J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 47(3): 1003-1010, 2023
Doi: 10.33483/jfpau.1344014

ORIGINAL ARTICLE / ÖZGÜN MAKALE

ELECTROCHEMICAL INVESTIGATION OF OTILONIUM BROMIDE USING BORON-DOPED DIAMOND AND GLASSY CARBON ELECTRODES

CAMSI KARBON VE BOR KATKILI ELMAS ELEKTROTLAR KULLANARAK OTİLONYUM BROMÜRÜN ELEKTROKİMYASAL DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: *Using cyclic (CV) and differential pulse (DPV) voltammetric techniques, the electrochemical research of otilonium bromide (OTB) was carried out over a wide pH range (0.3–12) at glassy carbon electrodes (GCE) and boron-doped diamond electrodes (BDDE). The typical electrochemical behavior of OTB was identified as being dependent on the type of working electrode and pH. This research aims to provide a brand-new electroanalytical technique for measuring OTB in buffer solutions.*

Material and Method: *All experiments employed the typical three-electrode cell of 10 ml capacity in conjunction with a platinum wire counter electrode, a BDDE and GCE working electrode, and an Ag/AgCl reference electrode. NOVA 1.8 software and an AUTOLAB 204 potentiostat/galvanostat were used for electrochemical measurements.*

Result and Discussion: *The electrochemical behavior of OTB, which belongs to a class of drugs called 'antispasmodics' (spasm and cramps reliever), primarily used to treat irritable bowel syndrome (IBS), and other gastrointestinal conditions characterized by motility problems, painful bowel spasms and distension (swelling and bloating in the belly area), was examined in 0.1 M H_2SO_4*

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Submitted / Gönderilme : 15.08.2023
Accepted / Kabul : 28.08.2023
Published / Yayınlanma : 20.09.2023

at BDDE and GCE. The electrooxidation mechanism was also investigated by conducting CV investigations at various pH levels throughout a broad pH range (pH 0.3-12.0). Understanding the mechanism was aided by scan rate investigations, which revealed that diffusion was controlled for both electrodes. The proposed technique was successfully used to determine OTB under optimal conditions.
Keywords: *Boron-doped diamond electrode, differential pulse voltammetry, drug assay, glassy carbon electrode, Otilonium bromide*

ÖZ

Amaç: *Döngüsel (CV) ve diferansiyel darbe (DPV) voltametrik teknikler kullanılarak, otilonyum bromürün (OTB) elektrokimyasal araştırması, bor katkılı elmas elektrotlarda (BDDE) ve camsı karbon elektrotlarda (GCE) geniş bir pH aralığında (0.3–12) gerçekleştirildi. OTB'nin tipik elektrokimyasal davranışının, çalışan elektrot tipine ve pH'a bağlı olduğu tespit edildi. Bu araştırmanın amacı, tampon çözeltilerde OTB'yi ölçmek için yepyeni bir elektroanalitik teknik sağlamaktır.*
Gereç ve Yöntem: *10 ml kapasiteli tipik tek bölmeli üç elektrotlu hücre, tüm çalışmalarda bir BDDE ve GCE çalışma elektrotu, bir platin tel karşıt elektrot ve bir Ag/AgCl referans elektrotu ile birlikte kullanıldı. Elektrokimyasal ölçümleri gerçekleştirmek için NOVA 1.8 yazılımı ve bir AUTOLAB 204 potansiyostat/galvanostat kullanıldı.*
Sonuç ve Tartışma: *"Antispazmodikler" (spazm ve kramp giderici) adı verilen bir ilaç sınıfına ait olan OTB'nin elektrokimyasal davranışı, öncelikle hassas bağırsak sendromunu (IBS) ve hareketlilik sorunları, ağrılı bağırsak spazmları ve şişkinliği (şişme) ile karakterize edilen diğer gastrointestinal rahatsızlıkları tedavi etmek için kullanılır. ve göbek bölgesinde şişkinlik), GCE ve BDDE'de 0.1 M H_2SO_4'te incelenmiştir. Geniş bir pH aralığı (pH 0.3-12.0) boyunca çeşitli pH seviyelerinde CV araştırmaları yapılarak elektrooksidasyon mekanizması da araştırıldı. Mekanizmanın anlaşılması, difüzyonun her iki elektrot için de kontrol edildiğini ortaya koyan tarama hızı araştırmalarıyla desteklendi. Önerilen yöntem, optimal koşullar altında OTB'yi belirlemek için başarıyla kullanılmıştır.*
Anahtar Kelimeler: *Bor katkılı elmas elektrot, camsı karbon elektrot, diferansiyel puls voltammetrisi, ilaç analizi, Otilonyum bromür*

