Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

SHORT COMMUNICATION

Carqueja (*Baccharis trimera*) Essential Oil Chronic Treatment Induces Ventricular Repolarization Disorder in Healthy Rats but Not in Type 2 Diabetic Rats

Alice Pereira Duque[1], Carole Sant'ana Massolar[1], Cristiane Barbosa Rocha[2], Ana Paula Machado Da Rocha[1], Ricardo Felipe Alves Moreira[3], Luiz Fernando Rodrigues Junior[1,4]

[1]Department of Physiological Sciences, Cardiovascular Biophysics Laboratory, Federal University of The State of Rio De Janeiro, [2]Department of Physiological Sciences, Laboratory of Medicinal Herb Studies, Federal University of The State of Rio De Janeiro, [3]Department of Collective Health, Laboratory of Food Composition and Aroma Studies, Federal University of The State of Rio De Janeiro, [4]Department of Education and Research, National Institute of Cardiology, Rio De Janeiro, Brazil

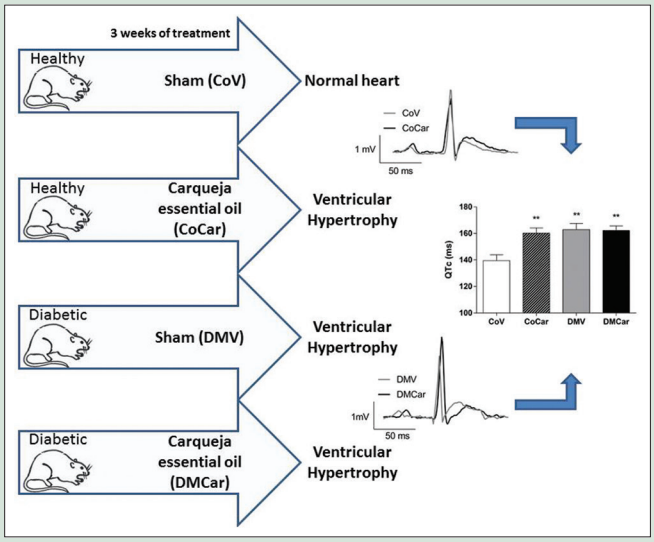
ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is a major risk factor for cardiovascular disease (CVD) development. The pharmacological treatment of T2DM can increase cardiovascular risk in diabetic patients. Carqueja (*Baccharis trimera*) is an antioxidant and hypoglycemic medicinal plant with promising action for T2DM non-pharmacological treatment. **Objectives:** The objective of this study is to investigate carqueja essential oil safety on the cardiovascular system of diabetic and non-diabetic rats. **Materials and Methods:** Four experimental groups were used to analyze the carqueja essential oil effects: control group (n = 5), carqueja-treated control group (n = 4), diabetic control group (n = 4), and carqueja-treated diabetic group (n = 5). T2DM was induced by hypercaloric diet followed by streptozotocin administration. Electrocardiogram parameters were used to analyze the alterations in the cardiovascular system. **Results:** Diabetic rats showed ventricular repolarization dysfunction with prolongation of QT and corrected QT intervals. The treatment increased ventricular repolarization duration in the control group. **Conclusion:** Carqueja essential oil treatment worsens ventricular repolarization in nondiabetic rats, increasing the arrhythmogenic risk.

Key words: Baccharis, cardiac hypertrophy, cardiovascular diseases, diabetes mellitus, electrocardiography, type 2

SUMMARY

- Carqueja (*Baccharis trimera*) is an antioxidant and hypoglycemic medicinal plant with promising action for type 2 diabetes mellitus non-pharmacological treatment
- There are no safety studies regarding the effects of carqueja essential oil consumption
- We investigated carqueja essential oil safety on the cardiovascular system of diabetic and nondiabetic rats
- Carqueja essential oil treatment induced heart hypertrophy and ventricular repolarization prolongation in nondiabetic rats, increasing the arrhythmogenic risk.



Abbreviations Used: T2DM: Type 2 diabetes mellitus, CVDs: Cardiovascular diseases, CoV: Control group, CoCar: Carqueja-treated control group, DMV: Diabetic control group, DMCar: Carqueja-treated diabetic group, QTc intervals: Corrected QT interval, CEUA: Ethics Committee on the Use of Animals, UNIRIO: Federal University of the State of Rio de Janeiro, ECG: Electrocardiogram recording, HW/BW: Heart weight body weight hypertrophy index, ANOVA: Analysis of variance, SEM: Standard error of the mean, Ito: Transient outward potassium current.

