

## **Modulation of ECG Alterations and Cardiac Hypertrophy by Fenofibrate in Isoproterenol Induced Heart Failure**

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### **Abstract**

The present study was designed to evaluate the cardioprotective effect of fenofibrate against isoproterenol (ISO) induced heart failure (HF) in rats by studying cardiac injury markers, left ventricular hypertrophy and electrocardiographic changes. Rat model of HF was induced by ISO administration (85 mg/kg, s.c.) at an interval of 24h on 0 and 1<sup>st</sup> day. Fifteen days post ISO injection, rats showed significant abnormalities in ECG profile along with increased serum levels of cardiac injury markers (creatin kinase-MB) and left ventricular hypertrophy. Treatment with fenofibrate (100mg/kg, orally) significantly improved ISO-induced ECG alterations, cardiac injury and left ventricular hypertrophy. In conclusion our results suggest that fenofibrate has ability to provide effective protection against electrical and structural changes developed in the heart of rat model of ISO-induced HF.

**Keywords:** Heart Failure; Isoproterenol; Fenofibrate; Electrocardiography; Hypertrophy

### **Abbreviations**

HF: Heart Failure; CVD: Cardiovascular Diseases; ISO: Isoproterenol; ECG: Electrocardiography; PPAR- $\alpha$ : Peroxisome Proliferator Activated Receptor- Alpha; CMC: Carboxymethylcellulose; CK-MB: Creatine Kinase-MB

### **Introduction**

Cardiovascular diseases (CVD) are the leading cause of mortality around the world, comprising of a range of disorders such as coronary heart diseases, peripheral arterial and rheumatic heart diseases, pulmonary embolism, deep vein thrombosis etc. According to WHO, an estimated 17.7 million people have died from CVD in 2015, which represents 31% of all global deaths [1]. Heart failure (HF) is the common final pathway for variety of these disease and is also reported to have an annual mortality rate of ~10% which results in a heavy economic burden on families and society [2,3]. Despite significant therapeutic advances, the morbidity and mortality of HF remains unacceptably high.

The rat model of isoproterenol (ISO)-induced HF is a reliable, reproducible, well-characterized and widely used model due to its similarity with clinically representative pathophysiological changes. ISO is a synthetic catecholamine and non-selective  $\beta$ -adrenoceptor agonist which at high concentrations has been reported to cause the development of necrotic lesions in the myocardium in experimental animals [4]. The pathophysiological and morphological aberrations produced in the heart of this myocardial necrotic rat model are com-

parable with those taking place in humans [5]. Among the various mechanisms proposed to explain the ISO induced cardiotoxicity, generation of highly cytotoxic free radicals through auto-oxidation of catecholamines has been implicated as one of the important causative factors. In a recent study by Yadav, et al. ISO caused marked abnormality in ECG which included elevation of ST segment, prolongation of QT segment and attenuation of PR, QRS and RR segment. These alterations reflect damage to integrity of myocardial cells and the function of heart [6,7]. Even with the advent of new technologies ECG still retains its position not only in the diagnosis of conditions responsible for heart failure, e.g. disorder of rhythm and conduction, but also for prognosis.

Fenofibrate is a well-known hypolipidemic drug and a potent activator of peroxisome proliferator-activated receptor-  $\alpha$  (PPAR- $\alpha$ ) and is used to treat hypertriglyceridemia, hypercholesterolemia and mixed dyslipidemia [8-12]. Apart from its lipid lowering effects fenofibrate also possesses numerous pleiotropic potentials such as antioxidant, anti-inflammatory, and antifibrotic actions on the heart thus affording myocardial protection [13,14].

Interestingly, the previous studies have not studied the effect of fenofibrate on electrical cardiac conduction in rats with ISO induced HF. Therefore the present study was done to investigate the effect of fenofibrate on ECG in ISO model of HF in Wistar rats.

### Materials and Methods

Wistar rats (300 - 350g) were obtained from the animal house of Jamia Hamdard Delhi. The care and use of laboratory animals were in accordance with the recommendations by National Accreditation Board of Testing and calibration Laboratories (NABL). All experimental protocols were approved by the Institutional Animal Ethical Committee, IGNOU, New Delhi, India, and experiments were performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. During the experimental study period rats were housed at constant room temperature, humidity, and light cycle (12: 12h light-dark), with free access to tap water and were fed with commercial standard chow *ad libitum*.