INTRODUCTION

Otilonium bromide (OTB; Figure 1) is an antispasmodic medicament used to treat Irritable Bowel Syndrome symptoms. The smooth muscles of the stomach and intestine contractions are relieved with OTB. The medicament relieves painful muscle spasms by relaxing the muscles in the intestine's walls. As a result, it lessens bloating, discomfort, and pain in the stomach caused by spasms, cramps, and gas development [1-4].

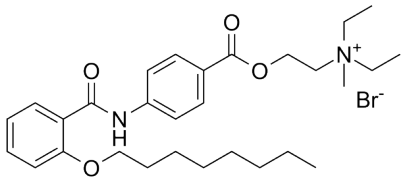

Figure 1. Structure of Otilonium Bromide (OTB)

In the 19th century, two main branches of natural sciences - chemistry and electrical science - merged to form electrochemical science. Examining pharmaceuticals can be done using an intriguing approach called electrochemical sensing. Electrochemical sensing techniques can be used for various purposes, including the quick and simultaneous examination of numerous samples with high sensitivity and low detection limits. All these investigations require extremely affordable devices. Thus, we have chosen electrochemical techniques over others because of their low cost. Other approaches include the use of many organic solvents and labor-intensive steps. In drug analysis, electrochemical techniques are

frequently employed. Voltammetric methods are popular because of their rapidness, sensitivity, and the fact that they only require simple equipment. This study employed differential pulse voltammetry (DPV), a method that is often utilized [5-9].

The glassy carbon electrode (GCE) is commonly considered for voltammetry and numerous applications in drug analysis [10]. The boron-doped diamond electrode (BDDE) has been of great interest as the electrode for different electroanalytical applications. It has advantages over conventionally used electrodes [11,12], such as its low background currents, large potential window (up to 3 V), thermal shock resistance, corrosion resistance towards aggressive media, and extreme electrochemical stability [5-9].

This study aimed to create novel pulse voltammetric methods for directly determining OTB using contemporary drug assay methods. Using CV and DPV, this work also aims to investigate the precise voltammetric oxidation mechanism of the OTB on BDDE and GCE.

MATERIAL AND METHOD

Apparatus

NOVA 1.8 software and an AUTOLAB 204 (Eco Chemie, Utrecht, and The Netherlands) potentiostat/galvanostat were used to conduct the electrochemical measurements. The typical three-electrode cell of 10 ml capacity was employed in conjunction with an Ag/AgCl reference electrode, a BDDE and GCE working electrode, a Pt wire counter electrode, and in all studies. Before each measurement, the BDDE or GCE was manually polished on a smooth polishing pad with slurries prepared from 0.01 m aluminum oxide, and it was then thoroughly cleaned with double-distilled water.

Chemicals

The following chemicals were purchased from Sigma-Aldrich: sodium phosphate monobasic, sulfuric acid, acetic acid, methanol, phosphoric acid, sodium hydroxide, sodium dihydrogen phosphate dihydrate, sodium acetate trihydrate, sodium phosphate, and acetonitrile. All the reagents were of analytical grade and utilized. All experiments were performed at room temperature; to prevent deterioration, all solutions were shielded from light and employed within 24 hours. The OTB utilized in the electrochemical studies was given by DEVA Holding AS. Istanbul/Turkey Analytical grade chemicals were employed to make solutions at all stages of the study, and they weren't further purified before use.

Electrochemical Study

For drawing the calibration graph of OTB using 0.1 M H_2SO_4 solutions (pH 0.3), the DPV technique was applied with the optimum parameters of scan rate 0.010071 mVs^{-1}, interval time 0.5 s, step potential of 0.005 V, modulation amplitude of 0.05 V, modulation time 0.05 s. CV was performed at a scan rate of 0.05 Vs^{-1}. All the electrochemical studies were applied at room temperature.

