Original Article
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Electrochemical Detection of Linagliptin and Its Interaction with DNA

Linagliptin'in Elektrokimyasal Tespiti ve DNA ile Etkileşimi

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ABSTRACT
INTRODUCTION: Linagliptin is a drug used for the cure of the type 2 diabetes mellitus. In this study, the electrochemical detection of Linagliptin and its interaction with DNA was analyzed for the first time with voltammetric methods by measuring the oxidation currents of adenine bases of DNA before and after the interaction with each other. In addition, the electrochemical properties of the Linagliptin was studied.
METHODS: The interaction between Linagliptin and DNA were evaluated by using Differential Pulse Voltammetry. Three electrode system which consists of pencil graphite electrode as the working electrode, a reference electrode (Ag/AgCl), and a platinum wire as the auxiliary electrode were used in electrochemical studies. Experimental conditions such as concentration, pH of the supporting electrolyte, and immobilization time were investigated to obtain the maximum analytical signals.
RESULTS: In our study, the adenine bases of DNA were evaluated as analytical signal obtained at approximately + 1.2 V vs. Ag/AgCl. After Linagliptin-DNA interaction, the oxidation currents of adenine decreased as a proof of interaction. There has been no literature for Linagliptin in connection with DNA. According to our results, diffusion controlled, irreversible redox process consisting of independent oxidation was revealed for Linagliptin. Under optimum conditions, limit of detection was found as 6.7 µg/mL for DNA, and 21.5 µg/mL for Linagliptin, respectively. According to the observations, Linagliptin has a toxic effect on DNA.
DISCUSSION AND CONCLUSION: We successfully demonstrated that Linagliptin interacts with DNA and its influence on DNA could have a role in the medicinal effect of the drug.
Keywords: DNA, Linagliptin, DNA-Drug Interaction, Electrochemistry, Voltammetry.

ÖZ

GİRİŞ ve AMAÇ: Linagliptin, tip 2 diabetes mellitus'un tedavisi için kullanılan bir ilaçtır. İlaçlar, ligandlar ve kimyasallar gibi küçük moleküller, kovalent ve kovalent olmayan etkileşimler yoluyla DNA ile etkileşime girebilmektedir. İlaçlar farmakolojik aktivitelerini farklı mekanizmalarla gösterdiklerinden, DNA ile etkileşimlerinin altında yatan mekanizmasını anlamak son derece önemlidir. Bu çalışmada Linagliptin'in elektrokimyasal tespiti ve ilk defa DNA ile etkileşimi çalışması yapılmıştır. Bu etkileşim süreci birbirleriyle etkileşim öncesi ve sonrasında DNA adenin bazlarının yükseltgenme akımları ölçülerek voltametrik yöntemlerle analiz edilmiştir. Ayrıca çalışmamızda Linagliptin'in elektrokimyasal özellikleri incelenmiştir.
YÖNTEM ve GEREÇLER: Linagliptin ve DNA arasındaki etkileşim, Diferansiyel Puls Voltametrisi kullanılarak değerlendirilmiştir. Bu elektrokimyasal temelli çalışmada; çalışma elektrodu olarak grafit uçlu kurşun kalem, bir referans elektrot (Ag/AgCl) ve yardımcı elektrot olarak bir platin telden oluşan üçlü elektrot sistemi kullanılmıştır. Maksimum analitik sinyalleri elde etmek için konsantrasyon, destekleyici elektrolitin pH'ı ve immobilizasyon süresi gibi deneysel koşullar araştırılmıştır.
BULGULAR: Çalışmamızda DNA-Linagliptin etkileşimi; DNA ve Linagliptin'in birbirleriyle etkileşimi öncesi ve sonrası adenine bazlarının Ag/AgCl referenas elektroda karşı + 1.2 V'da verdiği yükseltgenme akımları karşılaştırılarak değerlendirilmiştir. DNA etkileşiminden sonra, adenin yükseltgenme akımları azalmıştır. Linagliptin'in elektrokimyasal özelliklerinin araştırılması sonucu, bu molekül için difüzyon kontrollü, yükseltgenmeye bağlı ve geri dönüşümsüz bir redoks sürecinin meydana geldiği açığa çıkarılmıştır. Optimum koşullar altında, tespit sınırı DNA için sırasıyla 6.7 µg/mL ve Linagliptin için 21.5 µg/mL olarak bulunmuştur. Elde edilen sonuçlar değerlendirildiğinde, Linagliptin'in DNA üzerinde toksik bir etkiye sahip olduğu sonucuna varılmıştır
TARTIŞMA ve SONUÇ: Bu çalışmada elde edilen sonuçlar değerlendirildiğinde; elektrokimyasal metotlar kullanarak Linagliptin'in DNA ile etkileşime girdiği hızlı ve başarılı bir şekilde gösterilmiştir
Anahtar Kelimeler: DNA, Linagliptin, DNA-İlaç Etkileşimi, Elektrokimya, Voltametri.