### Induction of Heart failure

ISO hydrochloride was dissolved in freshly prepared normal saline (NaCl 0.9% w/v) and injected (85 mg/kg, sc) for 2 consecutive days (day 0 and day 1), and the rats were monitored for weight change, food/water intake for 14 days [15,16].

### Grouping of Animals

Fifty rats were divided into 5 groups each having 10 rats each. In group I (normal control), rats were maintained on standard chow and water, and no treatment was given. In group II (vehicle), rats were given carboxymethylcellulose (CMC) orally for the 14 days. In group III (Per se fenofibrate) rats were administered with fenofibrate suspended in CMC (100 mg/kg/day) by gavage for 14 days. In group IV (ISO) rats were administered with ISO hydrochloride (85 mg/kg/day) subcutaneously for two consecutive days and were kept for 14 days. In group V (Fenofibrate treatment) rats were administered with ISO (85 mg/kg/day) subcutaneously for the two consecutive days. Following the two days of ISO injection fenofibrate suspended in CMC (100 mg/kg/day,) was given orally by gavage for 14 days.

All the experiments were performed on the fifteenth day from the start of the experiment.

### Electrocardiography (ECG) and Heart Rate

After the end of experimental period i.e. on 15<sup>th</sup> day animals were anesthetized with urethane (1g/Kg) and ECG leads were connected to the dermal layer of both front paws and hind legs of animals from all the groups to PowerLab data acquisition system (Chart v.8.1.8, AD Instruments, Australia) for the recording of ECG [17].

### Assessment of Cardiac injury and Left ventricular Hypertrophy

The release of myocardial enzyme CK-MB into the circulation was estimated by using the commercially available enzymatic kit (Reckon India Pvt. Ltd.).

At the end of each experiment heart from rats was excised and weight of whole heart and left ventricle were measured to calculate the ratio of left ventricle weight to heart weight (LV/HW) was calculated for evaluation of left ventricular hypertrophy.

**Statistical Analysis**

The results were presented as mean ± S.E.M. All data were analysed by analysis of variance (ANOVA) followed by Tukey’s multiple comparison tests, for analysis between the groups. P value of less than 0.05 was considered as statistically significant.

