Effect of ivabradine on cardiac remodeling in experimentally induced heart failure in rats

Rawnaq Noel **Nidhal AK Mohammed Ali**

Abstract

Background: In heart failure (HF), myocardial dysfunction increase neurohormonal activity leading to progression of cardiac remodeling and worsening of clinical manifestations of HF. Ivabradine (IVA), selectively inhibits the funny current of the sinoatrial node and slow heart rate.

Objectives: The aim of this study was to investigate the effects of ivabradine on biomarkers of cardiac remodeling in rat model of induced HF.

Materials and Methods: Thirty rats were divided into five groups. Control, ivabradine (IVA), isoproterenol (ISO), ivabradine + isoproterenol (IVA-IHF) and digoxin+ isoproterenol (DIG-IHF) groups. HF was induced by administration of isoproterenol SC at 5mg/kg/day to all groups except control and co-administered with either ivabradine or digoxin in IVA-IHF and DIG-IHF groups respectively for fourteen days. Ivabradine was given orally to rats in IVA and IVA-IHF group at 10mg/kg/day whereas digoxin was given orally at 250μg/day to rats in DIG-IHF group for two weeks. Heart rate (HR) and blood pressure (BP) were recorded before and after drugs administration. Blood samples were taken for assessing NT-Pro BNP and MMP-9 levels. The rats were sacrificed and heart was taken for histopathology examination.

Results: The mean serum level of NT-Pro BNP and MMP-9 and HR were reduced significantly after ivabradine administration to IVA-IHF group compared to ISO group. BP was non-significantly changed after ivabradine administration to IVA-IHF compared to ISO group. Histopathological findings demonstrated a significant amelioration of myocardial damage in IVA-IHF group.

Conclusion: Ivabradine use was associated with decreased levels of cardiac biomarker, improved cardiac dysfunction and adverse remodeling in isoproterenol induced heart failure in rats.

Key words: Ivabradine, Heart failure, Heart rate, NT-Pro BNP, MMP-9.

Introduction

Cardiac remodeling contributes to poor prognosis in patients with coronary heart diseases and heart failure because of its association with ventricular dysfunction and malignant ventricular arrhythmias (1).

The pathophysiological aspects of cardiac remodeling in heart failure involve death of cardiomyocyte, oxidative stress, inflammation, collagen deposition and neurohormonal activation which leads to progressive ventricular dilatation, myocardial hypertrophy, fibrosis, deterioration of cardiac performance and subsequently heart failure (2).

Ivabradine is a selective hyperpolarization-activated cyclic nucleotide gated ion channel (HCN) blocker that blunts spontaneous pacemaker activity of the sinus node. This results in heart rate reduction with no significant hemodynamic effect (3).

The N-terminal pro-Brain Natriuretic Peptide (NT-Pro BNP) is released in response to ventricular dilation and pressure overload and numerous studies have highlighted the significance of monitoring the levels this biomarker as a guide in treatment of heart failure (4). MMP-9 is implicated in extracellular matrix (ECM) remodeling in heart failure and an elevated MMP-9 serum level in patients with heart failure is associated with increased LV diastolic dimensions and increased wall thickness and unfavorable progression of heart dysfunction (5). Elevated heart rate and volume overload are associated with unfavorable consequences in patients with heart failure therefore, the purpose of this study was to investigate the effects of ivabradine on biomarkers of cardiac remodeling and hemodynamic parameters in a rat model of heart failure.

Materials and Method

The study was carried out at the experimental animal house, College of Medicine, Hawler Medical University. Thirty male albino rats, 12 – 16 weeks old, weighing 200 – 300 grams, were used in the experiment and kept under normal room temperature (22 °C) with 12-hour light/dark cycles with rodent chow supplement and free excess to tap water.