1. INTRODUCTION

The interaction of small molecules such as drugs with DNA is very important in pharmaceutical sciences (1, 2). The interaction could happen in covalent or non-covalent way. In covalent interaction, the bonding of drug to DNA is irreversible and causes the cell death (3). In non-covalent interaction, the bonding of drug to DNA is reversible. The non-covalent interaction occurs mainly three ways: intercalation, groove binding, and electrostatic interaction (4). Among them, the intercalation is the most powerful interaction mechanism. In minor groove binding, a close interplay with wall of the groove occurs, and hydrogen bonds arise among drugs and DNA. In major groove, hydrogen binds to DNA and, a DNA triplex is comprised. In electrostatic interactions, the interaction occurs between the molecule and the negatively charge of phosphates of DNA (5). The interaction of drugs and DNA can be monitored with Voltammetry (6), Electrochemical Impedance Spectroscopy (EIS) (7), UV-Vis Spectroscopy (8), Fluorescence Spectroscopy (9), High Performance Liquid Chromatography (HPLC) (10), FT-IR and Raman Spectroscopy (11), Surface Plasmon Resonance (12), and Molecular Modelling methods (13). Among them, electrochemical methods such as voltammetry and impedimetry are generally preferred due to low cost, rapidness, and high sensitivity. In addition, compared with optical,

chromatographic or other transducers, the electrochemical transduction is more dynamic and tunable.

Linagliptin (Lin) is a drug that used for the cure of the type 2 diabetes mellitus (14). In the literature, Lin was electrochemically detected with Cyclic Voltammetry (CV) and Square Wave Voltammetry (SWV) by using Fe_2O_3 modified carbon paste electrodes (15), and Co_3O_4 nanoparticles and multi-walled carbon nanotubes modified carbon paste electrodes (16). There are also description of spectrofluorometric (17), and HPLC methods (18) for Lin quantification in human plasma and rat plasma, respectively. In addition, HPLC-DAD method for Lin quantitation in the presence of its degradation products in tablets was also studied (19).

Herein, we for the first time detected the interaction of Lin with DNA electrochemically by using Differential Pulse Voltammetry (DPV). In our study, electrochemical properties of Lin were analyzed at first step. Then, we examined the oxidation signals of adenine bases of DNA before and after the interaction with Lin. As DNA can acts as a molecular wire or a conductive bridge, intrinsic electro-activity of DNA bases such as adenine and guanine could be used as an indicator for drug-DNA interaction in a label-free assay. In our study, we explored how the oxidation of DNA bases of adenine was influenced by the presence of Lin. Upon the interaction with Lin, adenine oxidation currents decreased dramatically. Experimental parameters such as concentration, pH of the supporting electrolyte, and immobilization time were investigated to obtain the maximum current signals.

2. MATERIALS AND METHODS

2.1. Equipment: AUTOLAB apparatus connected to NOVA software (Metrohm, The Netherlands) were used for voltammetric measurements. In all experiments, Pencil Graphite Electrodes (PGEs), Ag/AgCl, and platinum wire were used as the working electrodes, the reference electrodes, and the auxiliary electrodes, respectively.

2.2. DNA and Lin: Fish sperm double stranded DNA (dsDNA) were purchased from Sigma-Aldrich (Germany). Stock solutions of DNA was prepared with tris-EDTA buffer (TE, pH: 8.0) and stored at -20 C. Lin was purchased from Sigma- Aldrich and 1 mg/mL of Lin stock solution were prepared with ultrapure water. 0.5 M acetate (ACB, pH: 4.8), 0.05 M phosphate (PBS, pH: 7.4), and 0.1 M sodium borate (BBS, pH: 8.1) buffers containing 0.02 M NaCl were used during the experiments.

