Exploration of proarrhythmogenic potential of rabeprazole in rat model

Thesis submitted in partial fulfilment of the requirement

for the degree of Master of Pharmacy

Under the guidance of

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Submitted by

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CERTIFICATION

This is to certify that Akash De, Final year Masters of Pharmaceutical Technology (M. Pharm) examination student of Department of Pharmaceutical Technology, Jadavpur University, Class Roll No. 002111402035, Registration No. 160263 of 2021-2022, Examination Roll No M4PHL23016 has completed the Project work titled, "**Exploration of proarrhythmogenic potential of rabeprazole in rat model"** under the guidance of Prof. Sanmoy Karmakar during his Master's Curriculum. This work has not been reported earlier anywhere and can be approved for submission in partial fulfilment of the course work.

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Declaration of Originality and Compliance of Academic Ethics

I hereby declare that this thesis contains literature survey and original research as part of my work on "**Exploration of proarrhythmogenic potential of rabeprazole in rat model**". All information in this document have been obtained and presented in accordance with academic rules and ethical conduct. I also declare that as required by these rules and conduct, I have fully cited and referenced all materials and results that are not original to this work.

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Dedicated to

My loving parents

and

My Lab-family

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INTRODUCTION

Although several aspects connected to cardiac arrhythmia have been thoroughly explored, arrhythmia still poses a challenge for medical professionals and researchers. There has been an increase in awareness of electrolyte imbalances and their impact on arrhythmia in recent decades(1) (2).Such research mostly focuses on the negative effects of long-term high potency diuretic usage(3). Furosemide (FUR), a routinely prescribed high potency diuretic, caused substantial hypokalaemia in experimental male Wister rats even at a dosage of 10 mg.kg-1, according to a previous publication. Higher doses of FUR may make laboratory rats more likely to get metabolic alkalosis, according to Akita et al. Thus, we believe that using our dosage of FUR to cause experimental hypokalaemia in the aforementioned animals is probably appropriate in order to reduce the likelihood of alkalosis. Since the early 1990s, drug-induced QT interval prolongation has been studied. More than 50 non-cardiac medications are known to lengthen the QT interval at this time. Numerous antimicrobials, particularly the oral macrolide family of antibiotics, are notorious for lengthening the cardiac repolarization time and are linked to case reports of arrhythmia. One such macrolide medicine that has undergone substantial research about its propensity to lengthen QT interval and is a known arrhythmogen is clarithromycin (CLA). Different regulatory agencies have established preclinical and clinical methodologies to evaluate the possible risk of QT interval prolonging of non-cardiac medicines since QT/QTc is a surrogate measure for identification of arrhythmogenesis. Finally, in 2005, the US-FDA made the cardiac assessment test a necessary component of the NCEs clinical evaluation.

While the primary focus of these recommendations is on evaluating novel agents potential to cause arrhythmias during clinical trials prior to marketing, there is always room for concern regarding already-approved medications for which such worries were not considered when they were approved. We believe that there is a connection between strong safety margin' and producing a long QT/QTc interval in the ECG in this situation. 'Strong safety margin' was preserved in the electrophysiology of cardiac myocytes, according to one of Biliczki's findings. He asserts that a network of many channels

together tightly regulates ventricular repolarization. Under typical circumstances, failure of repolarization will not always come from impairment or blockage of one type, but rather from repolarization reserve. However, he demonstrated that proarrhythmic lengthening of the ventricular APD occurs if this repolarization reserve is reduced by total potassium current suppression. In our earlier work, it was shown that administering FUR (10 mg kg-1) and CLA (80 mg kg-1) separately did not significantly alter QTc, but that doing so together caused a noticeable alteration in QT interval. In this situation, we believe that if we can artificially lower the potassium current by inducing hypokalemia, we can reduce the so-called "strong safety margin" by using a titrated dosage of an established arrhythmogen. Therefore, it was intriguing to look into the potential impact of a test agent on repolarization after the safety margin is reduced. We also believed that weaker QT prolongation tendencies of the test medication in experimental animals, which are otherwise not measurable by the present methodologies, may be exacerbated.

Aim and objective of Work

1. Aim

To explore the proarrhythmogenic potential of rabeprazole in rat model.

