BALNEO RESEARCH JOURNAL
English Edition
eISSN 2069-7619 pISSN 2069-7597 ROMANIAN ASSOCIATION OF BALNEOLOGY

Experimental model of acute myocardial infarction for evaluation of prevention and rehabilitation strategies in cardiovascular diseases – a pilot study

BOARESCU Paul-Mihai[1,2], BOARESCU Ioana[3], BOCȘAN Ioana Corina[4], POP Raluca Maria[4], GHEBAN Dan[5], BULBOACĂ Adriana Elena[1], DOGARU Gabriela[6] , BOLBOACĂ Sorana D.[2]

Corresponding Author: **Dan GHEBAN**, E-mail: dgheban@gmail.com

Balneo Research Journal DOI: http://dx.doi.org/10.12680/balneo.2019.270 Vol.10, No.3, September 2019 p: 288–293

1. "Iuliu-Haţieganu" University of Medicine and Pharmacy, Department of Pathophysiology, Cluj-Napoca, Romania
2. Iuliu-Haţieganu" University of Medicine and Pharmacy, Department of Medical Informatics and Biostatistic, Cluj-Napoca, Romania
3. County Clinical Emergency Hospital, Department of Neurology, Cluj-Napoca, Romania
4. "Iuliu-Haţieganu" University of Medicine and Pharmacy, Department of Pharmacology, Toxicology and Clinical Pharmacology, Cluj-Napoca, Romania
5. Iuliu-Haţieganu" University of Medicine and Pharmacy, Department of Pathological Anatomy, Cluj-Napoca, Romania
6. Iuliu-Haţieganu" University of Medicine and Pharmacy, Department of Medical Rehabilitation, Cluj-Napoca, Romania

Abstract

Introduction: Acute myocardial infarction (AMI) is an important acute disease of myocardial tissue, that occurs as a result of an imbalance between coronary blood supply and myocardial demand. Isoproterenol (ISO) is a synthetic catecholamine, a beta-adrenergic agonist that produces extensive biochemical, functional, and histological alterations in the heart, characteristic for AMI. The present study has been designed to identify the best dose of ISO that induces electrocardiogram (ECG) alterations, enzymatic reaction, and histopathological changes characteristic of AMI. **Material and method:** AMI was induced to Wistar-Bratislava white male rats, using three different subcutaneous doses of ISO (85 mg/kg bw, 100 mg/kg bw, and 150 mg/kg bw). ISO was administrated twice, with the second dose at 24h after the initial one. The ECGs were recorded at 24 hours after the last dose of ISO. Blood samples were collected for measurement of creatine kinase (CK), and CK-MB serum levels, and the hearts were excised and prepared for histopathologic examination. **Results and discussions:** All doses of ISO induced alterations in the ECG patterns such as increased heart rate and prolongation of QT and QTc intervals. Depression of the ST segment coupled with marked T wave inversion were observed at the doses of 100 mg/kg bw and 150 mg/kg bw of ISO. All doses of ISO induced an elevation of CK and CK-MB with highest levels observed for the dose of 150 mg/kg bw. Histopathologic examination revealed subendocardial AMI lesions for all doses tested. **Conclusions:** ISO in doses of 100 mg/kg and 150 mg/kg is useful for induction of infarct-like lesion on ECG, increased levels of myocardial necrosis enzymes and morphological changes characteristic for AMI.

Key words: *acute myocardial infarction, isoproterenol (ISO), dose, rats,*

Introduction

Cardiovascular diseases are the leading cause of deaths in many parts of the world, although modern drug therapies and strategies to both primary and secondary preventions are available for reducing their morbidity and mortality (1). Acute myocardial infarction (AMI) is an important acute disease of myocardial tissue, that occurs as a result of an imbalance between coronary blood supply and myocardial demand (2). This prolonged imbalance leads to cardiac ischemia, degeneration of cardiomyocytes, and eventually irreversible cardiac injury or death (3).

Catecholamines at low concentrations are considered to have a beneficial effect in regulating the heart function since they exert a positive inotropic effect, but their excess release from the endogenous stores or administration in high doses may deplete the energy reserve of cardiomyocytes, resulting in biochemical and structural changes and eventually development of irreversible cardiac damage (4).

