Anal. Bioanal. Electrochem., Vol. 12, No. 3, 2020, 365-375

Analytical & Bioanalytical Electrochemistry

2020 by CEE
www.abechem.com

Full Paper

Electrochemical Oxidation and Molecular Docking Studies of Leaves Extract of *Lemon Verbena* and Flowers Extract of *Echium Amoenum*: Green Antidotes for Treatment of Barbiturate Poisoning

Ameneh Amani,[1,*] and Mahdi Jamshidi[2]

[1]*Department of Chemistry, University of Nahavand, Nahavand, Iran*

[2]*Department of Toxicology and Pharmacology, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran*

*Corresponding Author, Tel.: +98-81 33493003; Fax: +98-81-33493003

E-Mail: amani.iran@gmail.com

Received: 9 February 2020 / Accepted with minor revisions: 16 March 2020 / Published online: 31 March 2020

Abstract- Electrochemical oxidation of leaves extract of lemon verbena and the flowers extract of echium amoenum have been studied in the absence and presence of barbituric acid and 1,3 dimethyl barbituric acidin aqueous solutions and biological pH, using cyclic voltammetry method. The results showed that the electrochemically generated compounds in leaves extract of lemon verbena and the flowers extract of echium amoenum, participate in the chemical reaction with barbituric acid and 1,3 dimethyl barbituric acid. Based on our results, leaves extractof lemon verbena and the flowers extract of echium amoenum have a high antioxidant activity in comparison with galic acid, salicylic acid and quercetin as standard antioxidants and simultaneous can be useful for the treatment of barbiturate poisoning before starting clinical treatments. The antioxidant activity of luteoline and verbascoside (as the main and natural compounds in lemon verbena) against some of the reactive oxygen species (ROS) generation enzymes, Cytochrome P450 3A4 (4D75), Myeloperoxidase (1DNW) and Thyosine (3nm8) has been performed through molecular docking studies. The results indicated that these natural compounds bound exclusively to the binding site of ROS generation enzymes and has a remarkable role in suppressing the destructive effects of oxidative stress in the biological system of the human body.

Keywords- Herb-drug interaction; Barbiturate poisoning; Electrochemical oxidation; Antioxidant activity

1. INTRODUCTION

Drug poisoning occurs when a drug used with a dose more than its recommendation. Barbiturates are an important case in poisoning and all of them are derivatives of barbituric acid [1]. This poisoning characterized by stupor or coma, areflexia and in late cases with severe respiratory depression and cardiovascular insufficiency. There is not an antidote for this toxicity and the management of the patients in this case is usually in the ICU with very close monitoring. Although today because of the replacing of these class of drugs with the other safety drugs, mortality rates associated with barbiturate toxicity is less than 2% but it is very important for each person to know how to manage this toxicity before the starting each clinical treatment [2-4]. It is obvious that medicinal plants used in the traditional medicine and healing are one of the sources of antioxidants and health care practitioners in 70% of population worldwide rely on herbal medicines in their primary health care. The dietary intake of antioxidants plays an important role in the protection of the human organism against free radicals. Radical reactive species (ROS) generated by our body by various endogenous systems by exposure to different physiochemical conditions or pathological states. A balance between free radicals and antioxidants is necessary for proper physiological function [5-7]. In using the medicinal plants as natural antioxidant due to the presence of multiple components in the herbal products, the effects arising from herb-herb or herb-drug interactions are often unpredictable and complicated. Various types of pharmacokinetic and pharmacogenomic interactions from herb-drug combinations have been described [8-10]. Lemon verbena from verbeaceae family, and *echium amoenum* from boraginaceae family, two important medicinal plant, are used as herbal remedy in all over the world .In this study at first the electrochemical oxidation of leaves extract of lemon verbena and the flowers extract of *echium amoenum* investigated. Because of the electrochemical oxidation very often parallels cytochrome P450 catalyzed oxidation in liver microsomes and the hepatic metabolism is the main route of endogenous clearance of all barbiturates [4], it is interesting to study the electrooxidation of leaves extract of lemon verbena and the flowers extract of *echium amoenum* in the presence of barbituric acid and 1,3 dimethyl barbituric acid. Our results showed that leaves extract of lemon verbena and the flowers extract of *echium amoenum* have high antioxidant activity and the products of their oxidation entered in the chemical reaction with barbiturates. This reaction reduces the concentration of the active form of the barbiturates and indicates an effective herb-drug interaction.