The animals were divided randomly and equally into 5 groups; Control group; received distilled water as a placebo. Ivabradine group (IVA) received only Ivabradine at 10mg/kg/day for a period of 14 days via oral gavage and 0.5 ml distilled water subcutaneously. Isoproterenol (ISO) group rats received 5mg/kg/day subcutaneous injection of isoproterenol (Sigma-Aldrich, Co. St. Louis USA) to induce heart failure for a period of 14 days. Treatment groups consisted of two groups represented the experimentally induced heart failure model. Group one rats were treated with Ivabradine (IVA-IHF) at 10mg/kg/day once daily along with S.C injections of Isoproterenol (5mg/kg/day) and the second group were treated with digoxin (DIG-IHF) at 250μg per day via oral gavage along with S.C injections of Isoproterenol (5mg/kg/day) for 14 days period.

Heart rate and blood pressure were recorded before the administration of any drug. After fourteen days of the last dose of each drug, rats were checked for their blood pressure and heart rate.

Heart rate and blood pressure were measured using a non-invasive blood pressure module (CODA) system (Kent Scientific Torrington, CT, USA). Blood samples were withdrawn from the heart after the rats were anesthetized with ketamine hydrochloride (75mg/kg) and xylazine (10mg/kg) intraperitoneally. Thereafter, the rats were prepared for dissection, heart was taken gently, washed with sterile saline, dried and weighed and then placed in formaldehyde solution for histopathological sectioning.

Analysis of serum samples were done using the enzyme linked immunosorbent assay kit of NT-Pro BNP (N-Terminal Pro-Brain Natriuretic Peptide) Elisa Kit (Elabscience, United states) and MMP-9 MICROPLATE (R&D, United states) Elisa Kit. The histopathological sections of heart were prepared and stained with hematoxylin and eosin (H&E).

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 22). Paired t test was used to compare the readings before and after treatment. One way analysis of variance (ANOVA) was used to compare means. A post hoc test (LSD) was used to compare means of each two groups (after doing the ANOVA test). A *p* value of ≤ 0.05 was considered statistically significant.

Results

The effect of ivabradine alone, ivabradine and digoxin co-administered with isoproterenol on serum concentrations of NT-Pro-BNP and MMP-9

Administration of ivabradine alone had no significant ($P \geq 0.885$) effect on the mean serum concentration of NT-Pro-BNP compared to that of control group, while it was significantly ($P \leq 0.001$) lower than that of ISO and DIG-IHF groups respectively and of IVA-IHF group with a P value of < 0.002 (Table 1).

Ivabradine administration to IVA-IHF group produced significant ($P < 0.001$) lower mean serum levels of NT-Pro-BNP compared to mean serum levels of ISO group and DIG-IHF group but was significantly ($P < 0.002$) higher than that of control and IVA group for both respectively (Table1).

The administration of digoxin +Isoproterenol significantly ($P < 0.001$) resulted in higher mean serum mean level of NT-Pro-BNP compared to control group, IVA, and IVA-IHF groups respectively whereas it was not significantly ($P > 0.076$) different than the mean serum mean level of ISOl group (Table1).

The administration of Ivabradine alone produced a non-significantly ($P > 0.747$) differences in the mean serum concentration of MMP-9 compared to that of control, but it was significantly ($P < 0.001$) lower than the mean serum MMP-9 concentration of ISO, DIG-IHF respectively and then that of IVA-IHF group ($P < 0.002$) as shown in table (1).

Co-administration of ivabradine with isoproterenol resulted in a significantly ($P \leq 0.001$) higher mean serum MMP-9 concentration compared to control, and to that of IVA group with a *P* value of ≤ 0.002 while it was lower than that of the ISO group (12.38 ± 4.04) and DIG-IHF group with a *P* value of $\leq 0.001$for both respectively (Table 1).