Correspondence:
Dr. Luiz Fernando Rodrigues Junior,
Department of Physiological Sciences,
Biomedical Institute, Federal University of the State of Rio De Janeiro/Unirio, Frei Caneca Nº 94, Centro, CEP: 20211-010, Rio De Janeiro, Brazil.
E-mail: luiz.junior@unirio.br
DOI: 10.4103/pr.pr_54_19

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is considered as a global epidemic.[1,2] Its prevalence was 4.7% (108 million people) in 1980 and increased to 8.5% in 2014, which represents 422 million people with diabetes.[3] It caused 1.6 million deaths in 2015, being the sixth-leading cause of death worldwide.[4] Furthermore, it is a major risk factor for cardiovascular diseases (CVDs), the leading cause of death in worldwide, responsible for 17.3 million deaths per year.[4,5]

Cite this article as: Duque AP, Massolar CS, Rocha CB, Da Rocha AP, Moreira RF, Rodrigues Junior LF. Carqueja (*Baccharis trimera*) essential oil chronic treatment induces ventricular repolarization disorder in healthy rats but not in type 2 diabetic rats. Phcog Res 2019;11:410-3.

Diabetic cardiomyopathy, one of the chronic complications of T2DM, is associated with cardiac hypertrophy and ventricular dysfunction, inducing to heart failure, also responsible for elevated mortality rates.[1,6] The adequate treatment of T2DM could decrease the progression of these complications, reducing mortality related to CVDs; however, it has been demonstrated the association between diabetes pharmacological treatment, like the sulphonylurea therapy, and the increase of cardiovascular risk, making the study of new therapeutic targets promising.[7]

In this regard, the medicinal plant carqueja (*Baccharis trimera*) stands out, since it acts as a hypoglycemic and antioxidant, probably due to its high flavonoid concentration on total extract and to high levels of carquejol and carquejyl acetate on essential oil.[8,9] Even though carqueja has been used as a very old therapeutic plant by the popular alternative medicine, there is a shortage of studies about this medicinal plant safety, mainly related to its essential oil fraction.[8] Hence, the objective of this study is to investigate the safety of carqueja essential oil for the cardiovascular system.

MATERIALS AND METHODS

Model and experimental design

This pilot study used 18 Wistar male rats weighing 250–350 g, which were randomly divided in four experimental groups: control group (CoV; n = 5), carqueja-treated control group (CoCar; n = 4), diabetic control group (DMV; n = 4), and carqueja-treated diabetic group (DMCar; n = 5). All procedures were approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal University of the State of Rio de Janeiro (UNIRIO), whose protocol is CEUA-UNIRIO/2012014-2.

Experimental diabetes induction

T2DM was induced by cafeteria diet produced using the powdered normal diet, chocolate, peanut, and biscuit (*ad libitum* for 3 weeks) followed by a single intraperitoneal injection of streptozotocin (35 mg/kg).[10] Concomitantly to this protocol, the animals in the control groups received a standard rodent diet (Nuvilab®, Brazil). T2DM was confirmed by glucose dosage (glucometer G-Tech®, Brazil) from capillary blood obtained from rat tail. The animals submitted to the protocol for DM2 induction were considered diabetic whether showed glycemia higher than 200 mg/dL. After confirmation of DM2, the rats of the DMV and DMCar groups received the standard rodent diet until the end of the protocol. The animals were weighed weekly.

Treatment

The CoCar and DMCar received carqueja essential oil (Laszlo®, Brazil) diluted in Tween 80 (Merck®, Germany) (co-emulsifier of oil in water) in 0.01% aqueous solution. The other groups (CoV and DMV) received only Tween 80 (co-emulsifier of oil in water) in 0.01% aqueous solution. Both administrations were made by gavage with a daily dose of 20 mg/kg for 3 weeks.

Electrocardiographic recordings

Electrocardiogram (ECG) recording was performed by the peripheral and bipolar DII lead. For this, three metallic electrodes were surgically implanted in the subcutaneous tissue of the animals, after general anesthesia with thiopental (40 mg/kg) ip. The ECG was recorded with awake animals, 24 h after the electrode implantation. Heart rate (HR), P-wave and T-wave amplitudes, P-wave duration, QRS complex, PR interval, and QT and corrected QT intervals were evaluated. For QT correction, standard Bazett's formula corrected QT (QTc) = QT/√RR was used. All records were analyzed using LabChart 7.0 software (ADInstruments, USA).