In addition, theoretical investigations (molecular analysis) conducted to study the interaction between ligands (natural compounds in these medicinal plants) and receptors (ROS generation enzymes). The results showed that these natural compounds reduced the oxidizing effect of these enzymes on macromolecules (DNA, lipids and proteins) in biological system.

2. METHODS

2.1. Apparatus and materials

Reaction equipment is described in earlier paper [11]. All chemicals were reagent-grade materials from E. Merck. These chemicals were used without further purification. The peak current ratios (I_{pC1}/I_{pA1}) were determined using the equation (1) given in Ref. [12]:

$$I_{pc}/I_{pa} = (I_{pc})_0/I_{pa} + 0.485(I_{sp})_0/I_{pa} + 0.086 \quad (1)$$

where $(I_{pc})_0$ and $(I_{sp})_0$ are cathodic peak current and "*switching potential*" current respect to the zero current, respectively. I_{pc} and I_{pa} have their usual meanings.

2.2. Preparation of aqueous leaves extract of lemon verbena and the flowers extract of *echium amoenum*

Lemon verbena leaves and *echium amoenum* flowers were collected from the campus at University of Nahavand and washed and dried in the shade at the room temperature (RT) for 72 hours. Then, they were grinded to make powdered. To prepare the aqueous extract, 1 g of the powdered herb was mixed with 50 ml distilled water warmed to 60 °C–70 °C and placed at RT for 15 minute. Then, the mixture was passed through filter paper (Whatman filter paper number 1) and left to be cooled. This extract was considered an aqueous extract of lemon verbena leaves and *echium amoenum* flowers.

2.3. Molecular Docking Analysis

To assessment the interactions between luteolin and verbascoside as the main and natural compounds in aqueous extract of *lemon verbena* leaves and ROS generation enzymes (Cytochrome P450 3A4 (4D75), Myeloperoxidase (1DNW) and Thyosine (3nm8)), molecular docking study was performed via AutoDock vina software [13]. The X-ray structure of cytochrome P450 3A4, 1DNW and3NM8 obtained from the online Protein Data Bank (PDB: https://www.rcsb.org). The ligands were draw and optimized with using ChemBio Ultra software (version: 16.0, Cambridge Soft) and Gaussian, respectively. The molecular docking was implemented by using AutoDock Tools (ADT, version: 1.5.6) and Discovery Studio 4.5 Client software.

3. RESULTS AND DISCUSSION

3.1. Electrooxidation of aqueous leaf extract of lemon verbena

The evaluation of the quality consistency of leaves extract of *lemon verbena* previously performed by the simple and reliable HPLC method. About eight peaks found in each

chromatogram of a *lemon verbena* extract. Some of these phenolic acids were luteolin, 7-diglucuronide, verbascoside, isoverbascoside, apigenin (Fig. 1) [14].