2.3. Method: Scheme for experimental steps is shown in Figure 1.

Activation: + 1.4 V was applied to PGEs for 30 s in ACB for activation.

DNA and its immobilization (when only DNA is immobilized)*:* The stock solutions of DNA was diluted with ACB. The activated electrodes were immersed in 200 µg/mL of DNA solution for 1 h and the electrodes were rinsed with ACB.

Lin and its immobilization (when only Lin is immobilized): The stock solutions of Lin was diluted with ACB. The activated electrodes were immersed in 600 µg/mL of the drug solution for 1 h in dark. Then, Lin immobilized electrodes were rinsed with ACB.

Interaction: 400 µg/mL of DNA and 1200 µg/mL of Lin were mixed with 1:1 ratio in ACB. Thus, 200 µg/ml of DNA and 600 µg/ml of Lin solutions were obtained. Then, 100 µl of solution was transferred into the vials. Then, the electrodes were immersed in these vials for 1h in dark. Then, the modified electrodes were rinsed with ACB.

Measurement: DPV measurements were carried out from + 0.4 V to + 1.4 V at 50 mV/s of scan rate in ACB.

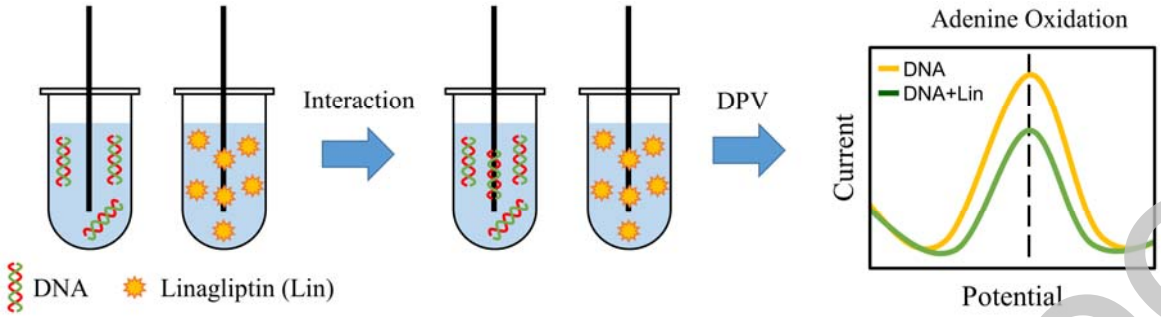

Figure 1. Experimental steps for the electrochemical detection of the interaction between DNA and Lin.

3. RESULTS and DISCUSSION

The electrochemical oxidation of DNA has been performed by carbon electrodes among which PGEs has been applied to the largest extent (20). It was found that DNA bases are oxidizable electrochemically at graphite electrodes, and give well separated oxidation peaks on differential pulse voltammograms. The purine bases, such as guanine and adenine are considered to have the negative charges, on the other hand in the pyrimidines, cytosine and thymine, the positive charges are located (21). As a result of that, the oxidation potential of guanine and adenine that is much lower when compared to the oxidation potential of cytosine and thymine (22). Generally, the more negative peak (+ 1.0 V vs Ag/AgCl) corresponds to the electro-oxidation of guanine, while the more positive peak (+ 1.2 V vs Ag/AgCl) belongs to electro-oxidation of adenine. A dramatic decrease/increase at the oxidation/reduction peak currents of drug (if drug has capability to be oxidized or reduced) or DNA, or potential shifts to the more positive or negative values can be used for the proof of interaction . The optimization studies for DNA such as concentration, immobilization time, and buffer prepared in were performed, and the results were shown in Figure 2.