Evaluation of cardiac hypertrophy

After the recordings, the animals were submitted to euthanasia through anesthesia with intraperitoneal sodium thiopental (80 mg/kg) and cardiac puncture. After euthanasia, each animal had the heart removed and weighed. The division of the heart weight value by body weight (HW/BW) was used as an index of cardiac hypertrophy.

Data analysis and statistics

The Shapiro–Wilk test was used to assess the distribution of the studied variables. For the comparison of the variables between experimental groups, it was used one-way ANOVA with a Newman–Keuls posttest, for those presenting Gaussian distribution (final body weight, heart weight, HW/BW, QT interval, QTc interval, HR, P-wave duration, PR interval, and P- and T -wave amplitude), and Kruskal–Wallis test with Dunn's posttest, for those with non-Gaussian distribution (QRS complex duration). All results are expressed as mean ± standard error of the mean, and $P < 0.05$ was considered significant.

RESULTS

Table 1 shows the results of biometry and electrocardiogram. As can be observed, comparing the four experimental groups, there are no differences in total heart weight at the end of the protocol. The animals of the DMV and DMCar groups presented lower ($P < 0.01$) body weight at the end of the 4th week when compared to the groups CoV and CoCar. DMV and DMCar groups showed an increased HW/BW (3.7 ± 0.1 mg/g and 3.6 ± 0.1 mg/g, respectively) [Figure 1b] compared to CoV (3.1 ± 0.1 mg/g) ($P < 0.001$)

Table 1: Biometric and electrocardiographic parameters

	CoV (n=5 rats)	CoCar (n=4 rats)	DMV (n=4 rats)	DMCar (n=5 rats)
Biometry				
Heart weight (mg)	1422.0±38.7	1500.0±99.1	1353.0±31.9	1288.0±24.9
Final body weight (g)	461.2±12.9	472.5±30.9	362.0±9.1**,##	353.4±5.0**,##
Electrocardiogram				
Heart rate (bpm)	365.1±17.8	362.1±9.8	299.6±9.7*,##	277.7±11.3**,##
P-wave duration (ms)	17.5±1.3	23.3±3.3	18.3±1.1	20.4±1.3
P-wave amplitude (μV)	174.8±29.1	223.6±11.2	242.7±16.0	144.6±47.4
T-wave amplitude (μV)	383.2±70.5	325.5±53.9	513.0±44.5	475.2±63.2
PR interval duration (ms)	47.4±0.7	54.2±2.7	48.9±2.1	46.4±2.1
QRS complex duration (ms)	15.2±0.2	15.6±0.7	16.6±1.0	18.9±1.8

Results are expressed as mean±SEM. Statistics analyses: Final body weight. Heart weight, P-wave duration, PR interval, and P- and T-wave amplitudes were evaluated with one-way ANOVA and Newman-Keuls posttest. The QRS complex duration was evaluated with Kruskal-Wallis test and Dunn's posttest. *P<0.01 versus CoV, **P<0.01 versus CoV, ##P<0.01 versus CoCar. ANOVA: Analysis of variance; SEM: Standard error of the mean; CoV: Control group; CoCar: Carqueja-treated control group; DMV: Diabetic control group; DMCar: Carqueja-treated diabetic group

and CoCar (3.2 ± 0.1 mg/g) ($P < 0.01$). Furthermore, the HW/BW was not altered in CoCar compared to CoV.

Representative ECG traces of each experimental group are exposed in Figure 1a. Concerning the ECG parameters, no differences were observed comparing the duration and amplitude of the P-wave, amplitude of the T-wave, duration of the QRS complex, and the PR interval between the groups CoV, CoCar, DMV, and DMCar. However, QT interval duration was significantly higher ($P < 0.01$) in CoCar (65.2 ± 1.5 ms) compared to CoV (57.8 ± 1.6 ms). Furthermore, the DMV and DMCar groups' QT intervals (72.8 ± 1.2 ms and 75.4 ± 1.2 ms, respectively) were higher compared to the CoV ($P < 0.0001$) and to the CoCar group ($P < 0.01$ and $P < 0.001$, respectively [Figure 1c]. The HR was significantly lower in diabetic rats from both DMV and DMCar (299.6 ± 9.7 and 277.7 ± 11.3 bpm, respectively) compared to control groups CoV and CoCar (365.1 ± 17.8 and 362.1 ± 9.8 bpm, respectively).