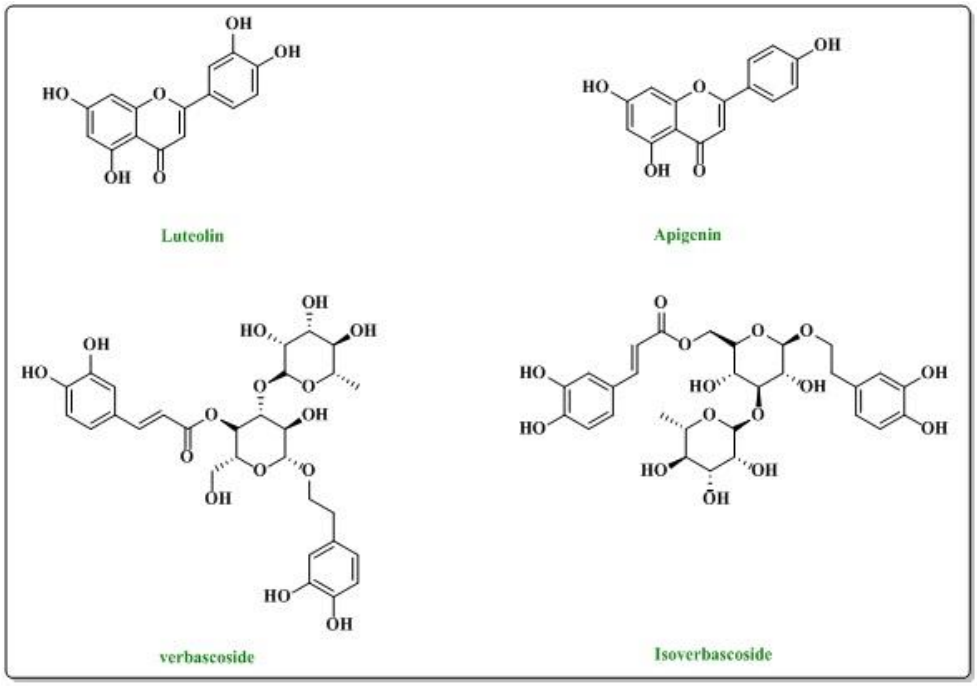

Fig. 1. The Structure of various phenolic compounds in *lemon verbena* leaves extract

The cyclic voltammogram of leaves extract of *lemon verbena* (3mL) in water (phosphate buffer, at the biological pH = 7.0, c = 0.2 mol/L) is shown in Figure 2 curve a. It shows one anodic peak (A_1) and its cathodic peak (C_1).It is obvious that proportional to the augmentation of the potential sweep rate (Fig. 2, curves a,b,c), the height of the A_1 and C_1 and the peak current ratio (I_p^{C1}, I_p^{A1}) increases (Fig. 2. Inset). This observation indicated that A_1 and C_1 are counterpart and related together [12]. Based on the previous papers [15, 16] we can conclude that these anodic and cathodic peaks are related to the electrochemical oxidation of luteolin in leaves extract of *lemon verbena*. Based on our HPLC analysis the quantity consistence of luteolin in this extract is 0.25 mg/g.

The cyclicvoltammogramsof galic acid, quercetin and salicylic acid as standard antioxidantsrecorded and the E_p^A values used for the estimation of antioxidant activity. It is seen a relationship between the oxidation potential measurement in cyclic voltammetry and ferric reducing antioxidant power. The increase in the antioxidant activity caused a decrease in E_p^A and observed an increased number on hydroxyl groups on the aromatic ring [17, 18]. As can be seen in Fig. 3, the leaves extract of *lemon verbena* and the flowers extract of *echium amoenum* have high antioxidant activity in comparison of galic acid, salicylic acid and quercetin as standard and synthesis antioxidants.

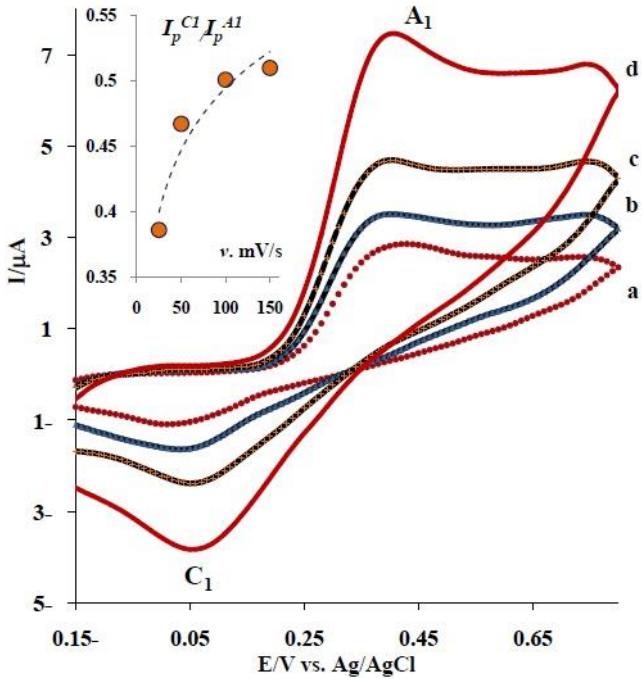

Fig. 2. Cyclic Voltammograms of 3 mL *lemon verbena* leaves extract at different scan rates (a) 25, (b) 50 (c) 100 and (d) 150 mV/s on glassy carbon eletctrodein aqueous solution (phosphate buffer, c = 0.2 mol/L, pH 7.0). Inset: The relationship between the peak current ratio and scan rate.scan rates: 25, 50 100 and 150mVs^{-1}

