing of matrix-induced waves allowed the ICP-MS approach to reach excellent sensitivity.

The validated ICP-MS method was applied to the whole blood of women. Table 3 lists the Pb concentrations at two different times. At the 0^{th} and 120^{th} minute, the average Pb concentrations were determined to be 12.763 µg/L and 15.823 µg/L, respectively (Figure 2, Table 4).

Table 3. Pb concentrations in whole blood samples at 0 and 120 minutes following EDG application (mean ± standard deviation, n=10)

Patients	0. minute (µg/L)	120. minute (µg/L)
1	12.763 ± 1.04	15.822 ± 3.04
2	14.363 ± 0.82	14.976 ± 2.19
3	11.999 ± 0.39	16.432 ± 1.38
4	14.379 ± 2.13	13.973 ± 0.76
5	13.337 ± 0.01	17.452 ± 1.51
6	12.975 ± 1.41	14.838 ± 1.68
7	12.273 ± 1.38	16.137 ± 0.43
8	11.678 ± 0.93	16.346 ± 0.69
9	11.869 ± 0.86	17.176 ± 1.14
10	11.974 ± 2.62	15.135 ± 1.76

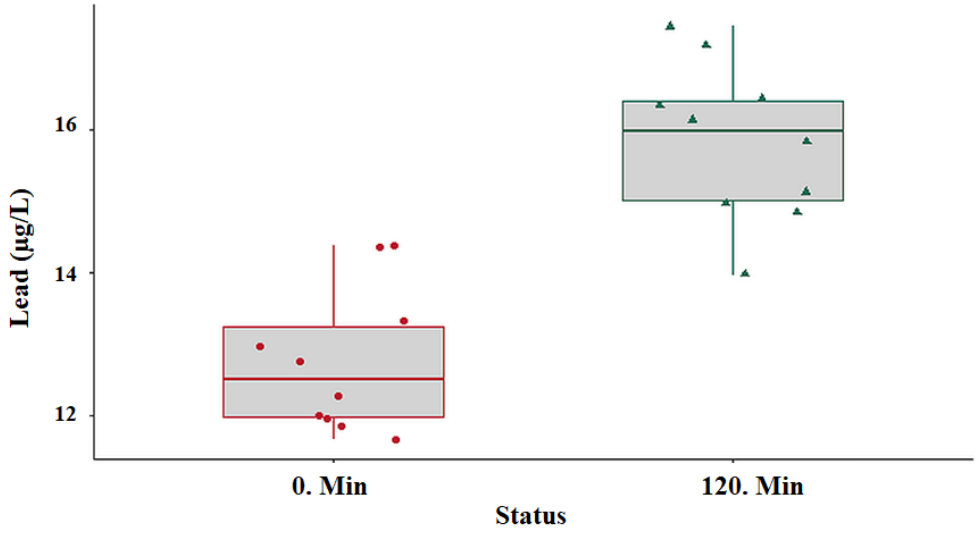

Figure 2. Boxplot showing change in the Pb concentrations with topical application of EDG

Table 4. Mean levels of Pb in whole blood of ten patients at 0. and 120. minutes

Paired Samples Statistics				
Groups	**Mean (µg/L)**	**n**	**Standard Deviation**	**Standard Error Mean**
0. minute	12.7630	10	8.25083	2.60914
120. minute	15.8225	10	10.30786	3.25963

The Paired Sample *t*-test was used to determine whether or not this increase was statistically significant. It was found that there was a considerable rise in Pb concentration following EDG treatment (<0.05, Table 5).

Table 5. Statistical analysis results with the Paired Samples *t*-test

Time	**Paired Differences**					***t***	**df**	**Sig. (2-tailed)**	**R***
	Mean	**SD***	**Std. Error Mean**	**95% CID***					
				Lower	**Upper**				
0. min/120. min	-3.059	2.719	.860	-5.005	-1.114	-3.557	9	.006	0.981

*R: Correlation coefficient, CID: Confidence Interval of the Difference, SD: Standard Deviation

Pb exposure is a global public health concern. Pb is the second most dangerous environmental toxic agent due to its high toxicity and widespread presence. According to the World Health Organization, the maximum acceptable concentration for Pb has not yet been determined. Additionally, it is stated that the estimated contribution of Pb to the "global burden of disease" is 0.6%. According to a number of studies, high blood Pb levels are associated with cognitive problems such as memory loss, difficulty focusing, learning challenges, and even reduced IQ, particularly in the developing brain [33,34].