2. Objective

I. To evaluate of Rabeprazole effects by measuring certain ECG parameters like- QT interval, RR interval, Corrected QT.

II. To measure serum electrolytes like magnesium, potassium.

Literature Review

The potentially fatal rhythm known as Torsades de Pointes (TdP) is linked to the extended QT interval, a common occurrence. Many different medications have been linked to prolonging the QT interval, despite the fact that it can happen spontaneously in the congenital form. Due to the rise in deadly polymorphic ventricular tachycardia, some of these medications have either been placed under restrictions or removed off the market. A current list of particular medications that lengthen the QT interval may be obtained at credible meds. The list of medications that induce QT prolongation is always expanding. The mechanism of drug-induced QT prolongation, risk factors for TdP, offending medicines, prevention and monitoring of prolonged drug-induced QT prolongation, and therapeutic approaches are the main topics of this study.

Electrical potential in nodal tissue:

The resting membrane potential of pacemaker (SA node) fibers is only between -55 and -60 mV, and it is not constant; rather, it slowly increases as gradual depolarization occurs. This gradual depolarization makes it take a very long time to reach the threshold level of -40 mV. quick depolarization up to +5 mV and quick repolarization, or action potential and impulse production, happen as soon as the threshold level of 40 mV is met. Once the resting membrane potential (phase 4 of the action potential) is attained following fast repolarization (phase 3 of the action potential), it is not stable and slowly rises to reach the threshold level necessary to generate the second impulse.

Ionic basis of pacemaker potential and action potential in SA node:

The myocardial cells present in the SA node and AV node are called slow fibres depending on the membrane potential and the shape and conduction velocity of the action potential.

1. A distinctive characteristic of the pacemaker tissue's slow fibers is that their resting membrane leaks sodium ions (as opposed to the resting membrane of fast fibers, which is mostly resistant to Na +). Under resting conditions, this results in a gradual diffusion

of Na + into the SA nodal fibers. Because non-selective channels are present, the slow entrance of Na + into the cells gradually elevates the potential to -55 mV (causing slow depolarization). The first portion of the pacemaker potential is formed by this gradual depolarization. Due to their unique activation during hyperpolarization, which is linked with enhanced permeability to both sodium and potassium, nodal tissues also have some funny (f) channels. However, sodium conductance has the dominating influence. These channels are also known as "h" channels since become active when the membrane is hyperpolarized (between -40 and -60 mV).

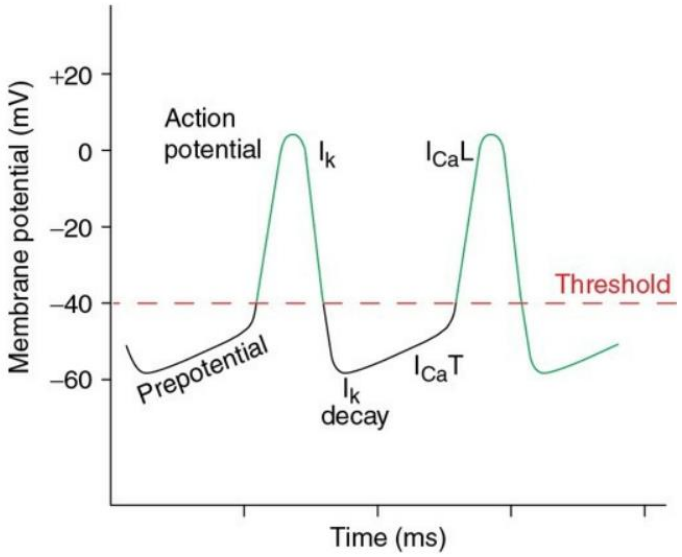


Fig.1 Pacemaker potential and its ionic basis. IcaT: Ca 2+ conductance through transient calcium channels; IcaL: Ca 2+ conductance through long lasting channels; Ik: potassium conductance

2. After then, the 'T' (transient) calcium channels open, allowing a sluggish inflow of Ca^{2+} to cause additional depolarization at a slower pace, up until a threshold level of -40 mV is achieved. The latter portion of the pacemaker potential is therefore formed by the calcium current (Ica) brought on by the opening of "T calcium channels.".
3. When the "long lasting calcium channels" open at the threshold level (-40 mV), the action potential begins with a fast depolarization caused by an influx of Ca 2+. It is crucial to remember that Ca 2+ rather than Na + is the primary cause of the depolarization in the SA node. As a result, the depolarization is less abrupt than it is in other cardiac fibers.