However, the mean serum levels of MMP-9 in DIG-IHF group was not significantly ($P \geq 0.289$) different than that of ISO group although significantly ($P \leq 0.001$) higher than that of control and IVA groups respectively as shown in table (1). **The effect of Ivabradine alone, Ivabradine and digoxin co-administered with Isoproterenol on the heart rate and blood pressure.**

No significant ($P > 0.05$) differences was found in the mean heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MBP) between all groups before intervention. However after starting drug administration (table2), the mean HR in IVA-IHF was significantly ($P < 0.001$) higher than that of control but significantly ($P < 0.003$) lower than of ISO group. Although the mean HR in DIG-IHF was significantly ($P < 0.001$) higher than of control and IVA-IHF group but was not significantly ($P > 0.220$) different than of ISO group (table2). Concerning the SBP, DBP and MBP, no significant ($P > 0.05$) differences were observed between all groups after administration of the drugs (Table 2).

The HR after administration of ivabradine only was significantly ($P < 0.0035$) lower than that of control group, and of ISO, IVA-IHF and DIG-IHF groups ($P < 0.001$) as shown in (Table2). Whereas, a non-significant ($P > 0.05$) differences were found between SBP, DBP and MBP of IVA group when compared with all other groups (table2).

Results of Histopathology

Microscopic examination of histological sections of myocardial tissue of isoproterenol (ISO) group revealed diffuse necrosis of myocardial muscles, extensive myofibrillar degeneration and severe inflammation (figure 1, C) in contrast to normal myocardial muscle fibers, with no inflammation in control (figure 1, A) and ivabradine (IVA) group (figure 1,B). Administration of ivabradine to isoproterenol induced heart failure group (figure1, D) showed reduced myocardial injury as focal myocyte damage with slight degree of inflammation was demonstrated that indicates improvements of myocardial remodeling while, the myocardial tissues of digoxin co-administered with isoproterenol group (Figure1, E) showed extensive myofibrillar degeneration with moderate inflammation.

Table 1. Mean serum concentrations of biomarkers, NT- Pro BNP and MMP-9 of the study groups.