IV is the most preferred method of administering large amounts of fluid into a vein via a catheter in hospitalized patients. However, this method causes complications such as phlebitis, thrombophlebitis, and edema [3]. For the well-being of the patient and the successful progression of treatment, topical Eau de Goulard solution is frequently used in hospitals [4–11]. Lead subacetate contained in the Eau de Goulard solution causes safety concerns with this method. Determination of Pb levels in patients after administration will provide important information about the reliability of EDG.

In this study, whole blood samples of patients who applied EDG around the catheter for 15 minutes were analyzed using the validated ICP-MS method. Intra-day and inter-day RSD% and RE% values of quality control solutions were below 15%. As a result, the results obtained from the ICP-MS method had high accuracy and precision. The sensitivity of the ICP-MS method was high because the LOD and LOQ values are at low levels such as 0.0418 and 0.1395 µg/L. The correlation coefficient found close to 1 showed that there was a strong positive correlation between the concentrations and the analysis results. Recovery values calculated by analysis of standard Pb samples at three different concentrations were within the acceptable range (80-120%).

The ICP-MS method had advantages compared to many studies reported in the literature. The method had a large linear range (1-500 µg/L) for determination of Pb [22,23,30]. Compared to other studies, the method allowed to determination with high accuracy (RE%: 1.54%) of Pb in the whole blood volume as low as 0.5 mL [22,24,28]. Since whole blood samples were used in the study, samples were prepared for ICP-MS analysis in a short time of approximately 1 hour, without the need for serum and plasma separation processes [22,24,28,29]. HNO_3:H_2O_2 (2:1) mixture was used for sample preparation and high recovery was achieved. To eliminate the masking problem and minimize the matrix effect in the determination of Pb, samples were prepared by diluting with distilled water as an analyte-free matrix compared to an acid or alkali mixture. In this way, the analysis of Pb in whole blood samples was performed with higher sensitivity (LOD: 0.0418 µg/L) [22,24,26,29,30].

EDG treatment increased the mean Pb concentration in the whole blood of patients by 3.05950 µg/L after 2 hours (Table 4). Statistical analysis of the analysis results with the Paired-Samples *t*-test showed that there is a significant correlation between Pb levels in the whole blood and the time elapsed after Pb exposure (R: 0.981; $p<0.05$, Table 5). It was determined that there was a significant difference in Pb concentrations before and after application (Figure 2). As a result, EDG, applied topically for 15 minutes to prevent phlebitis and edema, significantly increased the Pb concentration in the whole blood according to the Paired-Samples *t*-test ($p<0.05$, Table 5).

Percutaneous absorption is important in assessing the risk of exposure to toxic substances such as Pb. They are known to both accumulate in the skin and widely penetrate the body and cell membranes after long-term topical application. It is well-established that prolonged contact or topical application can lead to Pb absorption through the skin. However, it has not been reported in any study that Pb enters the systemic circulation after short-term topical application [35–38]. Unlike the literature, this study showed that short-term Pb exposure increased Pb concentration

in whole blood. Various factors also contribute to the increase of Pb concentration in whole blood by topical EDG application. Application duration and frequency, concentration of the EDG (lead subacetate) solution and physical properties of the skin are among these factors. Each of these factors can affect the absorption of Pb from the skin and subsequently lead to the accumulation of Pb in the bloodstream, tissue, and bones [38]. In this study, the effect of EDG solution applied to the skin for 15 minutes on Pb concentrations, especially in pregnant women was investigated. The results conclusively showed that this practice led to a significant increase in participants' whole blood Pb concentrations, threatening the health of pregnant women and fetuses.