4. Calcium channels close and potassium channels open as depolarization comes to an end. K+ diffuses out of the fibers as a consequence, causing a fast repolarization to between -55 and -60 mV.
5. Once more, because of the special property of the SA node's slow fibers (leaking of the resting membrane to Na+), the resting potential does not become stable. Instead, gradual depolarization begins as a result of the slow inflow of Na +, which forms the initial portion of the prepotential. And finally, new action potential is started as a result of repeating the previously outlined stages. In this approach, impulses (autorhythmicity) are produced at regular intervals of time.

Electrical potentials in cardiac muscle

Resting membrane potential

A typical cardiac muscle fiber's resting membrane potential (RMP) ranges from -85 to -95 mV (negative interior with respect to outside).

Action potential:

Each cardiac muscle fiber exhibits electrical activity known as a propagated action potential when it is activated. It differs from an electrocardiogram, which is an extracellular recording of the electrical activity generated by each heartbeat in all cardiac muscle fibers. A single cardiac muscle fiber's action potential is extremely lengthy and may be broken down into the following five separate phases:

1. **Phase 0: Rapid depolarization:**

Depolarization that occurs quickly during phase 0 (upstroke) is what distinguishes it from other phases. Skeletal muscle and nerves both exhibit overshoot during this phase. Depolarization lasts for around 2 ms in mammalian hearts. The potential amplitude in this phase might be as high as +20 to +30 mV (positive interior with respect to outside). base of ions. The quick opening of voltage-gated Na + channels and the rapid influx of Na + ions, which occur similarly to what happens in nerve and skeletal muscle, are what cause the initial rapid depolarization and the overshoot. The calcium channels also open up at membrane potentials of -30 to -40 mV, and the influx of Ca 2+ ions also play a role in this phase.

2. **Phase 1:**

Initial rapid repolarization: A very brief, modest, fast repolarization occurs after a rapid depolarization. During this phase, the membrane potential ranges from +30 mV to -10 mV.

Ionic basis. Na + channel closure and K+ channel opening cause the initial rapid repolarization, which results in a transient outward current.

3. **Phase 2, Plateau:**

   The heart muscle fiber stays depolarized during the plateau phase. During this phase, the membrane potential only slowly decreases to -40 mV. Plateau phase occurs for about 100–200 ms. The 5–15 times longer cardiac muscle contraction time than skeletal muscle can be attributed to this plateau in action potential.

Ionic basis. The slow pace inflow of Ca 2+ ions brought on by the opening of sarcolemmal L-type Ca 2+ channels and the closure of a particular subset of K+ channels known as the inward-rectifying K+ channels are the two causes of the plateau phase's extremely slow repolarization.

4. **Phase 3:**

   Repolarization: Complete repolarization takes place during this phase, and the membrane potential drops to around 80 mV, which is the resting state. This phase lasts for about 50 ms.

Ionic basis: The Ca 2+ channels close, and the following K+ channels open, causing a gradual repolarization. Delayed outward rectifying K+ channels are voltage-gated K+ channels that are activated very slowly. There are two types of rectifying K+ channels: rapid delayed rectifying K+ channel (Ikr) and slow delayed rectifying K+ channel (Iks). Ikr is responsible for initial phase of phase 3 repolarization, whereas late phase of phase 3 repolarization is controlled by Iks.