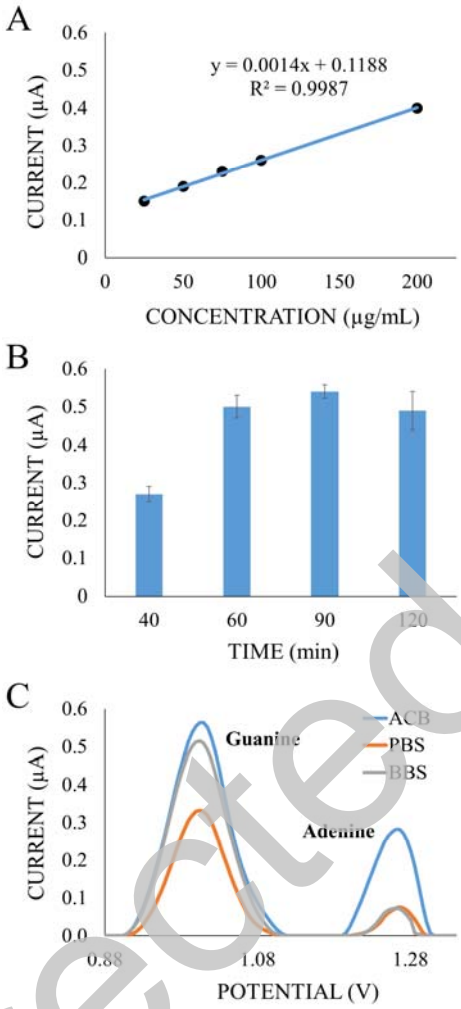

Figure 2. DNA optimization studies. **(A)** Calibration plot presenting adenine oxidation currents from 25 to 200 µg/mL of DNA. **(B)** Histograms for average adenine oxidation currents for different immobilization time of DNA on the activated PGEs surface, e.g., 40 to 120 min. **(C)** Differential Pulse Voltammograms of guanine and adenine oxidation currents obtained from different buffers where DNA prepared.

In order to obtain maximum surface coverage, 25-200 µg/mL of DNA were adsorbed on the PGEs. As it is clearly seen in Figure 2A, the peak currents of adenine increased with DNA concentrations (only linear values were showed in Figure 2A). A gradual increase was obtained until 200 µg/mL of DNA at PGE, and after this concentration there was almost no change in response. Thus, 200 µg/mL of DNA concentration was selected as the optimum DNA concentration.

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated in the concentration range between 25 and 200 µg/mL of DNA. According to the calibration plot (Figure 2A), LOD and LOQ for DNA was calculated as 6.7 µg/mL and 22.3 µg/mL, respectively using the equation LOD: 3 s/m, and LOQ: 10 s/m (s is the standard deviation for the blank solution and m is the slope of the related calibration curve) (23).

Figure 2B shows the histograms of adenine oxidation currents as a function of immobilization time of DNA on the electrodes from 40 to 120 min. Adenine oxidation currents slightly increased

with time and remained nearly unchanged after 60 min. As it is seen, the longer immobilization duration had no remarkable effect on the response. Thus, 60 min was selected.
The effect of the buffer solution where DNA prepared was investigated, and the results were shown in Figure 2C. As it is clearly seen, the highest guanine (+ 1.0 V) and adenine (+ 1.2 V) oxidation currents were obtained with ACB and further experiments were performed in ACB for preparation of DNA solution.

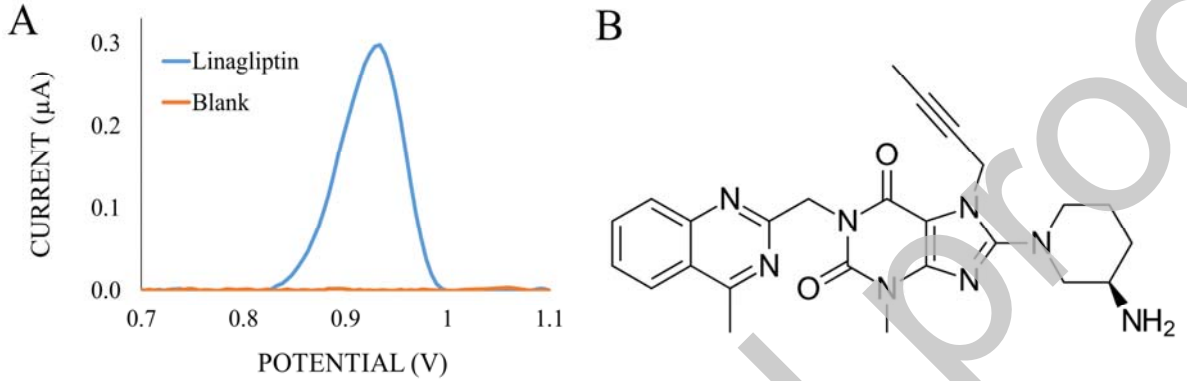

Figure 3. (A) Differential Pulse Voltammograms for oxidation currents of Lin obtained at nearly + 0.9 V in ACB. **(B)** Chemical structure of Lin molecule.
In Figure 3A, a differential pulse voltammogram is shown for the oxidation of Lin by scanning from + 0.7 V to +1.1 V vs. Ag/AgCl in ACB. As demonstrated in Figure 3A, Lin has an irreversible anodic peak at nearly + 0.9 V. Lin's chemical structure is also shown in Figure 3B. As shown previously, one proton could involve to the oxidation process aroused from the oxidation of amine group of pipyridine ring (16).