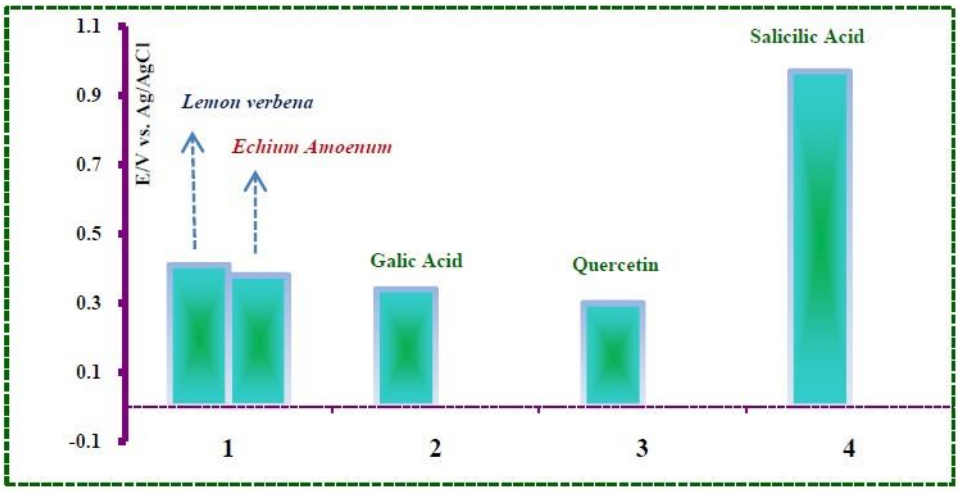

Fig. 3 . Comparsion of antioxidant activity (E_p^A) of *lemon verbena* leaves and *echiumamoenum* extract with galic acid, salicilic acid and quercetin

3.2. Investigation of antioxidant activity via molecular docking

The biological activity of some enzymes can produced reactive oxygen species (ROS) such as OH, H_2O_2 or HOCl. Leave extract of *lemon verbena* with good antioxidant properties can counteract with these oxidizing agents and prevent from some disorders and diseases. The

assessment of antioxidant activity of luteolin and Verbascoside (the major organic compound that exist in leaves extract of *lemon verbena*) was performed via molecular study or ligand-protein interactions in presence of three ROS generation enzymes cytochrome P450 3A4 (4D75), Myeloperoxidase (1DNW) and Thyosine (3NM8). As shown in Docking views (Fig 4, 5), a wide variety of interactions such as pi Alkyl, pi-cation, Amide pi-stacked, unfavorable donor-donor and pi-sigma are available for interaction of luteolin, Verbascoside and receptors.