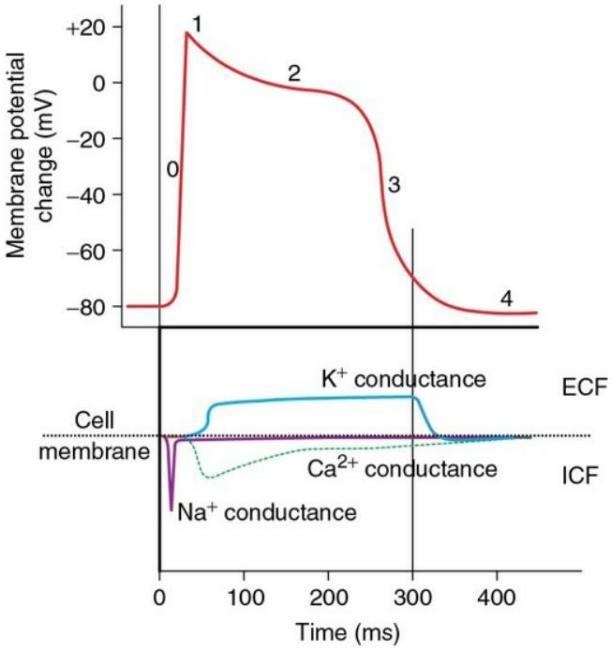
5. **Phase 4:**

   Resting potential: In this phase of RMP (also called as polarized state), the potential is maintained at −90 mV.

Ionic basis: The inward rectifying K+ current is the principal contributor to the resting K+ current, which maintains the RMP.

Duration of action potential:

At a heart rate of 75 beats per minute, the action potential (mainly repolarization) lasts around 250 ms. With a higher heart rate, the action potential's duration shortens (150 ms at 200 beats per minute). In the atrial muscle, it is shorter. Fast reaction action potentials are those that are present in the Cardiac myocyte cells of the ventricles.



Various phases of action potential and ion conductance: Phase 0 = depolarization; Phase 1 = rapid repolarization; Phase 2 = plateau phase; Phase 3 = late rapid repolarization and Phase 4 = resting potential.

Physiology of the QT interval and the mechanism of QT drug-induced prolongation

the surface-level QT interval EKG is made up of the ventricular myocytes action potentials (AP). The action potential is a reflection of the movement of ion currents across a cell membrane via specific protein complex-based channels. These protein channel malfunctions can cause either an increase in inward current or a decrease in outward current. As a result, the action potential duration lengthens, lengthening the QT interval.

Congenital long QT syndrome (LQTS) is caused by mutations in the IKr, IKs, and Na protein channel genes (4). The inward potassium rectifier (IKr) channel, commonly referred to as the hERG (ether a go go) channel, is virtually invariably blocked in acquired LQTS. It conducts a quick delayed rectifier potassium current (Ikr), which is important for the cardiac action

potential's phase 3 repolarization (5). Type 2 LQTS is brought on by inherited mutations (loss of function) of the hERG gene. The same hERG channel is affected by medications that lengthen the QT interval. The hERG channel has a unique molecular structure that makes it more drug-sensitive.

The structure of the hERG channel

The bacterial and mammalian K channel structures provide a solid foundation for understanding the structure of the hERG channel (6). The co-assembly of four alpha subunits, each of which includes six transmembrane spanning alpha-helical segments (S1-S6), is essentially what creates the hERG channel (6,7). Each segment has a voltage sensor domain (VSD) made up of the first four helices (S1–S4) that detects the transmembrane potential. The pore domain, which is made up of a short alpha helix (pore helix) and selectivity filter, is formed by the following two helices, S5 and S6. The movement of the potassium current is controlled by a central pore that is formed by four of them (one from each subunit). The pore opens out below the selectivity filter to create a central hollow. Many distinct aromatic residues that are missing from the majority of other K channels line its inside. The distinct binding sites for various pharmacologic drugs are heavily reliant on these strategically placed polar and aromatic residues (8–10). Drugs like arsenic oxide, pentamidine, and fluoxetine can disrupt KCNH2 protein trafficking and cause the loss of K channels. Cisapride can rescue the SCN5A channel and cause an increase in inward sodium current. Antimony can increase inward calcium current.

Table 1: Cardiac potassium (K+) channels in human cardiomyocytes include: (11)

Sl. No	Types of K^+ channel	Function
1	Transient outward K+ current (I(to1))	This channel contributes to the early phase of repolarization of the cardiac action potential.
2	Ultra-rapidly activating delayed rectifier current (IKur)	This channel is primarily found in atrial myocytes and contributes to the repolarization of the atrial action potential
3	Rapidly and slowly activating delayed rectifier currents (IKr) and I(Ks)	These channels contribute to the late phase of repolarization of the cardiac action potential.
4	Inward rectifier K+ current (IK1))	This channel helps to maintain the resting membrane potential of the cardiomyocyte.
5	Adenosine-5'-triphosphate (ATP)-sensitive K+ current (IKATP)	This channel is activated during metabolic stress, such as ischemia, to shorten the action potential duration and reduce energy consumption.
6	Acetylcholine-activated current (IKACh)	This channel is activated by acetylcholine and contributes to the slowing of the heart rate during parasympathetic stimulation.