Isoproterenol [1-(3, 4-dihydroxyphenyl)-2-isopropylamino ethanol hydrochloride] (ISO) is a synthetic catecholamine. It is widely known as a potent beta-adrenergic agonist that in high doses produces extensive biochemical, functional, and histological alterations in the heart, due to the production of substantially toxic free radicals and lipid peroxidation via an auto-oxidation process (2, 3).

ISO-induced myocardial necrosis is associated with alterations in membrane permeability as oxidative products of catecholamines stimulate lipid peroxidation and cause irreversible damage to the

myocardial membrane, leading to a loss of function and integrity of cardiomyocytes' membranes (5). This will cause increased release of cardiac marker enzymes into the bloodstream, alternated ischemic electrocardiograph changes, accumulated lipid peroxides, and damaged cardiac function (6,7). Apart from oxidative stress, inflammation also plays an important role in the pathophysiology of AMI (8,9,10).

The pathophysiological and morphologic alterations in the heart tissues of this drug-induced AMI experimental model are comparable with those taking place in human myocardial infarction (11,12). The standard model to study the beneficial effects of many drugs on cardiac function in AMI is a well-known (10).

The present study has been designed to identify the best dose of ISO that induces ECG alterations, enzymatic reaction and histopathological changes characteristic of AMI in rats.

2. Material and methods

2.1 Ethics statement

The experimental protocol was approved by the Ethics Committee of the „Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca (53/22.01.2018) and followed the Helsinki Declaration on Animal Studies.

2.2 Drugs

Isoprenaline hydrochloride (ISO) for AMI induction was purchased from Sigma–Aldrich (St. Louis, USA).

2.3 Experimental model

Four Wistar-Bratislava white male rats, with weights between 250 and 300 grams, from the Animal Department of the Faculty of Medicine, Iuliu Haţieganu University Cluj-Napoca, were housed in polypropylene cages, acclimated under a 12h/12h light/dark cycle at 22-24˚C at the Department of Pathophysiology. Animals were provided with free access to water and standard pellets (Cantacuzino Institute, Bucharest, Romania) *ad libitum*. To induce AMI, three different doses of ISO (85 mg/kg body weight (bw), 100 mg/kg bw, and 150 mg/kg bw) were administrated subcutaneously for two days at 24h interval. Rat 2 received the dose of 85 mg/kg bw, rat 3 received 100 mg/kg bw, and rat 3 received 150 mg/kg bw of ISO. Rat 1 received subcutaneously saline solution following the schedule of the rats with ISO.

2.4. Electrocardiography

Electrocardiography (ECG) was recorded 24h after the last injection of ISO or saline, using a Biopac MP36 system (Goleta, CA, USA) after the method described by Balea et al. (13). The rats were anesthetized with an intraperitoneal injection of ketamine (26 mg/kg bw) and xylazine (2.6 mg kg/bw). Fifteen minutes after anesthesia, electrodes were attached to the paw pads of each rat, and ECG was recorded in the lead II. RR and QT intervals (ms), PR segment (ms), QRS complex duration (ms), ST-segment changes (mV) were calculated from ECG recordings using the Biopac Student Lab 3.7.7 software (Goleta, CA, USA). Heart rates (HR, beats/min), were calculated from the RR intervals according to the following formula: HR = 60,000/RR (14). Corrected QT intervals (QTc) (ms) were calculated according to Bazett formula (14).

2.4 Biochemical tests

After ECG monitoring, while the rats were still under general anesthesia with ketamine and xylazine, blood samples were taken from the retro-orbital plexus. The creatine kinase (CK) and creatine kinase MB fraction (CK-MB) levels were measured by a Jasco V-530 UV-Vis spectrophotometer (Jasco International Co. Ltd., Tokyo, Japan) using commercially available kits.

2.5 Histopathological changes

The animals were sacrificed by administering an overdose of anesthetics. Hearts were excised and tissue was fixed in 10% formalin prepared in saline. After fixation, the heart tissues were embedded in paraffin. Sections were deparaffinized and stained by hematoxylin and eosin (H&E) for histopathologic examination using an electric light microscope.