Preparation of Standard and Calibration Solutions

For electrochemical experiments, various supporting electrolytes, including buffers of phosphate (pH 2.0 – 8.0), acetate (pH 3.7 – 5.7), H_2SO_4 solutions (0.1 and 0.5 M), and Britton Robinson buffers (pH 2.0 – 12.0), were created. Recordings of DPV voltammograms were made after each aliquot was added. Working solutions of OTB for the voltammetric studies were made from the stock solution (1 x 10^{-2} M) in bidistilled water by diluting it with the chosen supporting electrolyte.

RESULT AND DISCUSSION

At the GCE and BDDE, the CV approach was used to learn more about the extensive electrochemical analysis of OTB. The analysis of OTB for both GCE and BDDE used the DPV approach. To the best of our knowledge, there hasn't been any published research on OTB's electrochemical determination in the literature.

In our investigation, bare GCE and BDDE were used to conduct the first electrochemical

examination and OTB determination. The influence of pH values and supporting electrolytes on the electrochemical current peaks of OTB were studied using DPV and CV in the pH range of 0.30 to 12.00 utilizing GCE and BDDE in order to determine the most appropriate experimental settings.
On GCE (Figure 2A) and BDDE (Figure 2B), the maximum voltammetric peak for 1 x 10^{-3} M OTB was obtained using 0.1 M H_2SO_4 as the supporting electrolyte. Between pH 0.30 and 12.00, it was oxidized on both electrodes, yielding two distinct, irreversible oxidation peaks for GCE and BDDE. For GCE and BDDE, respectively, main peak potential values of roughly +1.00 V and +1.50 V were noted. This distinction clearly showed that OTB is simply oxidized on the GCE in comparison with the BDDE. The absence of a cathodic reaction at either electrode during reverse scanning revealed that the electrooxidation of OTB is irreversible.

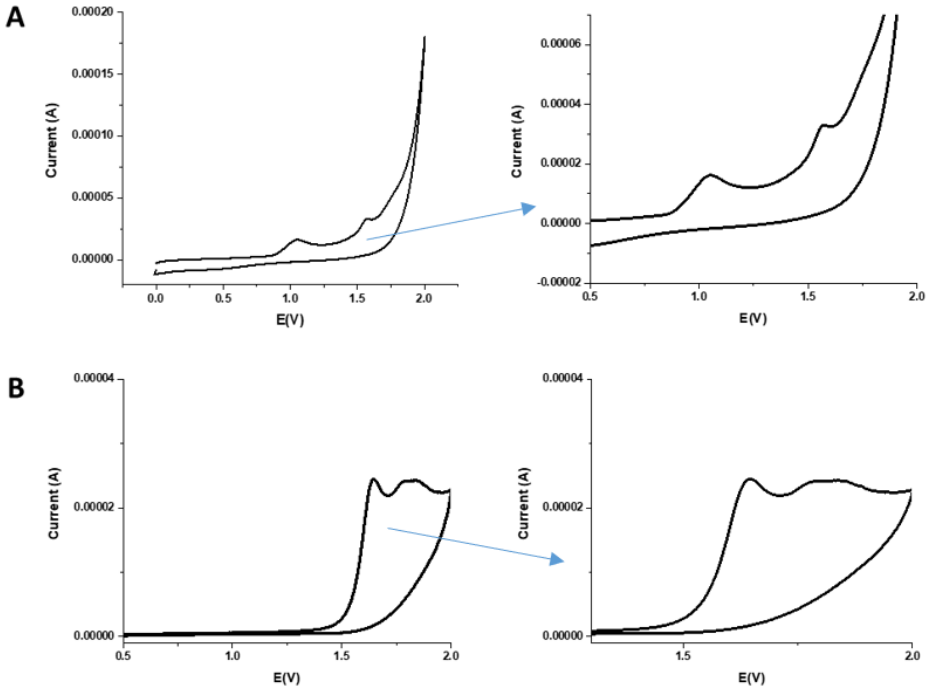

Figure 2. Cyclic voltammograms of 1x10^{-3} M OTB with GCE (A) and BDDE (B) in 0.1M H_2SO_4 at scan rate 0.5 V/s

To obtain distinctive information on the voltammetric behavior of OTB, the DPV method was applied by GCE and BDDE. It was observed that the Ep values of OTB for both electrodes shifted to less positive potential values. A higher peak value was observed for OTB using the BDDE compared to the GCE.