Repolarization reserve

The concept of repolarization reserve refers to the hearts ability to maintain repolarization when faced with challenges, like genetic mutations, drugs or environmental factors. It helps explain

why some people can handle conditions that affect repolarization without developing arrhythmias while others do experience them. The process of repolarization in the heart is intricate. Involves ion currents. If one current is affected others can. Provide a capacity for repolarization acting as a "reserve". However when this reserve is diminished the risk of arrhythmias increases. Repolarization reserve plays a role in conditions such as Long QT syndrome where there can be QT prolongation due to genetic or environmental factors. When the repolarization reserve is reduced in these cases it heightens the chances of developing arrhythmias [(12)].

Research has shown that individuals with heart disease tend to have less repolarization reserve compared to healthy individuals without any heart issues (13). Additionally studies have also highlighted that humans typically have a low repolarization reserve compared to dogs emphasizing how species specific factors influence the extent of their repolarization abilities(14). Understanding the concept of repolarization reserve becomes crucial when considering drug induced proarrhythmia. Some medications can impair one or more currents, for repolarizing the heart. If existing repolarization reserve of any individual is insufficient this disruption caused by drugs could lead to arrhythmias(15).

Transmural dispersion of repolarization and T wave generation

Transmural dispersion of repolarization (TDR) across the ventricular wall is defined by the difference in repolarization time (activation time +action potential duration [APD]) between the M cell and the epicardial cell.

The morphology of the T wave is thought to be a function of two opposing transmural currents as a result of the development of opposing voltage gradients during repolarization: between the epicardium and the M-cell region and between the M-cell region and endocardium, according to data from the arterially perfused left ventricular wedge preparation. The magnitude of the transmural currents is also modulated by the high tissue resistivity present between the epicardium and the M area. The end of the T wave in an ECG always occurs at the same time

as the repolarization of the longest M-cell action potential, whereas the peak of the T wave always occurs at the same time as the repolarization of the shortest epicardial action potential. Endocardial action potential repolarization typically falls somewhere between that of M cells and that of epicardial cells. Contrarily, repolarization of the subendocardial Purkinje fibers always lasts longer than that of the M cells, indicating that repolarization of Purkinje cells does not help the T waves manifest. The different T wave morphologies were attributed to the interaction of the three electrically distinct cell types that are found in the ventricular wall (epicardium, M, and endocardium), and it was hypothesized that LQTS is caused by the preferential prolongation of M cells by ion channel mutations, which helps to cause long QT intervals and TdP. It is known that exhibit electrical properties and reactions to medications that are compatible with the ECG symptoms associated with the development of long QT and TdP.

Classification of LQTS

LQTS can be classified in following types.

Congenital LQTS

A genetic heart condition known as congenital long QT syndrome (LQTS) is characterized by a protracted QT interval at basal ECG and a significant risk of potentially fatal arrhythmias. Nearly 1 in 2,500 live births are thought to be affected by a disease. Syncopal episodes, which can cause cardiac arrest and sudden cardiac death, and electrocardiographic abnormalities, such as a prolonged QT interval and aberrant T waves, are the two primary symptoms of LQTS. Congenital LQTS can manifest in more than ten distinct ways [(16–18)]. Most congenital LQTS instances are caused by one of the three LQT subtypes: LQT1, LQT2, or LQT3. 40–55% of instances of the LQTS are caused by LQT1(19,20). Mutations in the KVLQT1 gene, also known as KCNQ1, are to blame. Exercise-induced events are what make LQT1 unique. 35–45% of congenital LQTS instances are caused by LQT2 (19,20)

It is brought on by various mutations in the potassium channel gene hERG, which is found on chromosome 7 and is also known as KCNH2. The hERG channel's pore or nonpore region may be affected by the mutations. Nonpore mutations frequently result in Torsades de Pointes (TdP) in the context of hypokalemia, whereas pore mutations present a significant risk for cardiac

events and may impact young patients (21). 8–10% of instances (19,20) are accounted for by LQT3. SCN5A, a gene for sodium channels, is situated on chromosome 3 at positions 21–24 and is the source of the disease. Events that take place when you're at rest or asleep define it.