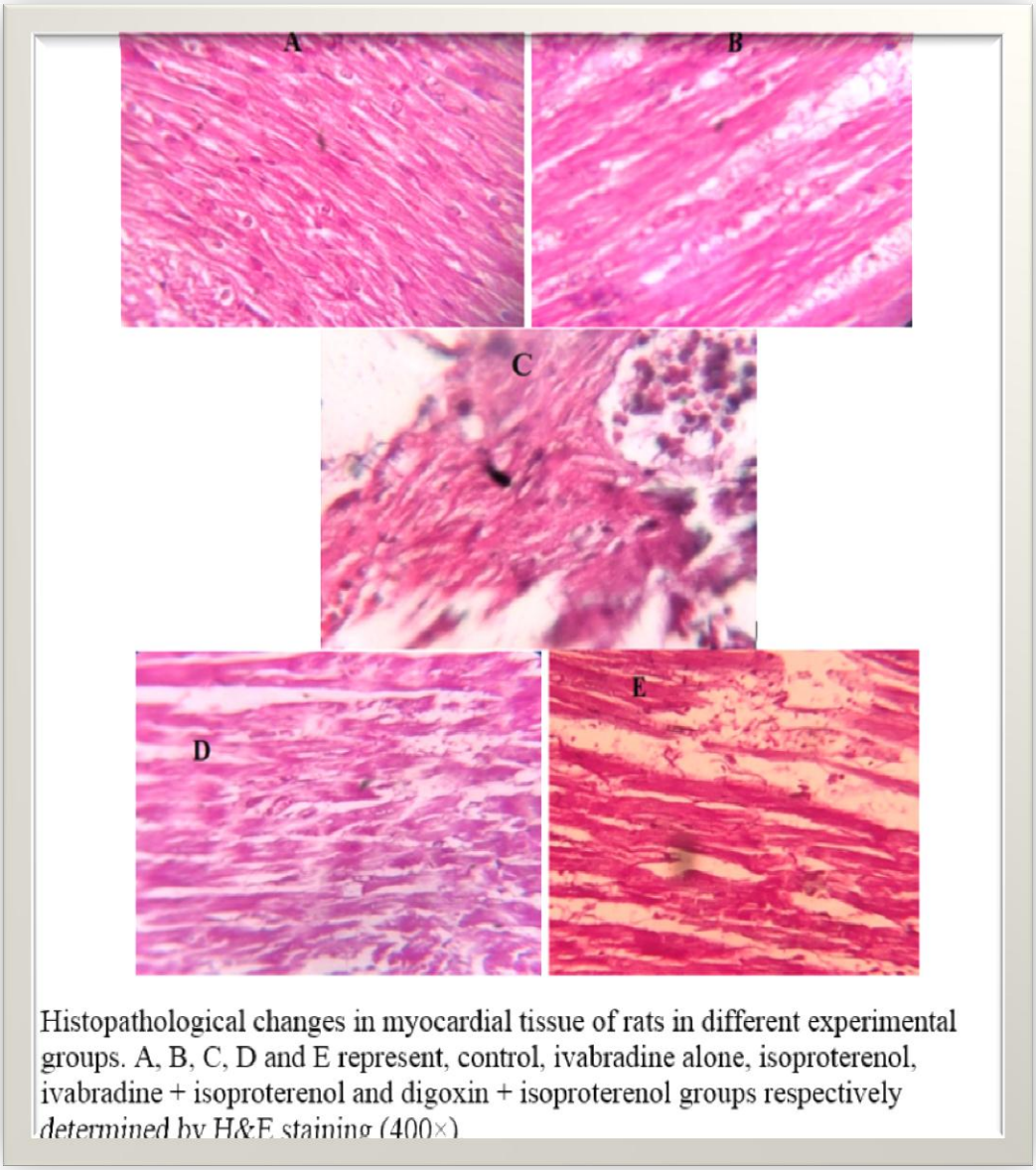
Biomarker	Group	Mean	(±SD)	p ANOVA	LSD groups	p (LSD)	LSD groups	p (LSD)
NT-Pro-BNP	A) Control	0.44	0.24		A X B	0.885	B X E	< 0.001
	B) Ivabradine alone	0.83	0.50		A X C	< 0.001	C X D	< 0.001
	C) Isoproterenol	21.89	6.02	< 0.001	A X D	0.002	C X E	0.076
	D)Ivabradine + Isoproterenol	9.97	7.49		A X E	< 0.001	D X E	< 0.001
	E)Digoxin + Isoproterenol	26.87	3.96		B X C	< 0.001		
					B X D	0.002		
MMP9	A) Control	0.58	0.41		A X B	0.747	B X E	< 0.001
	B) Ivabradine alone	1.03	0.45		A X C	< 0.001	C X D	< 0.001
	C) Isoproterenol	12.38	4.04		A X D	0.001	C X E	0.289
	D) Ivabradine + Isoproterenol	5.79	1.26	< 0.001	A X E	< 0.001	D X E	0.001
	E) Digoxin + Isoproterenol	10.89	3.21		B X C	< 0.001		
					B X D	0.002		

A p value of ≤ 0.05 was considered statistically significant.

Table 2. Means of the hemodynamic parameters of the study groups measured after the intervention.