The presence of Pb salts at parts per billion raises serious concerns about fetal health even if they are poorly soluble. Recent studies have demonstrated a strong correlation between cord blood Pb levels and maternal blood Pb levels [39]. There have been important scientific findings that Pb exposure can cause metabolic, neurological and psychological problems in pregnant women and fetuses. It has been reported that in cases where the maternal blood level is approximately 10 μg/dl, hypertension, spontaneous abortion and cognitive development of the fetus are negatively affected in pregnant women. Studies show that maternal Pb exposure is associated with serious outcomes such as low birth weight, preterm birth, stillbirth, spontaneous abortion, congenital defects and hypertension. Pb exposure in the intrauterine period can lead to serious health problems such as fetal growth retardation, nervous system disorders, postnatal mental retardation, motor skill disorders and renal dysfunction [39–43]. Studies conducted by the United States Centers for Disease Control and Prevention (CDC) have determined the acceptable blood Pb level as 50 ng/mL in children. However, no lower threshold has been reported for pregnant women. To avoid the reported effects of Pb, it is critical to identify the factors that may contribute to increased Pb exposure and immediately execute preventive measures against these causes. For example, the development of safer and more effective Pb-free treatment methods that can replace EDG is a critical part of this effort [44]. In the context of alternative treatment options, hot-cold and dry-wet compresses, nonsteroidal anti-inflammatory drugs (NSAIDs), topical products containing heparinoids and herbal creams (such as *Aesculus hippocastanum* and *Arnica montana*) have been shown in various studies to be effective in preventing phlebitis and relieving symptoms [45–47]. In addition, blood Pb tests for confirmation and monitoring purposes should be performed in accordance with the protocols determined by the CDC, and maternal blood or umbilical cord blood Pb levels should be measured during delivery. Women with blood Pb levels above 450 ng/mL should be treated by specialists experienced in the management of complications related to Pb toxicity and high-risk pregnancies [39,41–43].

It is known that skin thickness, contact time, and pregnancy can all influence lead absorption. Thicker skin may act as a barrier, reducing lead absorption, however longer contact times may enhance absorption. Furthermore, pregnancy can impact lead absorption and distribution in the body by generating metabolic changes such as hormonal alterations, higher blood pressure, and abnormalities in nutrient transport. Although this study did not directly investigate these factors, it is obvious that these limitations may have an impact on the generalizability of our findings and that more research into lead exposure is required.

4. Conclusion

In this study, the effect of topically applied Eau de Goulard (2% lead subacetate) solution on lead concentration in whole blood was investigated. A validated ICP-MS method was employed for whole blood samples in the study. Through this method, a notable elevation in lead concentrations within the whole blood of pregnant women was determined. The findings of the study drew attention to the potential threat that topical application of Eau de Goulard poses to both maternal and fetal health. The sensitivity of the ICP-MS method played a pivotal role in detecting even low concentrations of lead absorbed through the skin into the systemic circulation. Eau de Goulard can be applied topically for shorter periods and at longer intervals to reduce lead exposure and prevent lead from reaching the bloodstream. In addition, the use of Eau de Goulard solutions with lower lead concentrations may be a viable strategy. However, this study suggests that safer, lead-free treatment methods should be the first choice for pregnant women who are a vulnerable population, rather than Eau de Goulard, which has been shown to increase lead exposure. In conclusion, the study emphasizes the necessity for in-depth investigations into the toxic effects of topical Eau de Goulard application. This step is crucial for shedding light on the potential health risks associated with this common medical practice. Finally, the study represents an important step toward advocating for safer medical protocols and enhancing awareness about lead exposure, particularly among sensitive populations like pregnant women.

Limitation of the study

The small number of patients limits the study. Comprehensive studies with large numbers of participants are needed to more clearly determine the safety and toxicity of Eau de Goulard solution. In addition, the application of multivariate analyses that take into account factors such as age, BMI, and skin condition that may affect lead absorption through the skin, as well as the increasing number of participants, will provide stronger results.

Ethics in Publishing

Written informed consent was obtained from each patient. The study was carried out in accordance with the Declaration of Helsinki and approved by the Atatürk University Clinical Research Ethics Committee on 07.11.2019 (Permission Number: B.30.2.ATA.0.01.00, Decision Number: 65/482).

Conflict of Interest

The authors declare no competing interests.

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Author Contribution

A.A. conceived and designed the research. T.C.A. and D.S.S. conducted experiments and analyzed the data statistically. G.N.C.S. collected the whole blood samples. A.A. and T.C.A. evaluated the analysis results. T.C.A, D.S.S. and A.A. drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

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