LQT1

A reasonably selective IKs blocker called chromanol 293B imitates the LQT1 (and LQT5) condition by homogeneously prolonging the APD across the ventricular wall without widening the T wave or raising the TDR. The transmural dispersion of repolarization is increased by beta adrenergic stimulation with isoproterenol as a consequence of a reduction in the APD of epicardial and endocardial cells, but not by extending the M-ceil, resulting in a lengthy QT period with a broad-based T wave. Isoproterenol would be expected to augment any remaining IKs in epicardial and endocardial cells more than in M cells, where IKs is intrinsically weak. This would shorten the epicardial and endocardial responses but not the M cell's response, resulting in a broad-based T wave and a large TDR.

LQT2

IKr blocker D-Sotalo1 mimics both acquired (drug-induced) types of LQTS and LQT2. The effects of D-Sotalol, particularly in the presence of low potassium (2 mmol/L), include a preferential prolongation of the M-cell APD and a very significant slowing of phase 3 repolarization of the three cell types. These effects lead to a prolonged QT interval, an increased transmural dispersion of repolarization, and low amplitude T waves with a deeply notched or bifurcated appearance, which are frequently seen in patients with the LQT2.

LQT3

A substance called ATX-II, which increases late sodium current (INa), imitates the LQT3 condition (I6). Because late INa is more prominent in the M cell's APD, ATX-II significantly prolongs the QT interval, expands the T wave, and produces a rapid increase in TDR. Due to the drug's significant effects on epicardial and endocardial APD, ATX-II also causes a noticeable delay in the commencement of the T wave, which is consistent with the late-appearing T-wave pattern seen in patients with the LQT3 syndrome.

Acquired LQTS

Genetic differences may have an impact on an individual susceptibility in developing acquired QT interval prolongation.(22) This is corroborated by the estimate of 35% heritability of QT interval duration in the general population, apart from congenital LQTS patients(23). In addition, first-degree relatives of patients with congenital LQTS are more likely than unrelated people to experience drug-induced QT prolongation(24). Numerous genes linked to QT interval length have been found in genome-wide association studies (GWAS)(25). The nitric oxide synthase 1 adapter protein gene (NOS1AP), which is located on chromosome 1 (1q23.3) and inhibits L-type calcium channels and affects impulse propagation, has the largest correlation with QT interval duration(25,26).Other GWAS results revealed variants in genes linked to intracellular calcium handling, congenital LQTS gene mutations, and previously unknown genes that affect cardiac repolarization(27). In a recent research, 28% of patients with drug-induced LQTS were discovered to have disease-causing mutations, compared to more than 1000 individuals with congenital LQTS(28). It's noteworthy that the QTc of patients with drug-induced LQTS (453 39 ms) was substantially longer than that of control participants (406 26 ms) under baseline circumstances(28). Pharmacogenetics of drug-induced LQTS should be taken into account in relation to both pharmacokinetic and pharmacodynamic features(27). The body's reaction to a medication is called pharmacokinetics, and it may be divided into effects on drug distribution, absorption, metabolism, and elimination. Variation in the genes encoding drug-metabolizing cytochrome P450 or drug transporters like P-glycoprotein mostly defines pharmacokinetic genetic vulnerability. However, genes known to be linked to QT prolongation in the general population and genes where the causative mutations of congenital LQTS are found primarily define the pharmacodynamics component of genetic vulnerability(28).

Table 2: Drugs, responsible for acquired LQTS

CARDIAC	Antiarrhythmic drugs	**Class Ia** Quinidine, Procainamide, Disopromide
		Class III Dofetilide, Ibutilide, Sotalol
NON-CARDIAC	Antihistamines	Terfenadine, Astemizole
	Antipsychotic antidepressant agents	**Neuroleptic** Haloperidol, Droperidol, Thioridazine, Chlorpromazine
		Atypical antipsychotics Sertindole, Ziprasidone, Risperidone, Zimeldine, Citalopram
		Antidepressants Amitriptyline, Desipramine, Imipramine, Maprotiline, Doxepin, Fluoxetin
	Antibiotics	**Quinolone** Sparfloxacin, Levofloxacin, moxifloxacin, grepafloxacin
		Macrolide Erythromycin,Clarithromycin
	Antimalarials	Quinine, halofantrine)
	Antiprotozoal	Pentamidine
	Antifungal	Azole group
	Antimotility	Cisapride

