

Original Article

## ISOPROTERENOL TOXICITY INDUCED ECG ALTERATIONS IN WISTAR RATS: ROLE OF HISTAMINE H<sub>3</sub> RECEPTOR AGONIST IMETIT

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### ABSTRACT

**Objective:** The study was performed to evaluate protective role of histamine receptor (H<sub>3</sub>R) agonist on isoproterenol induced alterations of electrocardiography (ECG) segments and heart rate of rat which are characteristic of myocardial infarction.

**Methods:** We administered positive control carvedilol (10 mg/kg), H<sub>3</sub>R agonist imetit (5 and 10 mg/kg), H<sub>3</sub>R antagonist thioperamide (5mg/kg), imetit (5 and 10 mg/kg) with combination of thioperamide (5mg/kg) and vehicle (normal saline) for 7 days, with concurrent subcutaneous injections of isoproterenol (85 mg/kg) at 24 h interval on last two consecutive days. We have also taken control and *per se* groups of imetit, carvedilol and thioperamide to compared ECG and heart rate changes with control rats.

**Results:** Isoproterenol induced cardiac dysfunction is evidenced by significant alterations ( $p < 0.01$ ) of ECG segments (ST, PR, QRS, QT and RR) and augmentation of heart rate. Imetit groups except IMT 5 T ISO significant ( $p < 0.01$ ,  $p < 0.05$ ) restored isoproterenol induced ECG segment alterations and heart rate. Carvedilol also attenuated isoproterenol induced ( $p < 0.01$ ) ECG and heart rate alterations as compared ISO control group. Thioperamide augmented isoproterenol induced ST segment with no significant effect on other ECG segment alterations and heart rate whereas *per se* groups of all treatment showed no alterations of ECG and heart rate as compared to control group.

**Conclusion:** The present study demonstrates cardioprotective potential of imetit by attenuating isoproterenol-induced ECG and heart rate alterations which reflects improvement in integrity of myocardium. It may be linked to explore cardioprotective potential of H<sub>3</sub>R agonist in further investigation.

**Keywords:** Isoproterenol, imetit, carvedilol, ST elevation, ECG alteration.

### INTRODUCTION

The major health challenge of 21st century is leading death cause in both developed and developing countries is cardiovascular disease (CVD). Its toll will increase to 25 million people by 2030 mainly from heart diseases such as myocardial infarction (MI) and stroke [1]. MI demonstrated as heart disease occurs as an oxygen imbalance between blood supply and myocardial demand which can be evaluated by electrocardiography (ECG) [2, 3]. Alteration of ECG segments reflects MI which is a definite criterion for diagnosis of either clinical or experimental MI [4].

Isoproterenol (ISO), synthetic non-selective  $\beta$ -adrenoceptor agonist catecholamine causes pronounced pathological changes have been substantially characterized in myocardium like necrosis, fibrosis, migration of leukocytes and cell permeability [4]. It causes severe stress and necrosis similar to patient of MI [3]. ISO pronounced abnormality in rat ECG is characterized by 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 function of heart [5, 6]. It was found that  $\beta$ -blockers significant in declining the effect of ISO induced alterations.

Histamine receptor (H<sub>3</sub>R) presynaptic autoreceptors are abundantly found in cardiac tissue [7]. In previous study, H<sub>3</sub>R agonist imetit have shown cardioprotective effect by diminishing norepinephrine (NE) release in heart by a number of pathways which is main cause of cardiac dysfunction where as thioperamide aggravate the heart failure [8-10]. ISO raises myocardium sympathetic discharge which on auto-oxidation alters integrity of myocardium consequently change in ECG [11]. So, Imetit could have a protective role in ISO induced ECG changes in wistar rat.

Previous study revealed its protective action by in-vitro study, so this in-vivo study was designed is done to correlate its protective role in wistar rat by showing effect on ISO induced ECG variables and heart rate.

### MATERIALS AND METHODS

#### Laboratory animals

Male wistar rats were obtained from Central Animal House, Jamia Hamdard, New Delhi having body weight 180–200 g and age 6 weeks. They were maintained on standard conditions such as a natural dark light cycle controlled room (25±2°C temp, 50±10% humidity) and feeding rat chow with water *ad libitum*. Ethical clearance for handling the animals was obtained from the Institutional animal ethical committee prior to the beginning of the research work (Proposal number: 677/2010), according to prescribed guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), under the Ministry of animal welfare division, Government of India, New Delhi.