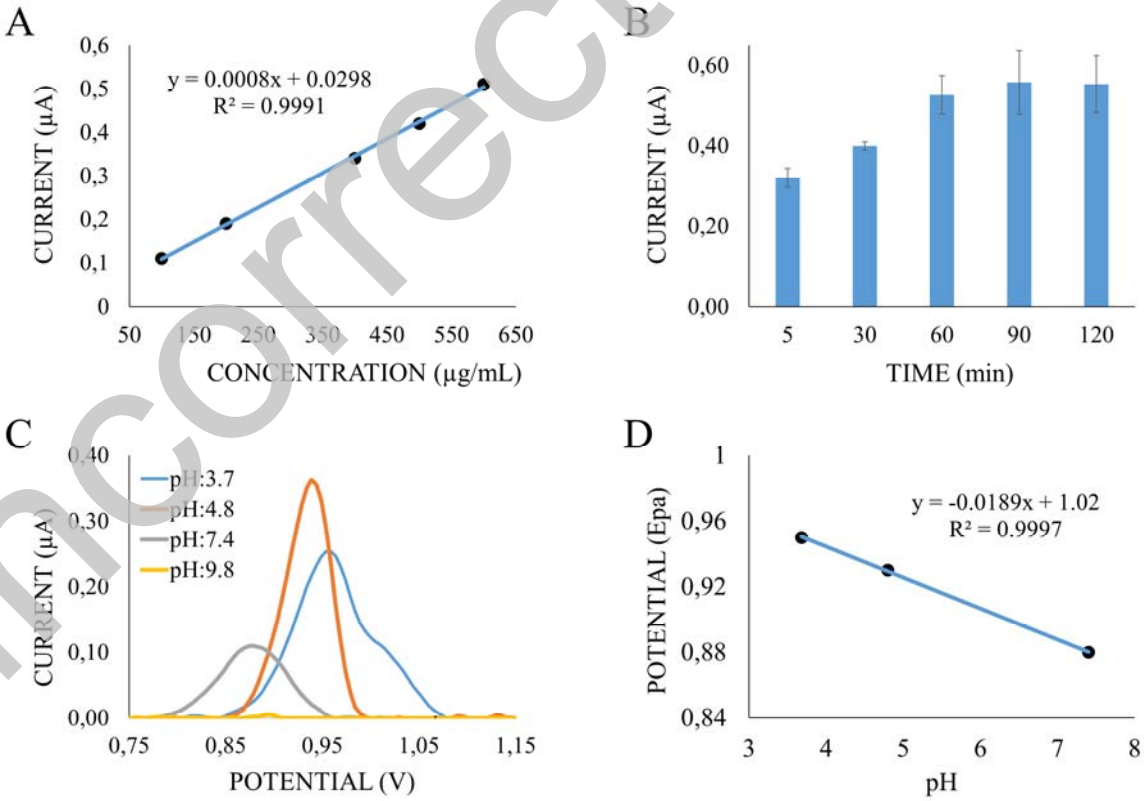

Figure 4. Lin optimization studies. **(A)** Calibration plot presenting Lin oxidation currents from 100 to 600 µg/mL of Lin. **(B)** Histograms for average Lin oxidation currents for different immobilization time of Lin on the activated PGEs surface, e.g., 5 to 120 min. **(C)** Differential Pulse Voltammograms of Lin oxidation currents from different supporting electrolytes ranging from pH 3.7 to pH 9.8. **(D)** Plots of Ep_a vs. pH.

In order to optimize oxidation signals of Lin, various concentrations from 100 µg/mL to 600 µg/mL of Lin were prepared, and their oxidation currents were measured with DPV (only calibration plot-linear values-were showed in Figure 4A). As it is seen from Figure 4A, Lin oxidation currents obtained from nearly +0.9 V signals were increased with the increased Lin concentration. The highest and reproducible Lin oxidation currents were measured in the presence of 600 µg/mL of Lin, and thus, 600 µg/mL of Lin concentration was selected as the optimum concentration.