The QTc interval [Figure 1d] was significantly increased ($P < 0.01$) in the DMV (162.9 ± 4.6 ms) and DMCar (162.2 ± 3.4 ms) groups when compared to the CoV group (139.5 ± 4.3 ms). Interestingly, the CoCar group QTc interval (160.2 ± 3.9 ms) was increased ($P < 0.01$) compared to the CoV group, matching with QTc of the diabetic groups.

DISCUSSION

The main finding of the present study is that chronic treatment with carqueja essential oil prolongs ventricular repolarization (evidenced by QT and QTc prolongation) in nondiabetic rats. Interestingly, it seems to exert no effect on ventricular repolarization of diabetic rats. Diabetic cardiomyopathy is associated with diastolic dysfunction, left ventricular hypertrophy, and repolarization dysfunction which may be evidenced by QTc interval prolongation.[1]

Our data suggest that diabetic groups developed diabetic cardiomyopathy, since diabetic rats presented cardiac hypertrophy, as already reported in the literature.[11] It has also been shown that treatment with carqueja essential oil does not reverse but does not aggravate this alteration. Although the HW/BW index is well accepted in the literature as a marker of cardiac hypertrophy, variations in animal weight may influence this result.[12] Hence, it was necessary to investigate whether the significant result evinced by this index is reliable, using more sensitive, specificity, and accuracy methods, such as electrocardiogram.[13]

The QT interval is directly associated with the time that electrical stimulus remains in the ventricles.[14] The prolongation of QT and QTc intervals without QRS alteration strongly suggests a ventricular repolarization disturbance that increases arrhythmogenic and the sudden death risks.[15] Hence, the identifying of increases in QT and QTc is a non-invasive method to predict the risk of cardiovascular mortality.[16] In the present study, we observed a prolongation of QT and QTc intervals on diabetic groups, a typical remodeling alteration found in T2DM patients.[15] We also noted a similar repolarization disturbance on the DMCar group, suggesting that carqueja essential oil treatment does not reverse but does not aggravate the already existing alterations in diabetic animals.

However, our data evidenced a prolongation of QT and QTc intervals in nondiabetic animals that received chronically treatment with carqueja oil. This suggests that the chronic consumption of this essential oil dosage by healthy individuals could be harmful, leading to a ventricular repolarization disorder. Furthermore, QTc prolongation, alone, is associated with autonomic dysfunction, generation of arrhythmias,