3. Results

Isoproterenol in doses of 100 mg/kg bw and 150 mg/kg bw induced significant alterations in ECG recordings, such as decreased RR intervals, increased HR, prolonged QT, and QTc intervals and depression of the ST-segment associated with marked T wave inversion (Figure 1, Table 1), which are characteristic for ISO-induced infarct-like lesion on ECG. Even more, these doses of ISO also prolonged the QRS complex (Figure 1, Table 1). Deepest ST-segment depression was obtained for the dose of 150 mg/kg bw (Figure 1, Table 1). The dose of 85 mg/kg bw of ISO decreased the RR interval and therefore increased HR, prolonged the QT and

QTc intervals but did not affect the QRS duration and ST-segment. ISO did not influence the PR segment. Our results showed increased serum levels CK and CK-MB after the ISO administration, in all doses (Table 2). Histopathological examinations revealed normal architecture of myocardial tissue of the rat 1, localized subendocardial myocytolysis with inflammatory infiltration in rat 2, diffuse subendocardial myocytolysis with inflammatory infiltration in rat 3 and extended subendocardial necrosis associated with diffuse infiltration with leukocytes in rat 4 (Figure 2).

Table 1. ECG characteristics

Rat+ISO	RR (ms)	HR (b/min)	PR (ms)	QRS (ms)	QT (ms)	QTc (ms)
1	190	316	49	39	63	56
2+ISO85	182	329	48	35	109	92
3+ISO100	170	352	46	42	115	98
4+ISO150	165	363	48	45	130	104

RR = RR interval; HR = heart rate; PR = PR interval; QRS = QRS complex, QT = QT interval; QTc = corrected QT interval; ST = ST segment; ISO = isoproterenol.

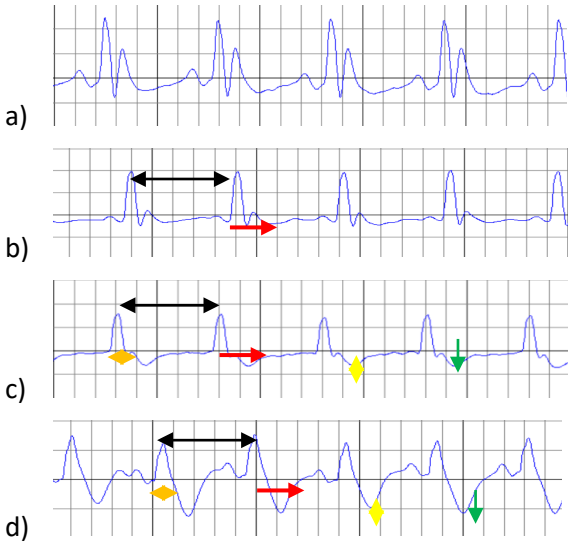

Fig. 1. ECGs after ISO administration. Decreased RR interval (black arrow), enlargement of the QRS complex (orange arrow), increased QT interval (red arrow), ST-segment depression (green arrow), T wave inversion (yellow arrow). (a) Control; (b) ISO in dose of 85 mg/kg bw; (c) ISO in dose of 100 mg/kg bw, (d) ISO in dose of 150 mg/kg bw; ISO = isoproterenol.