The effect of supporting electrolyte and pH value on the peak current and potential for 1 x 10^{-3} M OTB was examined for GCE and BDDE. The shifting of Ep values of OTB showed the presence of protons in the electrochemical oxidation procedure for GCE and BDDE. Using the DPV technique, the highest peak current was observed in the 0.1 M H_2SO_4 medium for both electrodes. The following equation indicates the influence of pH on the peak potential. For both electrodes, the dependency between Ep and pH can be stated with the following equations:

$$Ep\ (mV) = 986.27 - 19.721\ pH;\ r = 0.992 \text{ for GCE (pH 0.3 – 8.0)}$$

$$Ep\ (mV) = 1646.4 - 28.703\ pH;\ r = 0.964 \text{ for BDDE (pH 0.3 – 4.0)}$$

The slope values obtained from the above equation show that the number of electrons equals the

number of protons in the electrochemical oxidation process.

Scan Rate Study

The influence of scan rate works was performed to provide important knowledge on the electrochemical redox mechanism procedure and define whether the electrochemical process happened under the adsorption or diffusion control mechanism. The effect of the scan rate using CV between 0.01 and 1 V/s on the peak potential and current was examined in selected supporting electrolytes, where the maximum peak current was acquired in pH experiments with a GCE and BDDE.

The effect of scan rate (ν) on the Ip values of 1 x 10^{-3} M OTB has been investigated in 0.1 M H_2SO_4 for BDDE and GCE.

As illustrated in the following equations, the Ip was linear to the square root of scan rate ($\nu^{1/2}$) for BDDE and GCE in the between of 0.01 - 1.00 Vs^{-1}:

$$Ip\ (\mu A) = 0.5193\ \nu^{1/2}\ (Vs^{-1}) + 0.7304\ (r = 0.9656;\ n=10)\ \text{at GCE in 0.1 M } H_2SO_4$$

$$Ip\ (\mu A) = 2.5415\ \nu^{1/2}\ (Vs^{-1}) - 6.1322\ (r = 0.9763;\ n=10)\ \text{at BDDE in 0.1 M } H_2SO_4$$

According to the linear dependency of the Ip – $\nu^{1/2}$, the electrooxidation process of OTB is a diffusion-controlled process on GCE and BDDE.

The following equations showed a linear relationship between the logarithms of Ip (log Ip) and scan rate (log ν):

$$\log Ip\ (\mu A) = 0.562\ \log \nu\ \ (Vs^{-1}) - 0.3721\ (r = 0.9931;\ n = 10)\ \text{at GCE in 0.1 M } H_2SO_4$$

$$\log Ip\ (\mu A) = 0.501\ \log \nu\ (Vs^{-1}) + 0.1145\ (r = 0.9953;\ n = 10)\ \text{at BDDE in 0.1 M } H_2SO_4$$

The slope value of the linear relationship of the log Ip and the log ν was calculated as 0.562 and 0.501 for both electrodes in 0.1 M H_2SO_4, which is near the theoretical value of 0.50, obviously approving the diffusion-controlled mechanism.

For Ep, in 0.1 M H_2SO_4, at GCE and BDDE, the linear relationship of the Ep versus log ν was revealed as:

$$Ep\ (mV) = 0.0721\ \log \nu\ (Vs^{-1}) + 1.2474\ (r = 0.9952;\ n = 10)\ \text{at GCE in 0.1 M } H_2SO_4$$

$$Ep\ (mV) = 0.0374\ \log \nu\ (Vs^{-1}) + 1.7055\ (r = 0.9973;\ n = 10)\ \text{at BDDE in 0.1 M } H_2SO_4$$

When the scan rate was increased, the peak potential of the OTB's oxidation mechanism for GCE and BDDE was proven to be irreversible, and the peak potential values were moved to be more positive without the reverse reduction peak. The irreversible electrochemical mechanism shifts the Ep to around 30/n mV with greater positive potentials for a 10-fold improvement in scan rate. "α" is the anodic charge transfer coefficient, and "n" is the electron transfer number, respectively. Ep values were shifted to 72.1 and 37.4 mV in 0.1 M H_2SO_4 at GCE and BDDE, respectively. Since the value is typically regarded to be 0.5, the values of n = 2.40 (2) and 1.25 (1) for GCE and BDDE, respectively, were discovered.