Role of magnesium in developing LQTS

Magnesium (Mg), which is crucial for many cellular processes, is the fourth most abundant cation in the body and the second most abundant intracellular cation after potassium (K) (29). It is a crucial substance with activities in the neuromuscular, cardiovascular, immunological, and hormonal systems as well as controlling membrane stability. A lack of magnesium (Mg^{2+}) or Mg causes a number of metabolic problems and clinical effects (30). Between 8% to 30% of hospitalized individuals have hypomagnesemia (31). 33% of patients with diabetes mellitus (DM) and 19–44% of patients with chronic heart failure had hypomagnesemia, respectively (32). On the other hand, DM is also linked to Mg insufficiency, perhaps as a result of urinary Mg^{2+} loss(31). The Ito channel (Kv4.2) was shown by Ai et al to be downregulated in diabetic rat cardiac tissues(33). The lengthening of the QT interval is one of these disorder primary cardiac complications, however the underlying molecular pathways are still unclear. Mg^{2+} loss both in the extracellular and intracellular fluid has a direct impact on arrhythmias in Mg shortage, according to conventional wisdom. Natural Ca^{2+} channel blockers like Mg^{2+} are well-known(34). As a result, a $MgSO_4$ intravenous injection reduces Ca^{2+} channel currents, particularly the L-type Ca^{2+} current, which should shorten the QT interval. As a result, Mg^{2+} depletion ought to be accompanied by a rise in ICa.L, which may eventually result in QT lengthening (35). Activation of the Na^{+}-Ca^{2+} exchanger current or transient inward current (ITI) should generate a rise in ICa.L, which should raise the intracellular Ca^{2+} concentration and lengthen the QT interval. In fact, patients with hypomagnesemia experience more PVCs on average. In the chronic phase of hypomagnesemia, however, ICa.L augmentation is not required for the occurrence of aberrant because QT prolongation is frequently present, cardiac excitability together with an extension of the atrioventricular conduction (36). As opposed to this, intracellular Mg^{2+} values below the physiological range might result in QT lengthening, due to transitory IK1 almost reaching zero, reflecting the Mg^{2+} block is not present(37). The removal of the Mg^{2+} block, however may not be adequate to explain why QT intervals are getting longer. Mg deficiency in clinical and experimental settings for the the subsequent explanations as follows (38):

(1) APDs are noticeably extended in even though the cardiomyocytes came from Mg-deficient animals.
(2) Under normal intracellular Mg^{2+} circumstances, IK1 consistently decreases by 28–32% in Mg–deficient cardiomyocytes in voltage-clamp tests.
(3) Intracellular Mg^{2+} concentrations 50% of normal may not be possible in vivo.

Measurement of the QT interval

The QT interval on a 12-lead ECG is the distance between the start of the QRS complex and the end of the T wave as it returns to baseline. Leads II and V5 or V6 should be used to manually measure the QT interval, with the longest value being chosen. When it comes to identifying aberrant QT intervals, measurements made from these leads have the highest positive and negative predictive values [(39)]. At least three to four cardiac cycles should be used to get the mean value. At least three to four cardiac cycles should be used to get the mean value. The junction point between the tangent formed at the highest downslope of the T wave and the iso-electric line serves as a reliable indicator of the end of the T wave according to the slope technique. If the T wave is notched, the greatest slope should get the tangent. While bigger U waves that merge with T waves should be included in the measurements, smaller U waves (0.1 mV) should be removed (40). The interpretation of extended QT intervals from Holter or 24/48 h ambulatory monitoring recordings is not standardized. As a result, ambulatory QT assessment monitoring is not advised. The QT interval is influenced by a number of variables, including gender, heart rate, underlying rhythm, and conduction abnormalities. The patients' physiologic and metabolic states have an impact as well. There are several methods for adjusting QT intervals for heart rate, and each has advantages and drawbacks of its own. There is no agreement on which is the most efficient. However, Bazett's calculation (QTc = QT/RR in seconds), which offers a sufficient correction for heart rates ranging from 60 to 100 beats per minute, is the one that is most frequently used. However, for low and high heart rates, it, respectively, underestimates and overestimates the QT interval. Other correction techniques, such as Fredericia (QTc = QT/(RR)1/3) or Framingham (QTc = QT + 0.154(1 - RR)), should be used for heart rates beyond the normal range (41). These techniques of adjustment, however, do not take into account intra- or inter-individual variability because they are based on the population mean correction factor. The optimal HR adjustment for QT should be computed for each individual as there is now substantial evidence for significant inter-individual variability (42–44). The procedure of estimating each individual correction factor is laborious and time-consuming. Although it is recommended in clinical research, it cannot be used in actual clinical settings [(45)]. Based on a QT-RR cloud diagram created from human preclinical investigations [(46)], Fossa and associates offered a QT-HR nomogram that is easily used in the clinical context. The nomogram, which uses HR rather than RR interval, has been found to be secure, to have great sensitivity, and to be sufficiently precise to allow the identification of many