Table 2. Serum levels of cardiac enzymes

Rat+ISO	CK (U/L)	CK-MB (U/L)
1	78	10
2+ISO85	114	30
3+ISO100	129	34
4+ISO150	130	38

CK = Creatine kinase; ISO = isoproterenol

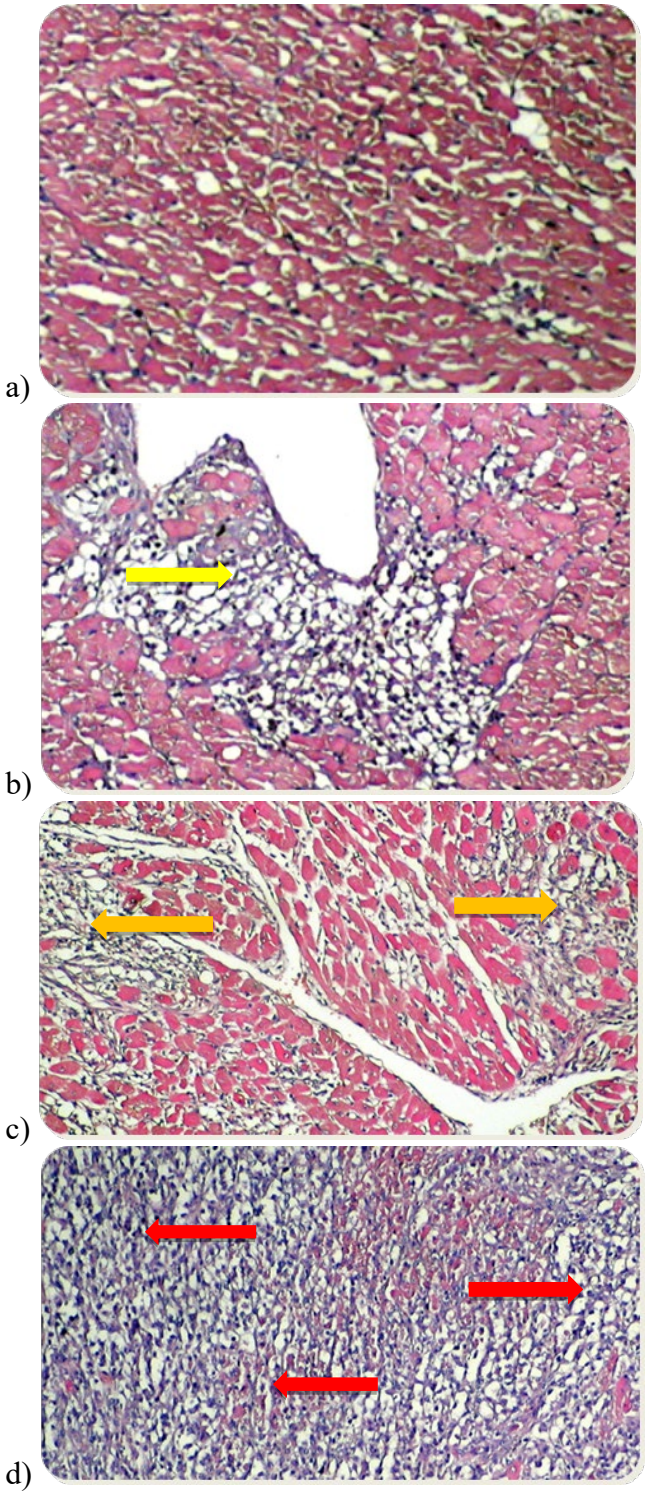

Fig. 2. Histopathology changes. (a) Rat1 - normal cardiac tissue; (b) Rat2+ISO85 (isoproterenol – ISO 85 mg/kg bw) - localised subendocardial myocytolysis with inflammatory tissue infiltration (yellow arrow); (c) Rat2+ISO100 (ISO 100 mg/kg bw) - diffuse subendocardial myocytolysis with inflammatory tissue infiltration (orange arrow); (d) Rat2+ISO150 (ISO 150 mg/kg bw) extensive sub-endocardial necrosis associated with diffuse infiltration with leukocytes (red arrow).

4. Discussion

ECG is a useful tool to assist in the diagnosis of AMI. After ISO administration in all doses, a reduction of RR interval and increased HR were observed on the ECG recordings (Table 1, Figure1). ISO is a beta-adrenoreceptor agonist, and the activation of beta-adrenergic receptors increases not only the HR but also the force of contraction of the left ventricle, stimulating the activity of the heart (13). By accelerating the heart rate, ISO induces impairment in perfusion of the subendocardial myocardium of the left ventricle with secondary subendocardial ischemia, necrosis, and associated ST depression on the ECG (15). In our study high doses of ISO (100 and 150 mg/kg bw) induced ST-segment depression coupled with marked T wave inversion, prolonged the QRS complex, increased QT and QTc intervals (Table 1, Figure 1). T-wave inversion was reported to be secondary to changes in action potential duration in cardiomyocytes after ISO administration (16). Slow ventricular conduction, due to ISO cardiotoxic effect, can explain the prolongation of the QRS complex and QT, QTc intervals after ISO administration (15). It is well known that ST-segment elevation and ST-segment depression are suggestive of AMI. None of the doses of ISO influenced the PR interval (Table 1). All ISO-induced ECG AMI-like alterations mentioned above could be due to the secondary loss of action potential in the cardyomyocites, as a result of oxidative stress (13). Oxidative stress plays an important role not only in AMI but also in many other cardiovascular disorders (17-19). Younis et al. reported that ISO in a dose of 85 mg/kg bw decreased in P wave, QRS complex, PR segment, and RR intervals and increased in the QT interval duration and ST-segment deviation, in comparison with the control rats (20). According to results of Soraya et al. rats from AMI group treated with ISO in a dose of 100 mg/kg bw showed a marked decrease in R-amplitude, a significant decrease in the RR interval and therefore, a significant increase in the heart rate (21). The same dose was reported by Sudha et al. to induce EKG abnormalities such as ST elevation in the ST segment, a higher heart rate, T wave inversion, prolonged QRS complex, and prolonged QT interval when compared to normal rats (22). Balea et al. reported that ISO in a dose of 150 mg/kg bw was observed to induce significant alterations in ECG patterns such as decreased HR, increased RR, QT, and QTc intervals, and ST segment depression coupled with marked T wave inversion, changes that reflect ISO-induced AMI-like lesions (13).