Analytical Applications

The relationship between OTB peak current and concentration was studied to assess the analytical applications of OTB using GCE and BDDE. The electrochemical oxidation process of OTB was monitored as a diffusion control mechanism on both electrodes. The fast and sensitive electrochemical technique, DPV, was applied to determine OTB in 0.1 M H_2SO_4 on BDDE and GCE. Under the optimum conditions, the corresponding characteristics of the linear regression analysis are calculated in Table 1 for both electrodes [13,14]. Both electrodes' performance in various concentrations of OTB is given in Figure 3.

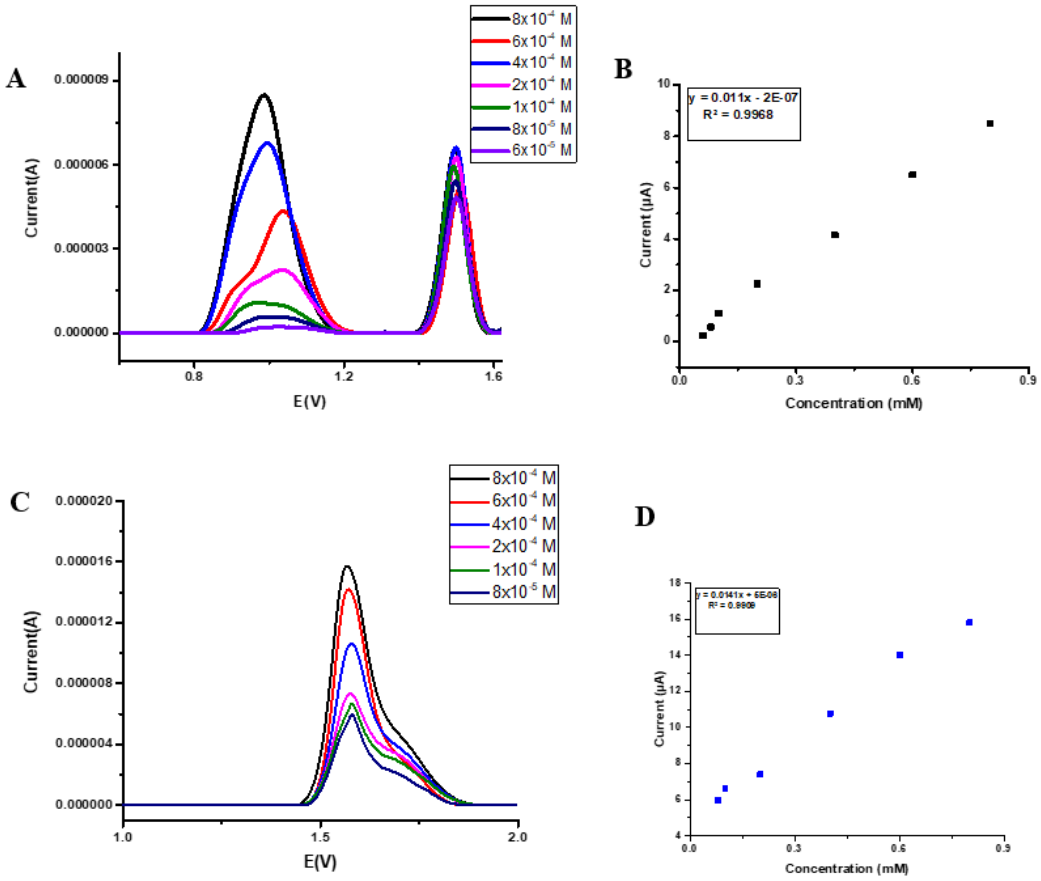

Figure 3. DP voltammograms of the developed sensor in different OTB concentrations in 0.1 M H_2SO_4 using A) GCE and B) BDDE

Based on the results of the experiments, GCE and BDDE were proposed for the detection of OTB using DPV under the optimum experimental conditions (step potential of 0.005 V, modulation amplitude of 0.05 V, modulation time 0.05 s, interval time 0.5 s, scan rate 0.01 mV s^{-1}, in 0.1 M H_2SO_4). As seen in Figure 3A, the anodic currents that appeared at 1022.2 mV were linearly increased in the range of concentrations from 6 x 10^{-5} – 8 x 10^{-4} M with a linear regression equation as follows:

$$Ip\ (\mu A) = 0.011\ C_{OTB}\ (\mu M) - 2 \times 10^{-7} (R^2 = 0.9968)$$ for GCE in 0.1 M H_2SO_4