#### Chemicals procurement

Imetit dihydrobromide, thioperamide maleate and isoproterenol hydrochloride were purchased from Sigma Aldrich Chemical Co., (St Louis, MO, USA). Carvedilol was procured as a gift sample from Cadila Pharmaceuticals, Gujarat (India).

#### Experimental design

Wistar albino rats were randomly assigned into eleven groups each containing eight animals. All groups animal were treated with oral dose of vehicle or drug (2ml/kg, 1% Sodium CMC suspension in normal saline) for 7 days whereas ISO groups were administered with ISO subcutaneous (s.c.) (85 mg/kg) at an interval of 24 h for last two consecutive days. The treatment schedule was as follows; Group-I normal control group (normal saline), Group-II ISO negative control group (vehicle for 7 days and then ISO for last two consecutive days), Group-III CRVD 10 *per se* positive control group (carvedilol orally for 7 days), Group-IV CRVD 10 ISO positive control group (carvedilol orally for 7 days & ISO), Group-V IMT 10 *per se* group (Imetit 10 mg/kg, pod. for 7 days), Group-VI IMT 10 ISO

group (Imetit 10 mg orally for 7 days & ISO), Group-VII IMT 10 T ISO group (Imetit 10 mg/kg and thioperamide 5 mg/kg orally for last 7 days with ISO), Group-VIII IMT 5 ISO group (Imetit 5 mg orally for 7 days and ISO), Group-IX IMT 5 T ISO group (Imetit 5 mg/kg, p.o. with thioperamide 5 mg/kg p.o.) for 7 days & ISO), Group-X THIO *per se* group (Thioperamide 5 mg/kg orally for 7 days) and Group-XI THIO ISO group (Thioperamide 5 mg/kg p.o. for 7 days with ISO).

#### Induction of myocardial injury

Isoproterenol was dissolved in normal saline and injected s.c to rats (85 mg/kg) for last two consecutive days at an interval of 24 h (i.e., on 6<sup>th</sup> and 7<sup>th</sup> day of treatment) to induce experimental myocardial infarction having ECG alterations [6].

#### Experimental Studies

##### Measurement of ECG and heart rate

Briefly, rats were anesthetized with light diethyl ether at the end of experimental period (after 24h of second ISO injection or 8th day of group/vehicle treatment) and leads were connected to the dermal layer of both front paws and hind legs to Powerlab data acquisition system (4/25, AD Instrument, Bella Vista, Australia) for the measurement of ECG and Heart rate (HR) [12].

##### Statistical analysis

Descriptive statistics such as mean and standard error of mean (SEM) was calculated for all variables for each group.

One-way analysis of variance (ANOVA) was applied for statistical analysis followed by Dunnett's test. A p value < 0.05 has been considered as statistical significance level.

#### RESULTS

##### ECG parameters estimation

##### ST segment evaluation

ISO negative control animals showed significant ( $p < 0.01$ ) ST segment elevation as compare to normal control group. The ST-segment elevation represents the ischemic and non-ischemic zones potential difference and the consequent loss of cell membrane function. Pretreatment of positive control group carvedilol (CRVD 10 ISO group) showed significant ( $p < 0.01$ ) cardioprotection with more than 50% decline in ST segment elevation of ECG as compared to ISO group.

Treatment groups such as IMT 10 ISO, IMT 5 ISO, IMT 10 T ISO except IMT 5 T ISO showed cardioprotection with decline in ISO induced ST elevation in ECG as compared to ISO control group ( $p < 0.01$ ). THIO ISO showed significant elevation of ST segment ( $p < 0.05$ ) as compared to ISO positive control group whereas *per se* groups of IMT 10, THIO and CRVD showed no significant ( $p > 0.05$ ) ST segment alteration as compared to normal control group (Table 1, Fig 1).