Rats with ISO-induced AMI had higher levels of CK, CK-MB, (Table 2) compared to normal rat. CK and CK-MB have an increased plasma level within 6 hours after the onset of AMI, a peak level at 24 hours and returns to normal within 2-3 days (23). The quantity of these cellular enzymes existing in plasma reflects changes in membrane integrity and/or permeability (24). When the cell membrane becomes permeable or in case of rupture, CK spread out from the damaged tissues into the bloodstream, serving as diagnostic markers of myocardial tissue injury (25, 26).

All rats with ISO had histological changes characteristic for AMI. Our study results reveal subendocardial AMI lesions for all doses: localized for the dose of 85 mg/kg bw, diffuse for the dose of 100 mg/kg bw and extensive for the dose of 150 mg/kg. Myocardial cells lesions vary from localized subendocardial myocytolysis with inflammatory tissue infiltration to necrosis associated with diffuse infiltration with leukocytes. Hassan et al. reported that histopathological evaluation of H&E stained sections of myocardial tissue from ISO-induced AMI group using a dose of 85 mg/kg bw, showed extensive loss of muscle fibers, several inflammatory cells and undamaged muscle fibers in the surrounding area (26). Soraya et al. observed that heart tissue of the rats treated with ISO in a dose of 100mg/kg showed intensive necrosis of cardiomyocytes and increased edematous intramuscular space (21). Similar results were also obtained by Reddy et al., who identified local confluent necrosis of muscle fibers edema and inflammatory cells infiltration for the same dose of ISO (27). Acikel at al. observed large areas of myofibrillary degeneration, related to marked infiltration with neutrophil granulocytes and severe interstitial edema in myocardial tissue for the dose of 150 mg/kg bw of ISO (28). The dose of 85 mg/kg bw of ISO was associated with a mortality of 7.69% (29). Filho et al. reported a mortality of 25% for the dose of 150 mg/kg bw (30), while 33% was reported by Acikel et al. (28).

5. Conclusion

ISO in doses of 100 mg/kg and 150 mg/kg is useful for induction of AMI since leads to infarct-like lesions on ECG, increased levels of myocardial necrosis enzymes and morphological changes characteristic for acute myocardial infarction. The use of these two ISO doses provide a useful experimental model of AMI for evaluating the antidoxidative and antinflamatory effects of differerent drugs or natural compounds. Moreover it can be used in evaluating different prevention and rehabilitation strategies in cardiovascular diseases.

Funding. This research was funded by the „Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, grant PCD number 1680/27/19.01.2018.

Acknowledgements. The authors would like to thank first of all to Ana Uifalean for helping with serum cardiac enzymes analysis and also to Molnar Mirel for helping with the handling of rats during the experiment.