As seen in Fig. 3B, the anodic currents that appeared at 1576 mV were linearly increased in the range of concentrations from 8x10^{-5} – 8x10^{-4} M with a linear regression equation as follows:

$$Ip\ (\mu A) = 0.0141\ C_{OTB}\ (\mu M) + 5 \times 10^{-6}\ (R^2 = 0.9909)$$ for BDDE in 0.1 M H_2SO_4

The other important validation data, including precision, LOD, LOQ, and linearity range, were figured out and are given in Table 1. The LOD and LOQ were figured out from the equation as follows:

LOD = 3.3 s/m

LOQ = 10 s/m

where "s" is the standard deviation of five experiments and "m" is the slope of the calibration graph. The LOD and LOQ were calculated to be 1.17 x 10^{-5} M and 3.86 x 10^{-6} M, 4.92 x 10^{-6} M, and 1.49x10^{-5} M, for GCE and BDDE, respectively, all cases in 0.1 M H_2SO_4. LOD results approved that the developed DPV technique using GCE is quite similar to the proposed DPV technique using BDDE.

The precision (reproducibility and repeatability) of the technique was assessed in terms of RSD% and connected results are given in Table 1. Repeatability RSD% results of peak current were found to

be 0.86 and 0.62, for GCE and BDDE, respectively, in 0.1 M H_2SO_4.

Table 1. Validation and regression information of the calibration graphs of Otilonium Bromide in 0.1 M H_2SO_4 with GCE and BDDE using DPV

	GCE	BDDE
Measured potential (V)	1.0222	1.5761
Linearity range (M)	$6x10^{-5} – 8x10^{-4}$	$8x10^{-5} – 8x10^{-4}$
Slope (µA M^{-1})	0.011	0.0141
Intercept (µA)	$2x10^{-7}$	$5x10^{-6}$
Correlation coefficient	0.9968	0.9909
LOD (M)	$1.17x10^{-5}$	$4.92x10^{-6}$
LOQ (M)	$3.86x10^{-6}$	$1.49x10^{-5}$
Repeatability of peak current (RSD %)*	0.86	0.62
Reproducibility of peak current (RSD %)*	1.19	0.95

*Each value is an average of five measurements

OTB has been assayed by various advanced analytical techniques such as high-performance liquid chromatography [15-17] , and capillary electrophoresis [18], and two methods based on derivative spectrophotometry are available [19,20], out of which one is estimation in combination with diazepam. In addition to this, an electroanalytical method is also not found in the literature review. Although chromatographic assay techniques allow simultaneous diagnosis of more than one analyte, they have some disadvantages compared to voltammetric techniques. It contains time-consuming sample preparation periods, undefined reaction time, is quite expensive, and less green assay. All these published methods are required highly sophisticated instrumentation. Otherwise, the electroanalytical techniques are low cost, sensitive, rapid analysis times, high accuracy and precision, selective, eco-friendly, practical methods.

The primary objective of this investigation has been to elucidate the electrochemical investigation and analysis of OTB using GCE and BDDE. This study demonstrated the proposed and validated DPV procedure as speedy, simple, accurate, and precise. It was performed directly as standard quality control for a tablet dosage form, eliminating the use of organic solvents or expensive devices. CV and DPV methods were developed to determine OTB with GCE and BDDE. The proposed method offers significant advantages such as low-cost, fast performance, and easy instrumentation is required.

ACKNOWLEDGEMENTS

The pharmaceutical firm DEVA Holding A.S. provided the information, for which the authors are grateful.

AUTHOR CONTRIBUTIONS

Concept: L.K., E.B.A., S.A.Ö.; Control: L.K., E.B.A., S.A.Ö.; Sources: L.K., E.B.A., S.A.Ö.; Data Collection and/or Processing: L.K., E.B.A., S.A.Ö.; Analysis and/or Interpretation: L.K., E.B.A., S.A.Ö.; Literature Review: L.K., E.B.A., S.A.Ö.; Manuscript Writing: L.K., E.B.A., S.A.Ö.; Critical Review: L.K., E.B.A., S.A.Ö.; Other: -

CONFLICT OF INTEREST

The authors declare that this article has no real, potential, or perceived conflict of interest.

ETHICS COMMITTEE APPROVAL

The authors declare that this study does not require the ethics committee's approval.

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