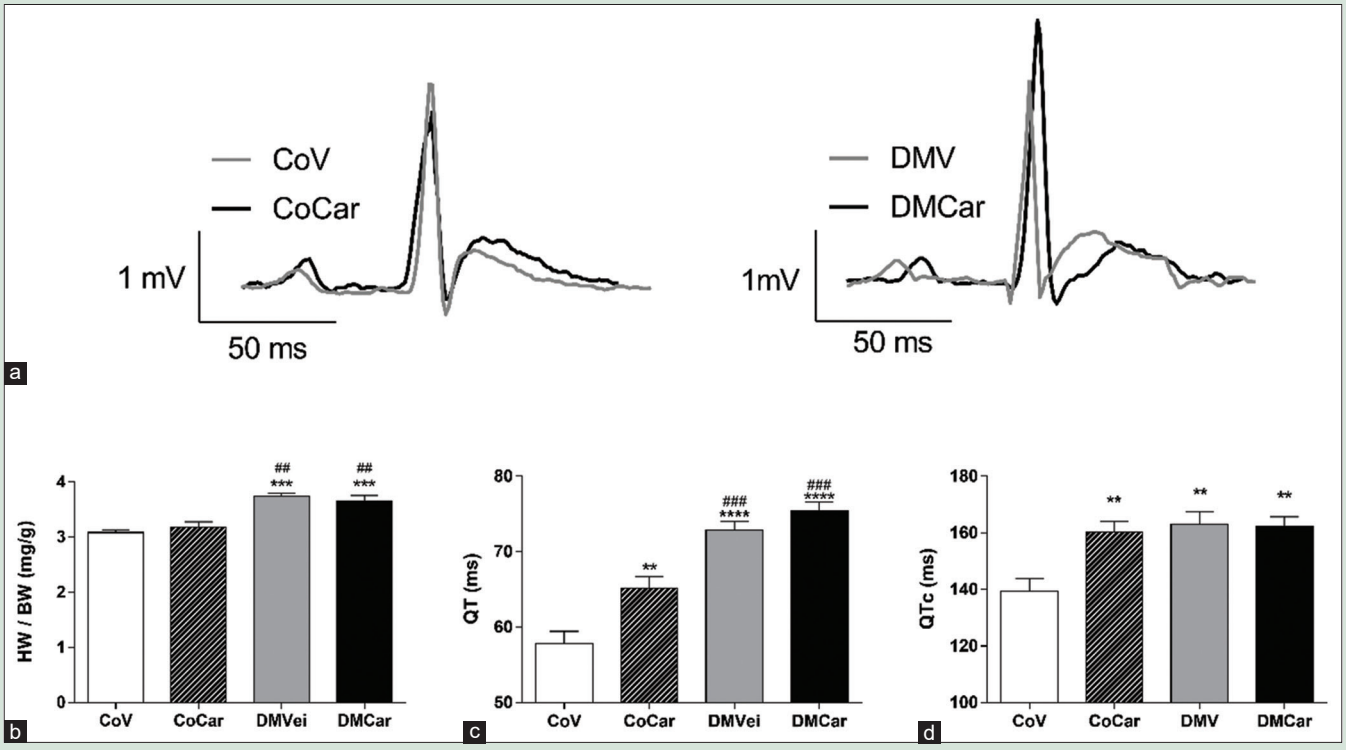


Figure 1: Heart hypertrophy and ventricular repolarization in control and diabetic rats treated with vehicle or carqueja essential oil. (a) Representative traces of electrocardiographic recorded in awake rats treated with vehicle or carqueja essential oil. (b) Cardiac hypertrophy index (HW/BW), the division of the heart weight value (mg) by body weight (g), HW/BW. (c) QT interval duration, in milliseconds. (d) corrected QT interval duration (millisecond), corrected by Bazett's formula: corrected QT = QT/√RR. The four experimental groups: CoV ($n = 5$ animals), CoCar ($n = 4$ animals), DMV ($n = 4$ animals) and DMCar ($n = 5$ animals). $^{**}P < 0.01$ versus CoV; $^{***}P < 0.001$ versus CoV; $^{****}P < 0.0001$ versus CoV; $^{\#\#}P < 0.01$ versus CoCar; $^{\#\#\#}P < 0.001$ versus CoCar. The parameters were evaluated with one-way ANOVA and Newman–Keuls posttest

mainly ventricular tachycardia and ventricular fibrillation, left ventricular hypertrophy, severe hypoglycemia, hyperglycemia, and increased mortality.[16-19]

The prolongation of QT and QTc intervals, without any other ECG alteration, suggests that a possible mechanism related to carqueja-induced ventricular repolarization disorder could be the reduction of transient outward potassium current (Ito), the main repolarization current present in rats heart.[20] However, other studies are necessary to evaluate whether carqueja essential oil consumption really affects I_{to} and in which manner it acts (by reducing I_{to} subunits expression, trafficking or conductance).

CONCLUSION

The present study demonstrates that chronic carqueja essential oil consumption by nondiabetic rats may prolong ventricular repolarization independently of left ventricular hypertrophy. Despite the limitations, the study alerts to the risk of inadvertent consumption of medicinal plants, drawing attention to the need to investigate its physiological effects and the safety of its consumption by individuals with some pathology and even by healthy individuals.

Limitations

This was a pilot study with a small sample size, but with important findings that should serve as a warning for the population about carqueja consumption. Hence, further studies are needed, with increased sample size to demonstrate the safety of this medicinal plant.

Acknowledgement

To Carlos Chagas Filho Research Support Foundation (FAPERJ) and National Council for Scientific and Technological Development (CNPq) for Financial support.