References

1. Thippeswamy BS, Thakker SP, Tubachi S, Kalyani GA, Netra MK, Patil U, et al. Cardioprotective effect of Cucumis trigonus Roxb on isoproterenol-induced myocardial infarction in rat. AJPT. 2009;4(2):29-37. DOI : 10.3844/ajptsp.2009.29.37.
2. Wang SB, Tian S, Yang F, Yang HG, Yang XY, Du GH. Cardioprotective effect of salvianolic acid A on isoproterenol-induced myocardial infarction in rats. Eur J Pharmacol. 2009 Aug 1;615(1-3):125-32. DOI: 10.1016/j.ejphar.2009.04.061.
3. Wei H, Li H, Wan SP, Zeng QT, Cheng LX, Jiang LL, et al. Cardioprotective effects of malvidin against isoproterenol-induced myocardial infarction in rats: A mechanistic study. Med Sci Monit. 2017 Apr 26;23:2007-2016. DOI: 10.12659/MSM.902196
4. Upaganlawar A, Gandhi H, Balaraman R. Isoproterenol induced myocardial infarction: Protective role of natural products. J Pharmacol Toxicol. 2011;6(1):1-7. DOI: 10.3923/jpt.2011.1.17.
5. Todd GL, Cullan GE, Cullan GM. Isoproterenol-induced myocardial necrosis and membrane permeability alterations in the isolated perfused rabbit heart. Exp Mol Pathol. 1980 Aug;33(1):43-54.
6. Rajadurai M, Prince PS. Preventive effect of naringin on isoproterenol-induced cardiotoxicity in Wistar rats: an in vivo and in vitro study. Toxicology. 2007 Apr 11;232(3):216-25. DOI: 10.1016/j.tox.2007.01.006
7. Rajadurai M, Prince PS. Preventive effect of naringin on lipid peroxides and antioxidants in isoproterenol-induced cardiotoxicity in Wistar rats: biochemical and histopathological evidences. Toxicology. 2006 Dec 7;228(2-3):259-68. DOI: 10.1016/j.tox.2006.09.005
8. Boarescu PM, Chirilă I, Bulboacă AE, Bocșan IC, Pop RM, Gheban D, et al. Effects of curcumin nanoparticles in isoproterenol-induced myocardial infarction. Oxid Med Cell Longev. 2019; 2019:7847142. DOI: 10.1155/2019/7847142
9. Bulboacă AE, Porfire AS, Tefas LR, Boarescu PM, Bolboacă SD, Stănescu IC, et al. Liposomal Curcumin is Better than Curcumin to Alleviate Complications in Experimental Diabetic Mellitus. Molecules. 2019 Jan;24(5):846. DOI: 10.3390/molecules24050846
10. Boarescu PM, Boarescu I, Bocșan IC, Pop RM, Gheban D, Bulboacă AE, et al. Curcumin Nanoparticles Protect against Isoproterenol Induced Myocardial Infarction by Alleviating Myocardial Tissue Oxidative Stress, Electrocardiogram, and Biological Changes. Molecules. 24(15):2802. DOI: 10.3390/molecules24152802
11. Panda VS, Naik SR. Cardioprotective activity of Ginkgo biloba phytosomes in isoproterenol-induced myocardial necrosis in rats: a biochemical and histoarchitectural evaluation. Exp Toxicol Pathol. 2008 Aug;60(4-5):397-404. DOI: 10.1016/j.etp.2008.03.010
12. Zhou R, Xu Q, Zheng P, Yan L, Zheng J, Dai G. Cardioprotective effect of fluvastatin on isoproterenol-induced myocardial infarction in rat. Eur J Pharmacol. 2008 May 31;586(1-3):244-50. DOI: 10.1016/j.ejphar.2008.02.057
13. Balea ȘS, Pârvu AE, Pop N, Marín FZ, Pârvu M. Polyphenolic Compounds, Antioxidant, and Cardioprotective Effects of Pomace Extracts from Fetească Neagră Cultivar. Oxid