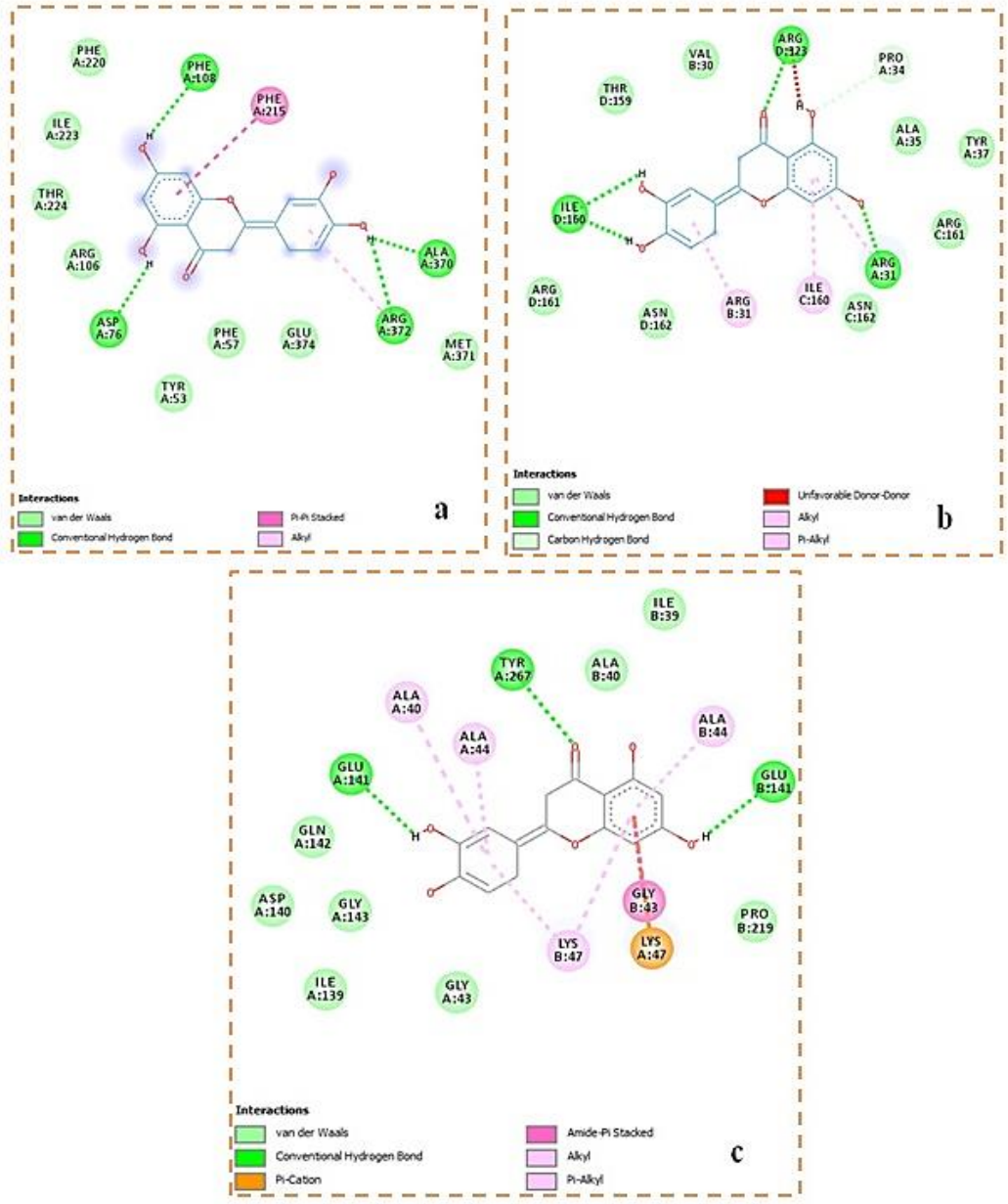

Fig. 4. 2D view of molecular study of (a) luteolin – cytochrome P450 3A4 (4D75) (b) luteolin – Myeloperoxidase (1DNW) (c) luteolin – Thyosine (3NM8) interacting complex