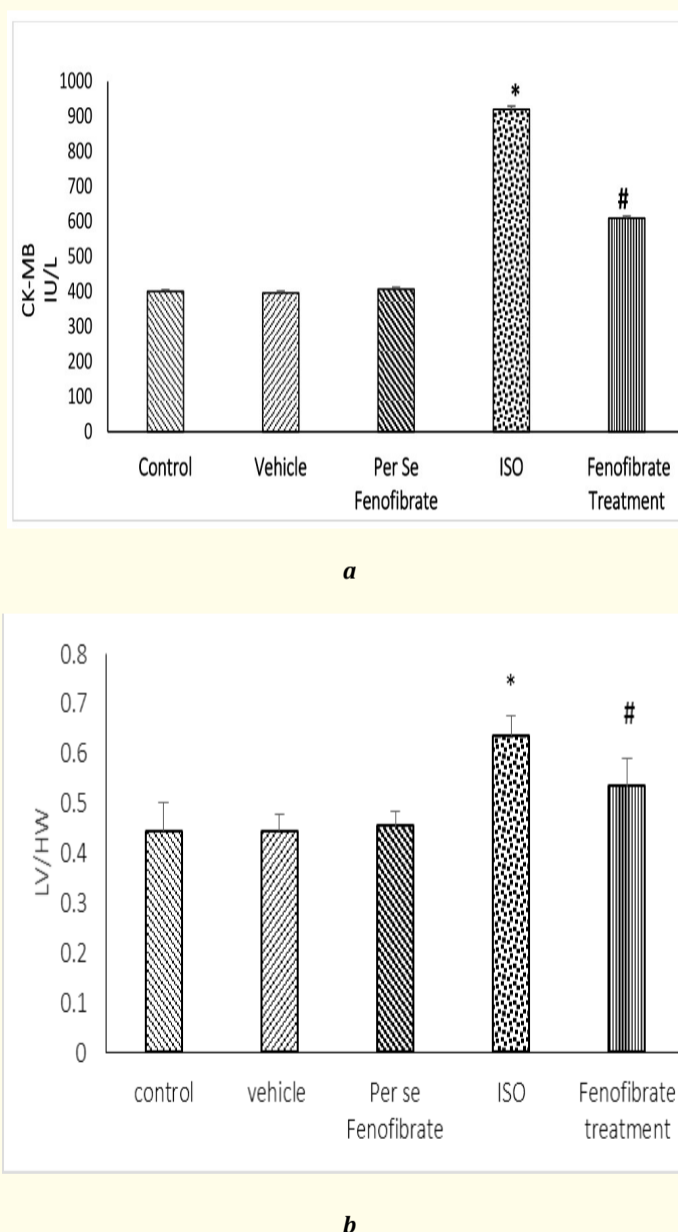
**Results**

No significant change in body weight was observed in animals of all the groups.

**Serum Marker of Cardiac injury and Left Ventricular Hypertrophy**

A marked elevation in serum markers of cardiac injury i.e. CK-MB was observed in ISO (P < 0.001) group as compared to normal control group (Figure 1a) signifying that ISO causes myocardial damage while treatment with fenofibrate significantly reduced the levels of CK-MB in rats administered with ISO indicating cardioprotective efficacy of fenofibrate. Fenofibrate per se and vehicle group did not show any significant changes as compared to control rats.

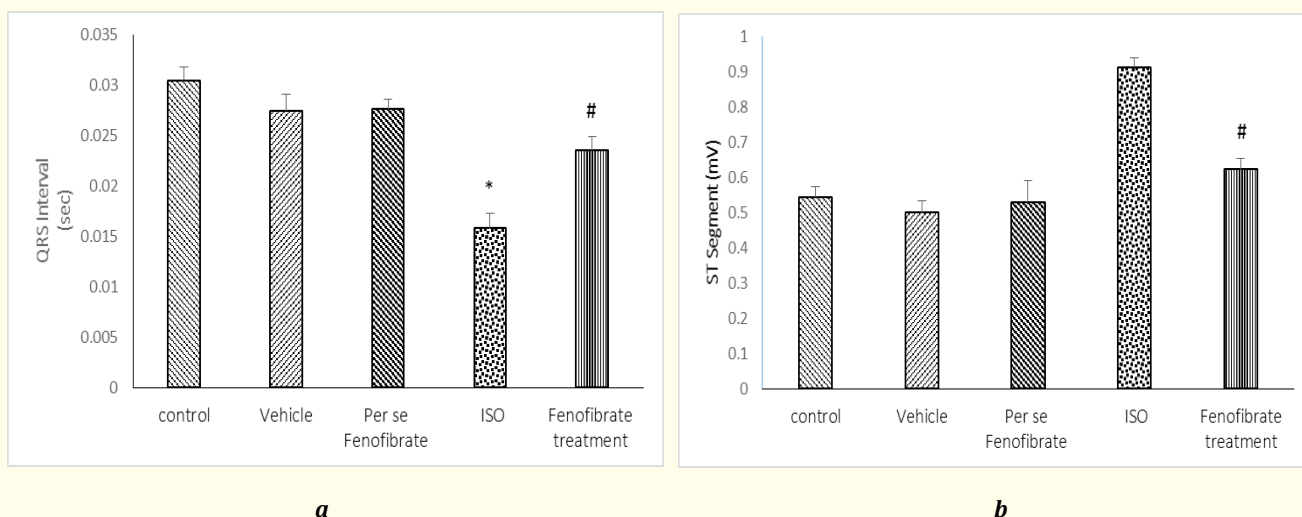
A significant increase in LV/HW ratio (Figure 1b) in comparison to control rats was noted indicating the development of left ventricle hypertrophy in ISO treated rats. Treatment with fenofibrate significantly attenuated the ISO induced ventricular hypertrophy. Fenofibrate per se and vehicle group showed no significant difference in LV/HW ratio as compared to control rats.



**Figure 1:** a) CK-MB serum levels. b) Left ventricular weight to heart weight ratio (LV/HW). Results represent mean ± SEM of ten animals per group. Results obtained are significantly different from control group (\*p < 0.05) and ISO (#p < 0.05) respectively.