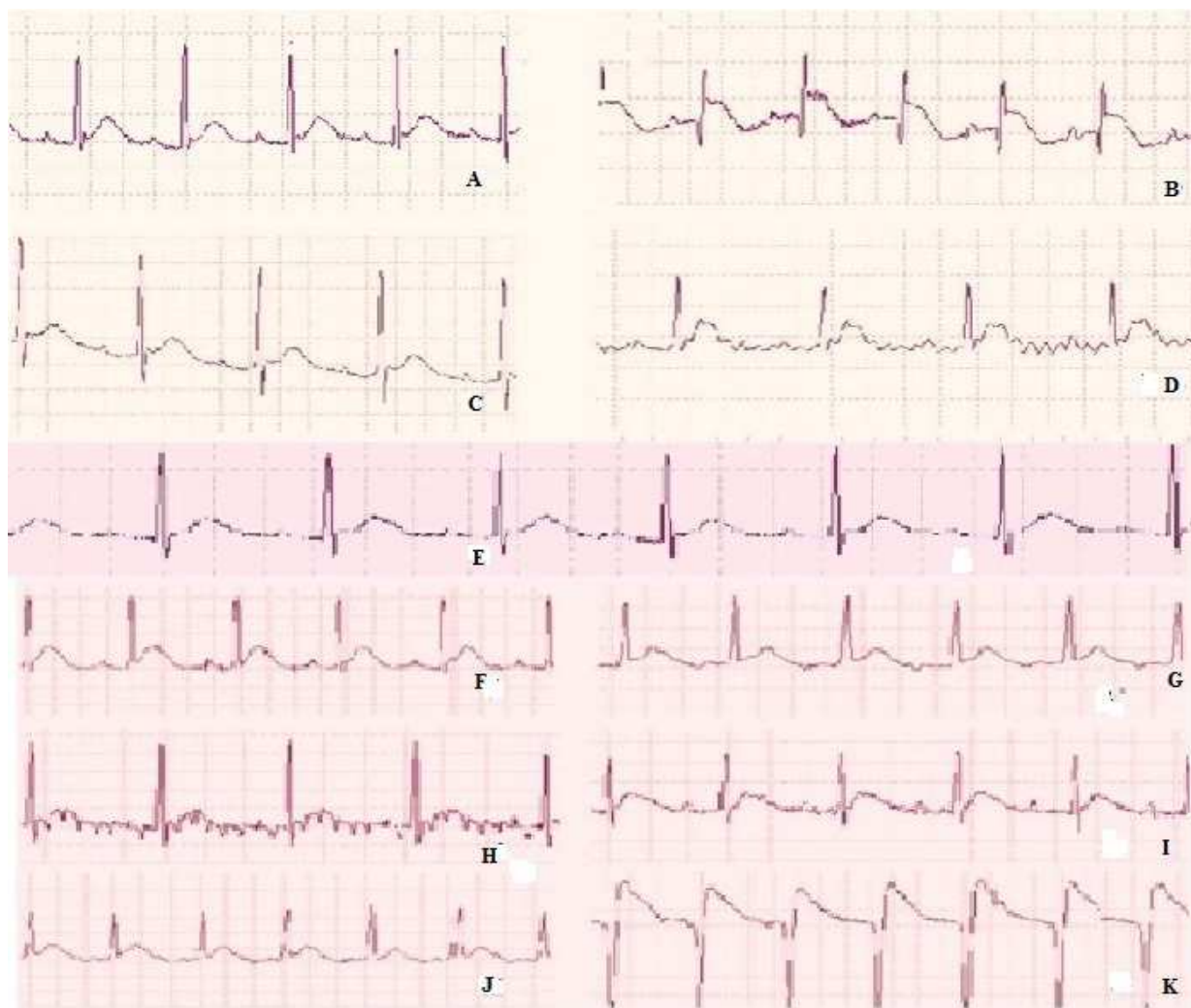
Table 1: Effect of Imetit on ISO induced ECG alteration in wistar rat

Treatment	Electrocardiography				
	ST elevation (mv)	Measurement PR segment (m sec)	QRS complex (m sec)	QT interval (m sec)	RR interval (m sec)
Normal control	0.176 ± 0.0034	31 ± 0.00041	432 ± 0.004	67 ± 1.21	162 ± 2.42
ISO negative	0.332 ± 0.0058##	22 ± 0.00032##	311 ± 0.0023 ##	81 ± 2.21##	144 ± .34##
CRVD positive <i>per se</i>	0.180 ± 0.0031 @	30 ± 0.0003 @	425 ± 0.0023@	64 ± 24.1@	166 ± 2.67@
CRVD positive ISO	0.236 ± 0.0043**	26 ± 0.00025*	385 ± 0.0043**	72 ± 1.68**	157 ± 2.35**
IMT 10 <i>per se</i>	0.169 ± 0.0026@	30 ± 0.00035@	417 ± 0.0028 @	65 ± 2.42@	167 ± 2.42@
IMT 10 ISO	0.248 ± 0.0037**	26 ± 0.00036*	362 ± 0.0035**	71 ± 2.51**	157 ± 1.86**
IMT 10 T ISO	0.267 ± 0.0039**	24 ± 0.00040@	343 ± 0.0029*	75 ± 2.43**	151 ± 2.73*
IMT 5 ISO	0.257 ± 0.0028**	25 ± 0.00027 *	342 ± 0.0027*	73 ± 2.34**	153 ± 1.91*
IMT 5 T ISO	0.296 ± 0.0052\$	23 ± 0.00025\$	332 ± 0.0034 \$	79 ± 2.61\$	148 ± 2.21\$
THIO <i>per se</i>	0.189 ± 0.0025@	28 ± 0.00043@	412 ± 0.0029 @	70 ± 1.12 @	157 ± 1.57@
THIO ISO	0.352 ± 0.0054 *	22 ± 0.00037\$	301 ± 0.0032 \$	83 ± 1.98 \$	141 ± 2.31\$