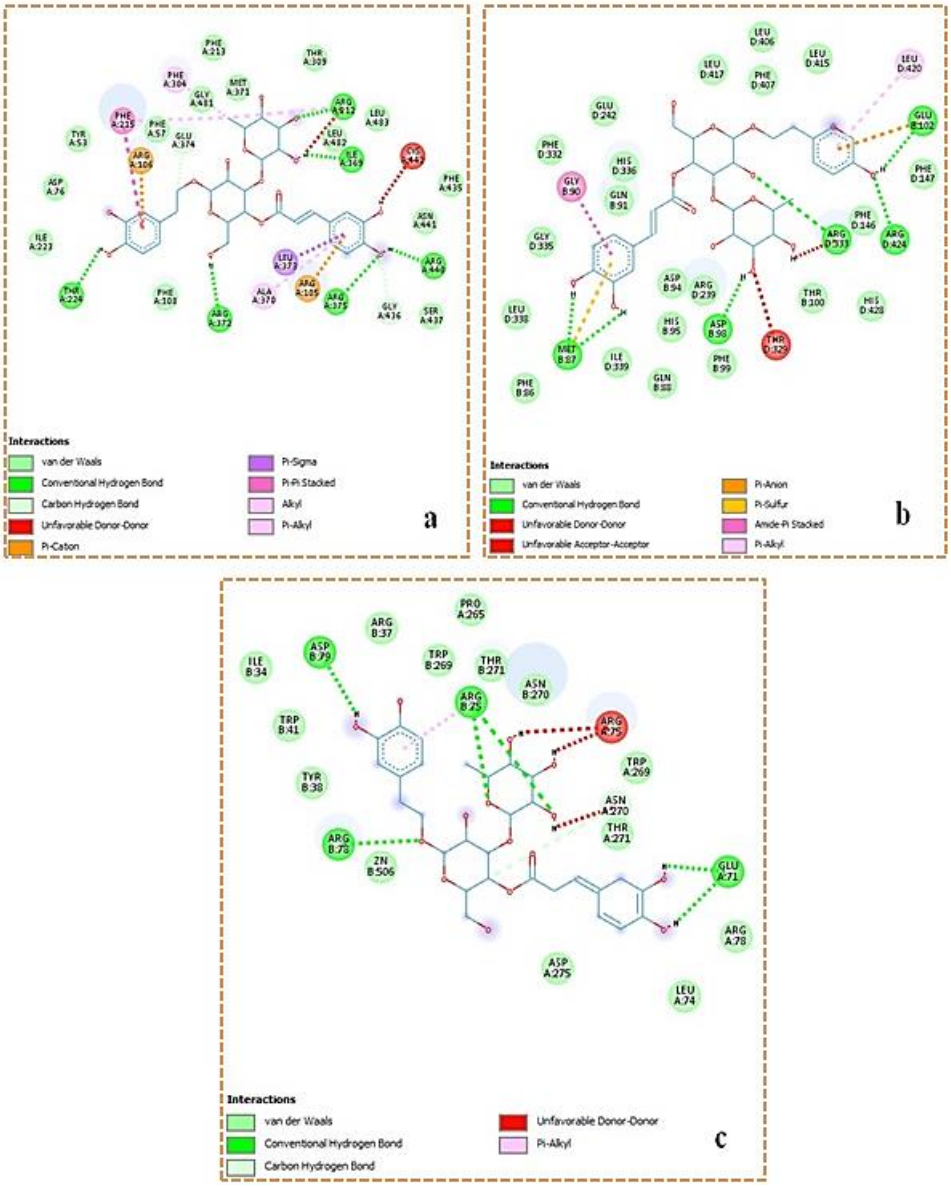

Fig. 5. 2D view of molecular study of Verbascoside– cytochrome P450 3A4 (4D75) (b) Verbascoside– Myeloperoxidase (1DNW) (c) Verbascoside– Thyosine (3NM8) interacting complex

Table 1. The binding affinity of luteolinand verbascoside in to different receptors

Organic and natural compounds	Cytochrome P450 3A4 (4D75)	Myeloperoxidase (1DNW)	Thyosine (3NM8)
Luteolin	-8.9 kj/mol	-8.5 kj/mol	-9.1 kj/mol
Verbascoside	-10.3 kj/mol	-11.1 kj/mol	-8.2 kj/mol

The results of molecular study and obtained affinity indicated that luteolin and verbascoside as ligand have positive effect on the stability and inactivation of ROS generation enzymes and subsequently, deactivating of oxidative stress process (Table 1).

3.3. Electrooxidation of aqueous leaf extract of lemon verbena in the presence of barbituric acids

The oxidation of aqueous leaf extract of *lemon verbena* (**1**) in the presence of barbituric acid (**3**) (1.0 mmol/L) was studied in some detail. Figure 6, curve b, shows the cyclic voltammogram obtained for 3 mL solution of *lemon verbena* leaves extract in the presence of barbituric acid (1.0 mmol/L). As can be seen the anodic peak A_1 increases and the cathodic peak (C_1) decreases. In this figure, curve c is the cyclic voltammogram of barbituric acid **(3)** and anodic peak A_2 is related to the oxidation of it.