LOD and LOQ were calculated in the concentration range between 100 and 600 µg/mL of Lin. According to the calibration plot (Figure 4A), LOD and LOQ for Lin was calculated as 21.5 µg/mL and 71.67 µg/mL, respectively.

The immobilization time, another parameter affecting the oxidation currents of Lin, was examined within the range of 5–120 min (Figure 4B). Lin oxidation current increased with time and remained nearly unchanged after 60 min. Therefore, Lin immobilization time was chosen as 60 min.

The effect of pH of the supporting electrolytes on the oxidation peak currents were investigated in the pH range from 3.7 to 9.8 (Figure 4 C). The Lin oxidation currents disappeared at pH 9.8. Therefore, we used three pH values (3.7, 4.8, and 7.4) to analyze effect of pH. As shown in Figure 4C, the peak potential shifted towards more negative values as pH increased, which demonstrated the involvement of protons during the oxidation process (24). The largest peak current was observed in pH: 4.8, and thus, this supporting electrolyte was chosen.

Over the pH range 3.7–7.4, the anodic peak potential (Ep_a) of Lin varied linearly as a function of pH (Figure 4D). According to Ep_a – pH behavior results, the equation is as follows:

$Ep_a = -0.0189\ pH + 1.02$ $\quad R^2 = 0.9997$ ***(Equation 1)***

The slope of Equation 1 (19 mV/pH) is far from ideal slope value of 59 mV/pH, which suggest that the number of transferred protons and electrons are not equal. According to the literature, the reason behind this results can be explained due to deprotonation or adsorption oxidation products that block electrode surface (25).

The effect of scan rate (ν) on Lin oxidation currents was also analyzed with CV. The peak currents of Lin increased with increasing scan rate (10 to 100 mV/s). According to the results, the equation is as follows:

$\log Ip_a = 0.6098 \log \nu + 0.746$ $\quad (R^2 = 0.9756)$ ***(Equation 2)***

According to Equation 2, the slope of equation (0.6) is close to the theoretical value of 0.5 which showed that the diffusion controlled electrode process was occurred (26).

The relationship of Ep_a between scan rate was analyzed with CV. According to the results, the equation is as follows:

$Ep_a = 0.0478 \log(\nu) + 1.0426$ $\quad (R^2 = 0.9618)$ ***(Equation 3)***

The peak potential shifted slightly positively with increasing scan rate (10 to 100 mV/s). This indicates that irreversible electrochemical process was occurred (27).

The relationship of the peak currents of Lin (Ip_a) between root of scan rate ($\nu^{1/2}$) was also analyzed. According to the results, the equation is as follows:

$Ip_a\ (\mu A) = 3.5219\ (\nu)^{1/2} + 0.0598$ $\quad (R^2 = 0.9808)$ ***(Equation 4)***

The linear increase of Ip_a with the $\nu^{1/2}$ indicates a diffusion controlled redox process (25).
The mechanism of interaction between drug molecules and DNA could be explored in three different ways. The first one is the evaluation of the changes of the electrochemical responses of DNA before and after the interaction with drugs. In addition, interaction could be assessed by obtaining dramatic decrease/increase at the oxidation/reduction peak currents of the drug which selectively binds to DNA. In general, the appearance or disappearance of redox signals in typical voltammograms of the drug of interest after the incubation with DNA in an electrochemical cell is preferred. At last, potential shifts to the more positive or negative side by the intercalation of nucleic acid-binding molecules into DNA could help to understand the underlying mechanism of the interaction.
The aim of our study is to investigate the interaction that occurs between DNA and Lin and to understand interaction mechanism. For these reasons, interaction studies were performed. In order to optimize DNA-Lin interaction, different interaction times from 30 to 90 min were performed, and the results were shown in Figure 5A. In the study, 400 µg/mL of DNA and 1200 µg/mL of Lin were mixed with 1:1 ratio in ACB. Thus, 200 µg/ml of DNA and 600 µg/ml of Lin solutions were obtained. Then, 100 µl of solution was transferred into the vials, and these vials and kept at different interaction times from 30 to 90 min. The adenine oxidation signal was measured as 0.51 (for 30 min), 0.72 (for 60 min), and 0.61 µA (for 90 min) before DNA-Lin interaction. After the interaction between DNA and Lin, the adenine oxidation signals were 0.25 (for 30 min), 0.32 (for 60 min), and 0.33 µA (for 90 min). The highest difference between before and after signal was observed with 60 min. Therefore, 60 min was chosen as the optimum interaction time.
After finding the optimum conditions, interaction between DNA and Lin was performed to investigate the behavior of Lin on DNA to understand how Lin could interact with DNA. (Figure 5B). The Lin-DNA interaction was investigated in comparison to the alterations in the adenine oxidation currents in the absence and presence of Lin. In our study, guanine currents were not analyzed due to the fact that their signals were close to Lin oxidation signals, and could interfere with each other.