Values are expressed as mean ± SEM of eight animals.  $p < 0.01$ , ##;  $p < 0.05$ , #; Significant compared with control group,  $p > 0.05$ , @; Non-significant compared with control group,  $p < 0.01$ , \*\*;  $p < 0.05$ , \*; Significant compared with ISO control group,  $p > 0.05$ , \$; Non-significant compared with ISO control group

Table 2: Effect of Imetit on ISO induced Heart rate (HR) in wistar rat.

Treatment Groups	Heart rate measurement Heart Rate
Normal control	380.47 ± 8.70
ISO negative control	423.34 ± 25.45##
CRVD positive <i>per se</i>	368.34 ± 31.34 @
CRVD positive ISO	378.73 ± 30.43**
IMT 10 <i>per se</i>	371.52 ± 27.13@
IMT 10 ISO	387.35 ± 26.43**
IMT 10 T ISO	401.74 ± 32.31*
IMT 5 ISO	394.24 ± 30.08**
IMT 5 T ISO	413.43 ± 26.38\$
THIO <i>per se</i>	386.23 ± 24.83@
THIO ISO	432.12 ± 35.67\$



Effect on imetit on ISO induced ECG segment in wistar rat

Fig. 1: reveals ECG evaluation of different groups: A). Control, B). ISO control, C). CRVD *per se*, D). CRVD 10 ISO, E). IMT 10 *per se*, F). IMT 10 ISO, G). IMT 10 T ISO, H). IMT 5 ISO, I). IMT 5 T ISO, J). THIO *per se*, K). THIO 10 ISO.

#### Segment PR, ORS, QT and RR evaluation

ISO negative control group showed significant decline ( $^{###}p < 0.01$ ) of PR, QRS & RR segment and prolongation of QT segment as compared to control group. CRVD 10 ISO pretreatment showed significant cardioprotection with restoration of PR, QRS, QT, and RR segment as compared to ISO negative control group. Treatment groups of imetit except IMT 5 T ISO showed cardioprotection by restoration of changes in ISO induced ECG segments as compared to ISO group ( $^{**}p < 0.01$ ,  $^{*}p < 0.05$ ). *Per se* groups of CRVD, THIO and IMT 10 showed no altered sign of ECG as compared to control group. Furthermore THIO ISO showed no alteration in ECG as compared to ISO control group. The data of the experimental study such as PR segment, QRS complex, QT interval and R-R interval are shown in (Table 1, Fig 1).

#### Effect on heart rate

ISO administration induced a significant rise in HR in ISO control group as compared to control group ( $^{###}p < 0.01$ ). Imetit groups pretreatment such as IMT 10 ISO, IMT 5 ISO, IMT 10 T ISO except IMT 5 T ISO restored HR significantly ( $^{**}p < 0.01$ ) in comparison to ISO control rats. *Per se* groups of CRVD, THIO and IMT 10 showed no significant changes in HR as compared to control where as CRVD 10 ISO found significant by attenuating HR as compared to ISO control group. THIO ISO group showed no significant alteration in HR as compared ISO positive control group (Table 2).

Values are expressed as mean+ SEM of eight animals:  $p < 0.01$ ,  $^{##}$ ; Significant compared with control group,  $p > 0.05$ , @; Non-significant compared with control group,  $p < 0.01$ ,  $^{**}$ ;  $p < 0.05$ ,  $^{*}$ ; Significant compared with ISO control group,  $p > 0.05$ ,  $^{\$}$ ; nonsignificant compared with ISO control group.