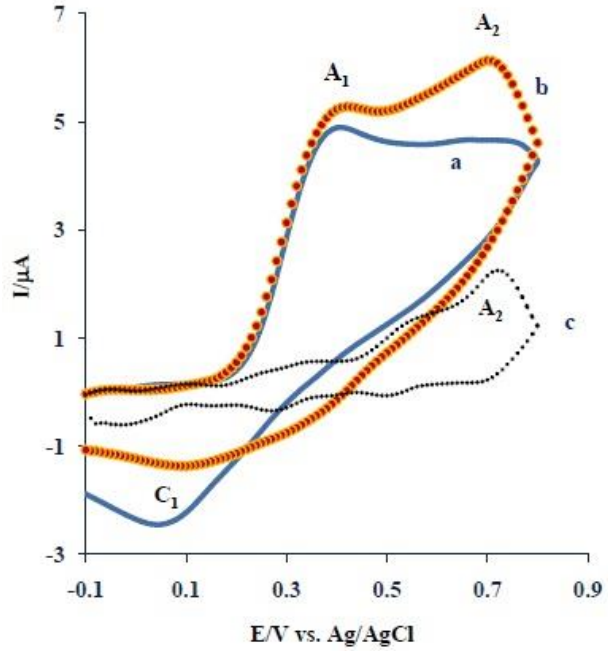

Fig. 6. (a) Cyclic voltammogram of 3mL *lemon verbena* leaves extract (**1**), in the absence, (b) in the presence of barbituric acid (1 mmol/L) and (c) barbituric acid (1 mmol/L) in the absence of **1** at a glassy carbon electrode in aqueous solution (phosphate buffer, c = 0.2 mol/L, pH 7.0). Scan rate: 50 mV/s. T = 25±1°C

More studies were performed by varying the potential sweep rate in 3mL solution of *lemon verbena* leaves extract in the presence of barbituric acid (**3**). Figure 7 shows the effect of potential sweep rate on the cyclic voltammograms of 3mL solution of *lemon verbena* leaves extract in the presence of barbituric acid (1.0 mmol/L). The results indicate that the peak current ratio (I_p^{C1}/ I_p^{A1}) strongly depends on the potential sweep rate and increases when the sweep rate

increases (Figure 7, inset).The same results obtained when the **3** to **1** concentration ratio is decreased.

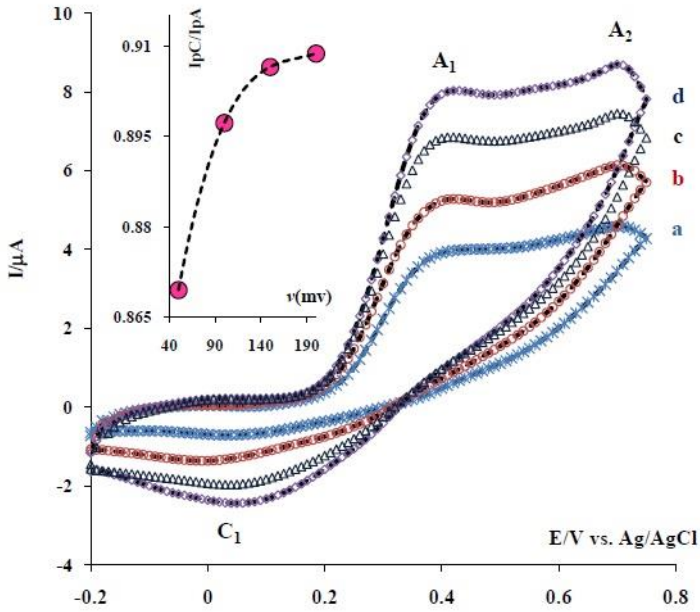

Fig. 7. Cyclic voltammogram of 3mL *lemon verbena* leaves extract (**1**) in the presence of barbituric acid (1 mmol/L) with various scan rates: (a) 50, (b) 100 and (c) 150 mV s^{-1}, (Inset) variation of peak current ratio versus scan rate at a glassy carbon electrode in aqueous solution (phosphate buffer, c = 0.2 mol/L, pH 7.0). T = 25±1°C