Parameter	Group	N	Mean	±SD	p (ANOA)	LSD groups	p (LSD)	LSD (groups)	p (LSD)
HR (bpm)	A) Control	6	357.17	11.53		A X B	0.035	B X D	< 0.001
	B) Ivabradine alone	6	318.17	12.16		A X C	< 0.001	B X E	< 0.001
	C) Isoproterenol	6	533.33	19.30	< 0.001	A X D	< 0.001	C X D	0.003
	D)Ivabradine+ Isoproterenol	6	474.83	58.61		A X E	< 0.001	C X E	0.220
	E)Digoxin+Isoproterenol	6	555.33	22.21		B X C	< 0.001	D X E	< 0.001
SBP (mmHg)	A) Control	6	121.50	2.88		A X B	0.318	B X D	0.830
	B) Ivabradine alone	6	109.00	12.54		A X C	0.128	B X E	0.788
	C) Isoproterenol	6	102.17	14.22	0.577	A X D	0.228	C X D	0.737
	D) Ivabradine + Isoproterenol	6	106.33	16.05		A X E	0.209	C X E	0.778
	E) Digoxin+Isoproterenol	6	105.67	40.39		B X C	0.582	D X E	0.957
DBP(mmHg)	A) Control	6	86.67	4.23		A X B	0.405	B X D	0.760
	B) Ivabradine alone	6	78.00	9.42		A X C	0.091	B X E	0.974
	C) Isoproterenol	6	68.67	10.69	0.528	A X D	0.596	C X D	0.233
	D) Ivabradine + Isoproterenol	6	81.17	16.18		A X E	0.423	C X E	0.354
	E) Digoxin+Isoproterenol	6	78.33	32.99		B X C	0.370	D X E	0.784
MBP(mmHg)	A) Control	6	98.00	2.76		A X B	0.376	B X D	0.914
	B) Ivabradine alone	6	88.33	10.42		A X C	0.129	B X E	0.914
	C) Isoproterenol	6	81.17	10.32	0.642	A X D	0.436	C X D	0.445
	D) Ivabradine + Isoproterenol	6	89.50	15.64		A X E	0.322	C X E	0.581
	E) Digoxin+ Isoproterenol	6	87.17	35.50		B X C	0.510	D X E	0.830

A p value of ≤ 0.05 was considered statistically significant.



Histopathological changes in myocardial tissue of rats in different experimental groups. A, B, C, D and E represent, control, ivabradine alone, isoproterenol, ivabradine + isoproterenol and digoxin + isoproterenol groups respectively determined by H&E staining (400×)

Discussion

The effect of ivabradine alone, ivabradine and digoxin co-administered with isoproterenol on NT-Pro-BNP and MMP-9 serum concentrations

Inhibiting cardiac remodeling is a primary goal for the treatment of CHF patients (6). The significant ($P \leq 0.001$) lower mean concentration of NT-Pro-BNP after administration of ivabradine to IVA-IHF demonstrate that ivabradine by virtue of inhibition of the **If** channel in the sinus node was capable in reducing cardiac overload and resulted in significantly attaining lower concentrations of this biomarker compared to ISO group. Similarly, serum levels of NT-pro BNP was shown to be decreased after ivabradine administration to patients with heart failure which was correlated with improved survival and promoted left ventricular remodeling (7, 8).

The significantly ($P < 0.001$) lower MMP-9 concentrations in IVA-IHF group compared to that of ISO group, demonstrate that ivabradine by significantly reducing MMP-9 concentrations most likely attenuated myocardial pressure overload through decreasing

extracellular matrix alteration which causes inflammation, fibrosis and progression of heart failure (9).Studies illustrated that ivabradine attenuate myocardial stiffness through reducing the mRNA expression of MMP-2, MMP-9 and collagen I and III gene and protein expression in heart failure (10,11) and by inhibiting the inflammatory responses and cardiac apoptosis by inhibiting pro-inflammatory cytokine as TNFα and interleukin (IL)-6, which are capable of up regulating MMP-2 and MMP-9 implicated in atherosclerosis, cardiac fibrosis and remodeling (11,12) and by reducing protein expression of mRNA of angiotensin II type 1 receptor (AT1 receptor) levels that contributes to extracellular matrix remodeling (13,14).

The non-significant ($P > 0.289$) difference in the mean MMP-9 levels in DIG-IHF group compared to ISO group and the significantly ($P< 0.001$) higher mean MMP-9 serum levels compared to IVA-IHF group indicates the inability of digoxin to alleviate the progression of myocardial dysfunction induced by ISO in contrast to the significant ($P< 0.001$) reduced MMP-9 mean serum levels following ivabradine which is beneficial in improving myocyte function.