#### DISCUSSION

The prime objective was to explore the protective role of  $H_2R$  agonist imetit pre-treatment on ISO induced ECG alteration characteristics of mild heart failure / cardiotoxicity in wistar rats. ISO, a synthetic  $\beta$ -adrenergic agent, causes ischemic necrosis by alteration of membrane integrity, sympathetic discharge of NE and marked inotropic and chronotropic actions resulting in greater oxygen demand [13]. Experimental ISO-induced MI model in which ISO, a  $\beta$ -adrenergic (85 mg/kg s.c for last two consecutive days of treatment) produces oxidative stress via formation of adrenochromes through its auto-oxidation, implicated for generation of highly toxic oxygen derived free radicals [14]. It causes ischemic necrosis leads alteration in ECG segments thereby causes several physiological and functional changes in heart [6] and resembles to the syndromes of human MI and sudden death in humans [3, 8]. Previous studies reported for imetit cardioprotective effects through inhibition of catecholamine amine (NE, higher discharge in cardiac tissue leads heart failure) exocytosis by a number of pathways [8-10].

Previous studies showed significant ISO induced alteration of rat ECG as compared to normal control group [11]. ECG ST-segment elevation in patients with acute myocardial ischemia is similar characteristic in ISO-induced experimental MI in rats [5, 6]. It is characterised by consecutive loss of cellular membrane fluidity, permeability and damage due to oxidative stress in injured myocardium [6].

The other characteristic findings of ISO-induced MI are attenuation in the PR segment, QRS complex, RR interval and prolongation of QT interval. The PR segment of ECG represents AV conduction where as QRS shows total duration of ventricular depolarisation, their alteration reflects abnormality of heart function. The QT interval represents ventricular repolarisation and determined by inward sodium and calcium current and outward potassium and chloride currents [4]. It also represents the period of electric systole of which is a method of determining the functional integrity of the myocardium [6].

The QT interval prolongation related with cardiac vagal dysfunction represents cardiac toxic potential such as indication of arrhythmias, cardiac dysfunction and sudden cardiac collapse [18]. In this study, negative control group i.e ISO group showed alterations in ECG variables and HR. Imetit administration showed a protective role against ISO-induced altered ECG pattern and eliminated the acute fatal complications by protecting the cell membrane damage. *Per se* groups of IMT 10, CRVD and THIO showed no alteration in integrity of myocardium reflected by normal ECG segments. ISO showed attenuation in R-R interval (which reflects myocardial oedema and membrane integrity) and raised heart rate of wistar rat [6].

We observed pretreatment with imetit except IMT 5 T ISO markedly diminished ISO induced elevation of ST-segment showed its cell membrane protecting effects. H<sub>3</sub>R agonist imetit groups except IMT 5 T ISO group pretreatment showed cardioprotection by significant restoration of PR segment, QRS complex, R-R interval and QT interval as compared to ISO control group. By restoring these parameters, imetit action might be related for maintaining integrity of cellular membrane. Whereas H<sub>3</sub>R antagonist Thioperamide diminished the protective effect in IMT 5 T ISO and augment ISO induced cardiac failure with ECG alterations. Thioperamide showed significant ISO induced ST-segment elevation reflects pathologic alteration in cell membrane integrity. However thioperamide found no significant alteration in other ECG parameters. Reference drug carvedilol administration showed its cardioprotective effects by significant attenuating ISO induced ECG alterations. It may be due to its antioxidant property which maintained integrity and permeability of cellular membrane [19-22]. In wistar rat, ISO raised HR which causes cardiac dysfunction and contractile failure in heart as reported in other experimental studies [23]. It may be due to deterioration of mitochondrial energetic and suppression of Ca<sup>2+</sup> transport which causes intracellular Ca<sup>2+</sup> overload and also deranged sympathetic and parasympathetic input to heart [24]. Another H<sub>3</sub>R agonist R- $\alpha$  methyl histamine (R- $\alpha$  MH) has been reported to possess sympatho-inhibitory action by attenuating HR that suggest it's potential clinical use in condition associated with myocardial ischemia or heart failure [25]. By attenuating HR, imetit similar to carvedilol showed its cardioprotective effect as increased HR is responsible for augmented oxygen consumption increased work load which leads to myocardial necrosis. *Per se* groups of carvedilol, imetit and thioperamide showed no significant alteration in rat ECG and HR.

Previous studies of imetit showed its cardioprotection by linking a number of pathways related to attenuate sympathetic discharge. Present study findings shed light on imetit protective role in ISO induced myocardial infarcted rats by restoring ECG parameter alterations and maintains integrity of cellular membrane.

## CONCLUSION

This study reveals cardioprotective potential of imetit which emphasizes role of H<sub>3</sub>R in ischemic injury. This in-vivo study explore new scientific information will help to link cardioprotective potential in further studies related to imetit and other H<sub>3</sub>R agonists.

## Declaration of Conflicting Interests

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

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