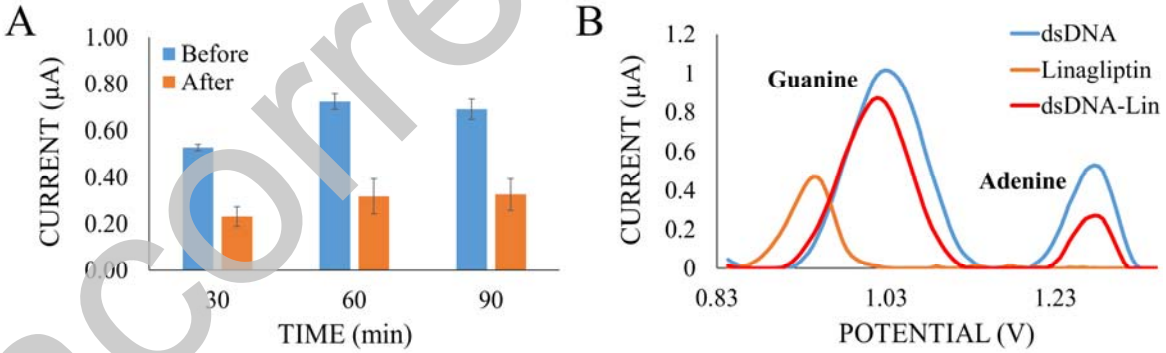

Figure 5. Interaction of DNA and Lin. **(A)** Histograms for average adenine oxidation signals for different interaction times between Lin and DNA e.g., 30 to 90 min. **(B)** Differential pulse voltammograms of guanine and adenine oxidation currents after interaction with Lin.
After the interaction with Lin, adenine oxidation signals were decreased. The adenine peak potential did not shift after the interaction. We assumed that the interaction of DNA with Lin leading to conformational changes in the DNA structure followed by the interaction of Lin to the adenine bases, which attenuates the electrochemical signal of adenine. This phenomenon could

be explained by the shielding of the oxidizable groups of adenine or fewer base molecules being available for oxidation (28).

According to the results based on adenine signals obtained from Figure 5B, Lin's toxicity effect (S%) on DNA was calculated according to the Equation 5 (29):

$S\% = (S_a/S_b) \times 100$ ***(Equation 5)***

S%: Percentage of the adenine peak current change

S_a: The height of the adenine peak current after the interaction with Lin

S_b: The height of the adenine peak current before the interaction with Lin

In general, if the %S value is more than 85, it is assumed to be non-toxic. If this value is between 50 and 85, it could be moderately toxic and if less than 50, it is considered as toxic. Using this equation, we calculated our S% value as 44% demonstrates Lin's toxicity to DNA.

Based on our voltammetric measurements and toxicity calculation, Lin could have toxic effects on DNA.

4. CONCLUSION

This is the first study for the demonstration of electrochemical detection of the interaction between DNA and Lin by using the electrochemical techniques. Electrochemical properties of Lin was investigated, and the effect of DNA-Lin interaction have been explored in comparison to the alterations in the adenine oxidation peak. The interaction that occurred in solution phase was characterized by the change in the adenine oxidation peak current before and after the incubation with each other. According to our study, diffusion controlled, irreversible redox process consisting of independent oxidation was revealed for Lin. After the interaction with Lin, adenine oxidation signals of DNA were decreased as a proof of interaction. The adenine peak potential did not shift after the interaction with Lin. Our results also showed that Lin is toxic to dsDNA.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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