Financial support and sponsorship

The study was supported by FAPERJ and CNPq.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Hölscher ME, Bode C, Bugger H. Diabetic cardiomyopathy: Does the type of diabetes matter? Int J Mol Sci 2016;17. pii: E2136.
2. Unnikrishnan R, Pradeepa R, Joshi SR, Mohan V. Type 2 diabetes: Demystifying the global epidemic. Diabetes 2017;66:1432-42.
3. Zghebi SS, Steinke DT, Carr MJ, Rutter MK, Emsley RA, Ashcroft DM. Examining trends in type 2 diabetes incidence, prevalence and mortality in the UK between 2004 and 2014. Diabetes Obes Metab 2017;19:1537-45.
4. World Health Organization. Top Ten Causes of Death Worldwide. Geneva: World Health Organization; May, 2018. Available from: https://www.who.int/en/news-room/fact-sheets/detail/the-top-10-causes-of-death. [Last accessed on 2019 May 26].
5. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, *et al.* Heart disease and stroke statistics – 2015 update: A report from the American Heart Association. Circulation 2015;131:e29-322.
6. Bui AL, Horwich TB, Fonarow GC. Epidemiology and risk profile of heart failure. Nat Rev Cardiol 2011;8:30-41.
7. Bain S, Druyts E, Balijepalli C, Baxter CA, Currie CJ, Das R, *et al.* Cardiovascular events and all-cause mortality associated with sulphonylureas compared with other antihyperglycaemic drugs: A Bayesian meta-analysis of survival data. Diabetes Obes Metab 2017;19:329-35.
8. Rabelo AC, Araújo GR, Lúcio KD, Araújo CM, Miranda PH, Silva BD, *et al.* Aqueous extract of *Baccharis trimera* improves redox status and decreases the severity of alcoholic hepatotoxicity. Rev Bras Farmacogn 2017;27:729-38.
9. Gomes AL, Souza WF, Souza GS, Terra AM, Carvalho RS, Barbosa C, *et al.* Chemical Characterization of a Commercial Essential oil of Carqueja (*Baccharis trimera*). Proceedings of the Food and Nutrition Symposium; 2018. Available from: https://proceedings.science/sian/papers/chemical-characterization-of-a-commercial-essential-oil-of-carqueja-%28baccharis-trimera%29. [Last accessed on 2019 May 30].
10. Terra AM, Souza WF, Souza GS, Gomes AL, Carvalho RS, Netto CC, *et al.* Induction of type 2 Diabetes in rats by in-House Prepared High-Fat Diet and Low Streptozotocin Dose. Campinas: Proceedings of the Food and Nutrition Symposium; 2018. Available from: https://proceedings.science/sian/papers/induction-of-type-2-diabetes-in-rats-by-in-house-prepared-high-fat-diet-and-low-streptozotocin-dose. [Last accessed on 2019 May 30].
11. Radovits T, Korkmaz S, Mátyás C, Oláh A, Németh BT, Páli S, *et al.* An altered pattern of myocardial histopathological and molecular changes underlies the different characteristics of Type-1 and Type-2 diabetic cardiac dysfunction. J Diabet Res 2015;2015:1-12.
12. Kumar NT, Liestøl K, Løberg EM, Reims HM, Mæhlen J. Postmortem heart weight: Relation to body size and effects of cardiovascular disease and cancer. Cardiovasc Pathol 2014;23:5-11.
13. Bacharova L, Schocken D, Estes EH, Strauss D. The role of ECG in the diagnosis of left ventricular hypertrophy. Curr Cardiol Rev 2014;10:257-61.
14. Konopelski P, Ufnal M. Electrocardiography in rats: A comparison to human. Physiol Res 2016;65:717-25.
15. Ninkovic VM, Ninkovic SM, Miloradovic V, Stanojevic D, Babic M, Giga V, *et al.* Prevalence and risk factors for prolonged QT interval and QT dispersion in patients with type 2 diabetes. Acta Diabetol 2016;53:737-44.
16. Kittnar O. Electrocardiographic changes in diabetes mellitus. Physiol Res 2015;64 Suppl 5:S559-66.
17. Haugaa KH, Bos JM, Borkenhagen EJ, Tarrell RF, Morlan BW, Caraballo PJ, *et al.* Impact of left ventricular hypertrophy on QT prolongation and associated mortality. Heart Rhythm 2014;11:1957-65.
18. Miki T, Tobisawa T, Sato T, Tanno M, Yano T, Akasaka H, *et al.* Does glycemic control reverse dispersion of ventricular repolarization in type 2 diabetes? Cardiovasc Diabetol 2014;13:125.
19. Pickham D, Flowers E, Drew BJ. Hyperglycemia is associated with corrected QT prolongation and mortality in acutely ill patients. J Cardiovasc Nurs 2014;29:264-70.
20. Rodrigues Junior LF, de Azevedo Carvalho AC, Pimentel EB, Mill JG, Nascimento JH. Chronic enalapril treatment increases transient outward potassium current in cardiomyocytes isolated from right ventricle of spontaneously hypertensive rats. Naunyn Schmiedebergs Arch Pharmacol 2017;390:225-34.