**ECG Parameters**

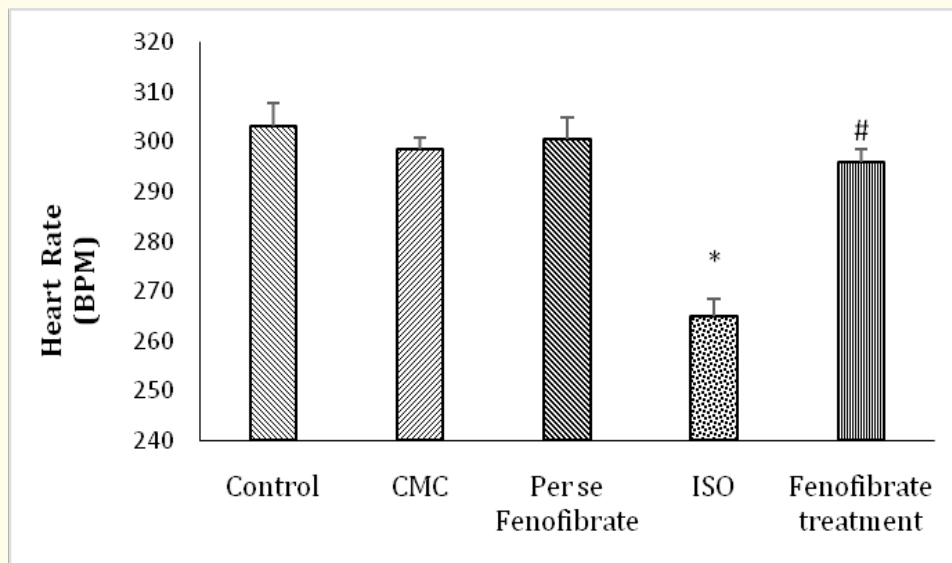
In ISO treated rats decline of QRS interval, ST segment elevation (Figure 2a and 2b), decrease in P amplitude and Q amplitude along with prolongation of QT interval and PR interval was observed in comparison to control rats (Table 1). The ST segment represents the interval between ventricular repolarisation and depolarisation and its elevation represents the ischemic and non-ischemic zones potential difference and the consequent loss of cell membrane function. While increase in QT and PR interval with a decrease in QRS interval in ISO rats reveals remarkable alteration in electrical activity occurring inside the myocardium of HF rats. Fenofibrate treatment significantly improved the ECG abnormalities suggesting its cardioprotective efficacy (Table 1). No significant change was observed in fenofibrate per se and vehicle group in comparison to control group. A significant decrease in HR was observed in ISO administered rats as compared to control group (Figure 3). Fenofibrate treatment in ISO administered rat restored the HR to normal. No significant change was observed in fenofibrate per se and vehicle group in comparison to control group.



**Figure 2:** a) QRS interval and b) ST segment. Results represent mean ± SEM of ten animals per group. Results obtained are significantly different from control group (\*p < 0.05) and ISO (#p < 0.05) respectively.

Group	P Amplitude (mV)	R Amplitude (mV)	PR Interval (s)	QT Interval (s)
Control	0.1012 ± 0.0051	1.6391 ± 0.2894	0.0279 ± 0.0035	0.0454 ± 0.0084
Vehicle	0.1083 ± 0.0066	1.5866 ± 0.1494	0.0315 ± 0.0036	0.0389 ± 0.0015
Perse Fenofibrate	0.1007 ± 0.0018	1.5941 ± 0.09794	0.0351 ± 0.0043	0.03937 ± 0.0013
ISO	0.0770 ± 0.0040*	0.7358 ± 0.0055*	0.0539 ± 0.0011*	0.0954 ± 0.0004*
Fenofibrate Treatment	0.0964 ± 0.0039#	1.1053 ± 0.0073#	0.0256 ± 0.00526#	0.0753 ± 0.0032#

**Table 1:** ECG Parameters: P amplitude, Q Amplitude, RR interval and QT Interval. Results represent mean ± SEM of ten animals per group. Results obtained are significantly different from control group (\*p < 0.05).and ISO (#p < 0.05) respectively.



**Figure 3:** Heart rate beats per minute (BPM). Results represent mean  $\pm$  SEM of ten animals per group. Results obtained are significantly different from control group ( $*p < 0.05$ ) and ISO ( $#p < 0.05$ ) respectively.