The existence of a subsequent chemical reaction between electrochemically generated compounds of*lemon verbena* leaves extract and barbituric acid (**3**) is supported by the following evidences (a) Decreasing of I_p^{C1} during the reverse scan and increasing of I_p^{A1} in the presence of **3** (Fig. 6 curve b). This could be indicative of the fact that electrochemically generated compounds in *lemon verbena* leaves extract are removed partially from surface electrode by chemical reaction with barbituric acid (**3**). (b) Dependency of peak current ratio (I_p^{C1}/ I_p^{A1}) on potential sweep rate (Fig. 7). In this case, for lower sweep rates, the peak current ratio (I_p^{C1}/ I_p^{A1}) is nearly zero and increases with increasing sweep rate (Figure 7, inset). This is indicative of departure from intermediate reign and arrival to diffusion reign with increasing sweep rate. The same results were obtained in the cyclic voltammetry of 3mL *lemon verbena* leaves extract in the presence of 1,3-dimethyl barbituric acid (**4**). The only difference between **3** and **4** is the higher reaction rate of 1,3-dimethylbarbituric acid (**4**) with electrochemically generated compounds of **1**. Also electrochemical oxidation of 3 mL *echium amoenum* flowers extract in the presence of barbituric acid and 1,3-dimethyl barbituric acid has performed and the same results obtained. These results suggests that leaves extract of *lemon verbena* and the flowers extract of *echium amoenum* oxidize at low potentials and have high antioxidant activity. The results of cyclic voltammetry shows that the electrogeneratedcompounds of

oxidation these extracts are stable and can react and reduce the concentration of barbituric acids. Our results indicate that theleaves extract of *lemon verbena* and the flowers extract of *echium amoenum* can be used asantidote in barbiturate poisoning before the start of clinical treatments. It can be seen clearlysynergism between antioxidant activity and treatment of barbiturate poisoning in aqueous leaves extract of *lemon verbena* and the flowers extract of *echium amoenum*.

4. CONCLUSION

The results of this work show that the leaves extract of *lemon verbena* and the flowers extract of *echium amoenum* have high antioxidant activity in comparison with some of synthesis antioxidants. The components produced by the oxidation of them are effective antidotes for barbiturate poisoning before starting clinical treatments. In the other words, we think our results are a new report about the effectiveness of the herb-drug interaction.

Acknowledgements

Support of this investigation by University of Nahavand Research Councilis gratefully acknowledged.

REFERENCES

[1] A. J. McBay, N. Eng. J. Med. 273 (1965) 38.
[2] K. Shashi, and B. C. Nandini, Indian J. Anaesth. 46 (2002) 480.
[3] A.A. Izzo, Med. Princ. Pract. 21 (2012) 404.
[4] R. Mactier, M. Laliberte´, J. Mardini, M. Ghannoum, V. Lavergne, S. Gosselin, R. S. Hoffman, and T. D. Nolin, Am. J. Kidney Dis. 64 (2014) 347.
[5] S. Chatterjee, Z. Niaz, S. Gautam, S. Adhikari, P. S. Variyar, and A. Sharma, Food Chem, 101 (2007) 515.
[6] A. Simić, D. Manojlović, D. Šegan, and M. Todorović, Molecules 12 (2007) 2327.
[7] Duh Pin-Der, J. Am. Oil Chem. Soc. 75 (1998) 455.
[8] R. Hermann, and O. von Richter. Planta Med. 78 (2012) 1458.
[9] B. J. Gurley, E.K. Fifer, and Z. Gardner. Planta Med. 78 (2012) 1490.
[10] T. CheCh, Z. J. Wang, M. S. S. Chow, and C. W. K. Lam, Molecules 18 (2013) 5125.
[11] A. Amani, and D. Nematollahi. J. Iran. Chem. Soc. 15 (2018) 2669.
[12] J. Bard, and L. R. Faulkner, Electrochemical Methods, 2nd ed.; Wiley: New York (2001).
[13] O. Trott, and A. J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, J. Comput. Chem. 31 (2010) 455.

[14] A. Felgines, D. Fraisse, C. Besson, M. P. Vasson, and O. Texier, British J. Nutrition 111 (2014) 1773.
[15] A. Liu, S. Zhang, L. Huang, Y. Cao, H. Yao, W. Chen, and X. Lin, Chem. Pharm. Bull. 56 (2008) 745 .
[16] E. D. S. Gil1, T. A. Enache, and A. M. Oliveira-Brett, Comb. Chem. High Throughput Screen. 16 (2013) 92.
[17] J. Sochor, J. Dobes, O. Krystofova, B. Ruttkay-Nedecky, P. Babula, M. Pohanka, T. Jurikova, O. Zitka, V. Adam, B. Klejdus, and R. Kizek, Int. J. Electrochem. Sci, 8 (2013) 8464.
[18] P. A. Kilmartin, Antioxid. Redox Signal. 3 (2001) 941.