Med Cell Longev 2018; 2018:8194721. DOI: 10.1155/2018/8194721
14. Konopelski P, Ufnal M. Electrocardiography in rats, a comparison to human. Physiol. Res. 2016 Oct 1;65(5).
15. Collins P, Billings CG. Isoprenaline-induced changes in regional myocardial perfusion in the pathogenesis of myocardial necrosis. Br. J. Exp. Pathol. 1976 Dec;57(6):637.
16. Shipsey SJ, Bryant SM, Hart G. Effects of hypertrophy on regional action potential characteristics in the rat left ventricle: a cellular basis for T-wave inversion?. Circulation. 1997 Sep 16;96(6):2061-8. DOI: 10.1161/01.CIR.96.6.2061
17. Dogaru G, Ispas A, Bulboacă A, Motricală M, Stănescu I. Influence of balnear therapy at Băile Tușnad on quality of life of post-stroke patients. Balneo Research Journal. 2017;8(4):201-205. DOI: 10.12680/balneo.2017.151
18. Dogaru G, Scripcă AS, Croitoru AE, Motricală M, Bulboacă A, Stănescu I. A clinical study regarding the improvement of symptoms and the time efficacy of treatments performed in Băile Tușnad balneoclimatic resort. Balneo Research Journal. 2018;9(2):76-81. DOI: 10.12680/balneo.2018.177
19. Bulboaca A, Bulboaca A, Stanescu I, Blidaru M, Bulboaca AI, Nicula C, Dogaru G. Post-stroke visual rehabilitation - the impact on life quality of the stroke survival patients - a brief review. Balneo Research Journal. 2019;10(1):17-23. DOI: 10.12680/balneo.2019.233
20. Younis NS, Mohamed ME. β-Caryophyllene as a Potential Protective Agent Against Myocardial Injury: The Role of Toll-Like Receptors. Molecules. 2019 Jan;24(10):1929. DOI: 10.3390/molecules24101929
21. Soraya H, Khorrami A, Garjani A, Maleki-Dizaji N, Garjani A. Acute treatment with metformin improves cardiac function following isoproterenol induced myocardial infarction in rats. Pharmacol Rep. 2012;64(6):1476-84.
22. Sudha M, Rajkumar D, Muthiah R, Colledge M, Nagar A. Effect of taurine pretreatement on ECG changes in isoproterenol induced myocardial infarction in male albino rats. Sch. J. App. Med. Sci.2016; 4(3F):1052-5.
23. Nigam PK. Biochemical markers of myocardial injury. Indian J Clin Biochem. 2007 Mar 1;22(1):10-7. DOI: 10.1007/BF02912874
24. Mehdizadeh R, Parizadeh MR, Khooei AR, Mehri S, Hosseinzadeh H. Cardioprotective effect of saffron extract and safranal in isoproterenol-induced myocardial infarction in wistar rats. Iran J Basic Med Sci. 2013 Jan;16(1):56.
25. Sabeena Farvin KH, Anandan R, Kumar SH, Shiny KS, Sankar TV, Thankappan TK. Effect of squalene on tissue defense system in isoproterenolinduced myocardial infarction in rats. Pharmacol Res. 2004 Sep 1;50(3):231-6. DOI: 10.1016/j.phrs.2004.03.004
26. Hassan MQ, Akhtar M, Ahmed S, Ahmad A, Najmi AK. Nigella sativa protects against isoproterenol-induced myocardial infarction by alleviating oxidative stress, biochemical alterations and histological damage. Asian Pac J Trop Biomed. 2017 Apr 1;7(4):294-9. DOI: 10.1016/j.apjtb.2016.12.020
27. Reddy NM, Mahajan UB, Patil CR, Agrawal YO, Ojha S, Goyal SN. Eplerenone attenuates cardiac dysfunction and oxidative stress in β-receptor stimulated myocardial infarcted rats. Am J Transl Res. 2015 Sep 15;7(9):1602-11.
28. Acikel M, Buyukokuroglu ME, Erdogan F, Aksoy H, Bozkurt E, Senocak H. Protective effects of dantrolene against myocardial injury induced by isoproterenol in rats: biochemical and histological findings. Int J Cardiol. 2005 Feb 28;98(3):389-94. DOI: 10.1016/j.ijcard.2003.10.054
29. Campos EC, Romano MMD, Prado CM, Rossi MA. Isoproterenol induces primary loss of dystrophin in rat hearts: correlation with myocardial injury. Int J Exp Pathol. 2008 Oct; 89(5):367-81. DOI: 10.1111/j.1365-2613.2008.00604.x
30. Lobo Filho HG, Ferreira NL, Sousa RB de, et al. Modelo experimental de infarto do miocárdio induzido por isoproterenol em ratos. Rev Bras Cir Cardiovasc. 2011;26(3):469-76. DOI: 10.5935/1678-9741.20110024.