The non-significant ($P> 0.747$) differences in mean serum levels of MMP-9 in ivabradine group reflects that ivabradine has no harmful impact on cardiac tissue and that ivabradine in addition to heart rate reduction has pleiotropic effects such as enhancing nitric oxide liberation that aids in improving left ventricular function, reducing hypertrophy, decreasing cardiac fibrosis, and increase capillary density and cardio-protection in myocardial infarction and heart failure (15).

The effects of Ivabradine alone, Ivabradine and digoxin co-administered with Isoproterenol on the heart rate and blood pressure.

Inhibiting HCN channels by ivabradine in IVA-IHF group most likely blunted the sympathetic activity of ISO and resulted in significantly ($P< 0.003$) lower heart rate compared to ISO group. Lowering the heart rate reduces myocardial oxygen supply and cardiac workload and prevent development of heart failure and tachycardia-mediated cardiomyopathy which is one of the main goals for the management of heart failure. Ivabradine by blocking these channels that plays a key role in controlling heart rate resulted in (10.91% and 10. 97% bpm) lower heart rate in ivabradine (IVA) and IVA-IHF groups respectively compared to control. Similarly, heart rate reduction by ivabradine has been also reported in other studies (12).

The significantly ($P< 0.001$) lower mean heart rate in IVA-IHF group compared to that of DIG-IHF group could be contributed to the efficient action of ivabradine in blunting the β-adrenergic activation by ISO whereas digoxin failed to do so which is related to differences in digoxin and ivabradine mechanism of action and their effects on left ventricular function (16). By this effect, ivabradine benefits heart failure patients and provides better management of tachycardia-mediated cardiomyopathy than digoxin as observed in different studies (17). Digoxin is an old cardiac drug but still is used, however questions regarding its efficacy and safety in the management of patients with HF remain controversial and even digoxin in therapeutic range had demonstrated sympathomimetic activity (18) which may explain the higher mean heart rate (+ 4.13% bpm) than the mean heart rate of ISO group.

The non-significant difference in systolic, diastolic and mean atrial pressure between IVA-IHF group and control, ISO, IVA, and Dig-IHF groups shows that IVA has no negative effect on blood pressure which is similarly described in experimental studies and human subjects which explains why ivabradine is useful as part of the management of heart failure patients with low blood pressure and elevated heart rate or given with β-blockers (19).

Histopathological changes

Histological examinations showed that isoproterenol induced excessive myocardial damage was highly attenuated by ivabradine treatment in IVA-IHF rats presumably attributed to the significant reduction of NT-Pro BNP, MMP-9 and heart rate. A potential mechanism of this effect can be that IVA promoted modifications in the extracellular matrix as a consequence of HR reduction, and / or inhibition of inflammatory cytokines and induced nitric oxide liberation (20).

Conclusion

Administration of IVA demonstrated protective effects in ISO-induced heart failure through lowering the levels of cardiac remodeling biomarkers, NT-Pro BNP, MMP-9 and reducing elevated heart rate with no significant effects on blood pressure indices. These results was further supported by the histological findings of improvements in myocardial remodeling.

Conflicts of Interest

The authors declare that they have no conflict of interest.

References

1. Azevedo PS, Polegato BF, Minicucci MF, Paiva SA and Zornoff LA. Cardiac remodeling: concepts, clinical impact, pathophysiological mechanisms and pharmacologic treatment. Arq Bras Cardiol. 2016; 106, 62-9.
2. Nishida K, Otsu K. Autophagy during cardiac remodeling. J Mol Cell Cardiol. 2016; 95: 11-18.
3. Abed HS, Fulcher J, Kilborn MJ, Keech AC. Inappropriate Sinus Tachycardia: Focus on Ivabradine. Internal Medicine Journal 2016; 46(8): 875-83.