## Discussion

The results of the present study clearly demonstrate that the treatment with fenofibrate improves the altered cardiac electrical conduction, reduces cardiac injury and ventricular hypertrophy in ISO- induced HF rats suggesting its cardioprotective action.

In ISO induced HF, ISO causes overstimulation of  $\beta$  adrenergic receptor on the heart resulting in cardiac overwork leading to diffused myocardial necrosis and interstitial fibrosis in rats. The initial myocardial necrosis progresses to cardiac remodelling with left ventricular hypertrophy [5,15,18].

In the present study, rats administered with ISO showed significant increases in the levels of CK-MB in serum, which is in accordance with several previous studies, indicating ISO-induced necrotic damage of the myocardium and leakiness of the plasma membrane [19-21]. During myocardial injury or death, the myocardial cells containing CK-MB isoenzyme are damaged and the integrity of cell membrane gets disordered and it become more permeable or ruptures and CK-MB isozyme leaks out from myocardium into the blood stream, the amount of the enzymes appearing in serum is reported to be proportional to the number of necrotic cells [22], which reflects the alterations in plasma membrane integrity and/or permeability [23] as a response to  $\beta$ -adrenergic overstimulation [24]. Left ventricular hypertrophy is a compensatory response developed after myocardial injury occurs and due to other stress that increases myocardium workload [25]. Left ventricular hypertrophy is a process of remodeling of ventricle myocardial structure which cause deterioration of cardiac functioning and progresses into heart failure at the later stage [26]. In the present study ISO- administration resulted in the development of ventricular hypertrophy which is evident by increased LV/HW ratio. Fenofibrate treatment significantly lowered the ISO-induced increase in CK-MB levels, however could not restore it to the normal levels [27,28]. Fenofibrate also significantly restored the ISO- induced hypertrophy in HF rats suggesting that fenofibrate which is also a potent antioxidant restricts the leakage of enzymes as it helps in maintaining membrane integrity and a consequence reduces ventricular hypertrophy.

ISO treated rats showed disturbed ECG parameters in the form of reductions in the duration of QRS complex, prolongation of QT and PR interval. There was also significant elevation in the ST segment along with reduction in P and R amplitude. These ECG findings indicate disturbance in the conduction system of the heart caused by chronic overstimulation of  $\beta$  adrenergic receptor. Such abnormalities in the ISO-induced HF rats might occur due to ISO-induced generation of free radicals. Increased oxidative stress causes loss of cell membrane function leading to cardiac conduction disturbances [29]. The reduction in duration of QRS complex in ISO induced HF indicates shorter duration of ventricular depolarisation, and thus ventricular dysfunction. The PR segment and QT interval which were prolonged in ISO induced HF represents the prolonged period of AV conduction and electrical systole of the heart which is determined by inward  $\text{Na}^+$  and  $\text{Ca}^{2+}$  current and outward  $\text{K}^+$  and  $\text{Cl}^-$  currents [30] and dysfunctional functional integrity of the myocardium [7]. The QT interval prolongation in ISO induced HF may also be related to cardiac vagal dysfunction and represents cardiac toxic potential such as indication of arrhythmias, cardiac dysfunction and sudden cardiac collapse [31]. Elevation in ST segment represent ISO induced myocardial ischemia [32]. Fenofibrate treatment significantly restored the ISO induced elevation of ST-segment showing its cell membrane protecting effects. Treatment with fenofibrate also showed significant restoration of PR segment, QRS complex, QT interval along with P and Q wave and HR as compared to ISO group. Thus, fenofibrate showed a protective role against ISO-induced altered ECG pattern by protecting the cell membrane damage, and improving hypertrophy. Efficacy of fenofibrate to improve cardiovascular functions could be due to its antioxidant property. To understand the mechanism behind the beneficial effects of fenofibrate further studies are needed to be done [33].

### Conclusion

In conclusion, present study provides the experimental evidence that fenofibrate improves cardiac electric conduction in rat model of HF by reducing the levels of CK-MB, restoring the ventricular hypertrophy and improving the electrocardiographical alterations.

### Conflict of Interest

The author(s) declared no conflicts of interest with respect to the authorship and/or publication of this article.

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