4. Magnussen C and S. Blankenberg. Biomarkers for heart failure: small molecules with high clinical relevance. J Intern Med. 2018; 283: 530- 43.
5. Morishita T, Uzui H, Mitsuke Y, Amaya N, Kaseno K, Ishida K. Association between matrix metalloproteinase-9 and worsening heart failure events in patients with chronic heart failure. ESC Heart Fail. 2017; 4(3): 321-30.
6. Konstam MA, Kramer DG, Patel AR, Maron MS, Udelson JE. Left ventricular remodeling in heart failure: current concepts in clinical significance and assessment. JACC Cardiocasv Imaging. 2011; 4(1): 98-108.
7. Parsons S, Clark AL and Cleland JGF. The remarkable effect of ivabradine in two adolescents with dilated cardiomyopathy. Clin Res Cardiol. 2014; 103(10): 847-49.
8. Ordu S, Yildiz BS, Alihanoglu YI, Ozsoy A, Tosun M, Evrengul H Et al. Effects of ivabradine therapy on heart failure biomarkers. Cardiology Journal. 2015; 22(5): 501- 09.
9. Wang S, Li S, Wang B, Liu J, and Tang Q. Matrix Metalloproteinase-9 Is a Predictive Factor for Systematic Hypertension and Heart Dysfunction in Patients with Obstructive Sleep Apnea Syndrome. BioMed Res Int. 2018; 2018: 1-11.
10. Sabbah HN, Gupta RC, Kohli S, Wang M, Zhang K, Rastogi S. Heart rate reduction with ivabradine improves left ventricular function and reverses multiple pathological maladaptations in dogs with chronic heart failure. ESC Heart Failure. 2014; 1: 94-102
11. Fang L, Murphy AJ and Dart AM. A Clinical Perspective of Anti-Fibrotic Therapies for Cardiovascular Disease. Front. Pharmacol. 2017;8: 1-8.
12. Simko F, Baka T, Poglitsch M, Repova K, Aziriova S, Krajcirovicova K Et al. Effect of Ivabradine on a Hypertensive Heart and the Renin-Angiotensin-Aldosterone System in L-NAME-Induced Hypertension. Int J Mol Sci. 2018; 19(10): 1-15.
13. Becher PM, Lindner D, Miteva K, Savvatis K, Zietsch C, Schmack B, et al. Role of heart rate reduction in the prevention of experimental heart failure: comparison between If channel blockade and beta-receptor blockade. Hypertension. 2012; 59: 949-57.
14. Lijnen PJ, Petrov VV. Role of intracardiac renin-angiotensin-aldosterone system in extracellular matrix remodeling. Methods Find Exp Clin Pharmacol. 2003; 25(7): 541-64.
15. Kaski JC, Gloekler S, Ferrari R, Fox K, Levy BI, Komajda M Et al. Role of ivabradine in management of stable angina in patients with different clinical profiles. Open Heart. 2018; 5(1): 1-13.
16. Castagno D, Petrie MC, Claggett B, McMurray J. Should we SHIFT our thinking about digoxin? Observations on ivabradine and heart rate reduction in heart failure. Eur Heart J. 2012; 33(9): 1137-41.
17. Borer JS, Le Heuzey JY. Characterization of the heart rate-lowering action of ivabradine, a selective I(f) current inhibitor. Am J Ther. 2008; 15: 461-73.
18. Al-Khateeb M, Qureshi WT, Odeh R, Ahmed AM, Sakr S, Elshawi R et al .The impact of digoxin on mortality in patients with chronic systolic heart failure: A propensity-matched cohort study. Int J Cardiol. 2017; 228: 214-18.
19. Komajda M. Heart rate in chronic heart failure: an overlooked risk factor. Eur Heart J. 2015; 36: 648– 49.
20. Rohm I, Kretzschmar D, Pistulli R, Franz M, Schulze PC, Stumpf C et al. Impact of Ivabradine on Inflammatory Markers in Chronic Heart Failure. J Immunol Res. 2016; 2016: 1-12.