patients as "not at risk" for drug-induced TdP and, as a result, to not need cardiac monitoring (46)Bateman looked explored how well the nomogram performed in individuals who had taken an excessive number of antidepressants but did not have arrhythmia. Comparing the QT nomogram to other frequently used QTc criteria, a reduced false-positive rate was found. Patients with heart rates between 30 and 60 beats per minute showed the highest disparity between the nomogram and QTc techniques. In contrast to venlafaxine and mirtazapine overdose, individuals with citalopram overdoses had QT values above the nomogram, indicating a greater risk of developing an arrhythmia and the need for vigilant cardiac monitoring (47). According to Bazett's corrected QTc value, a QT interval in adult men that is larger than 450 ms is regarded as protracted, while one that is between 430 and 450 ms is regarded as borderline. According to Goldenberg et al. (2006), a QT interval in females more than 470 ms is regarded as protracted, while one between 450 and 470 ms is regarded as borderline.

Table 3. Methodologies for correcting QT intervals

Correction	Formula
Bazzet	QTc = QT/√RR
Fredericia	QTc = QT/(RR)1/3
Framingham	QTc = QT + 0.154(1 – RR)

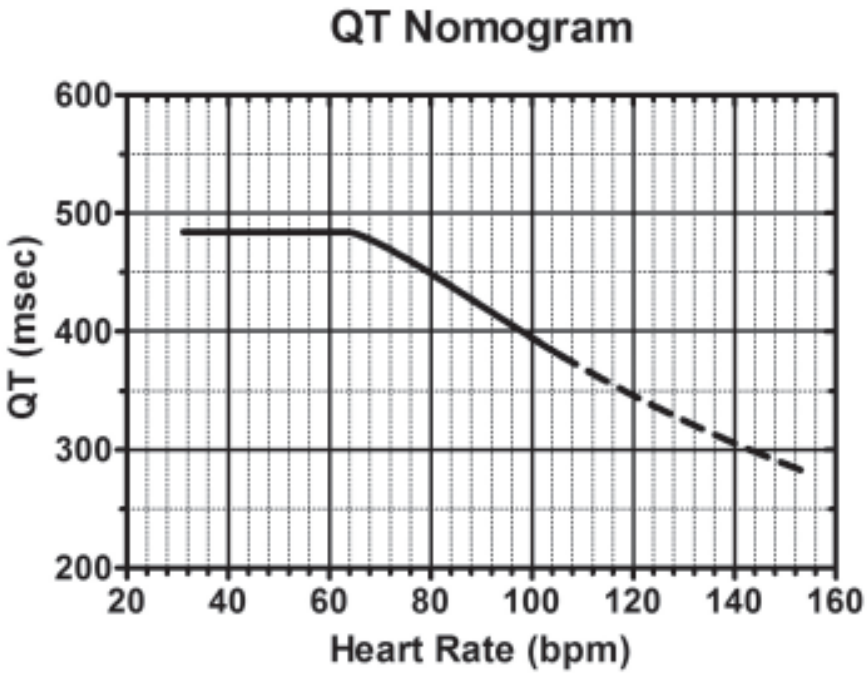


Figure 2. QT interval nomogram for "at risk" assessment from a single 12-lead ECG, QT-HR pairings. Use: On a 12-lead ECG, the QT interval should be manually recorded from the start of the Q wave to the end of the T wave.

The mechanism of TdP in light of LQTS

A prolonged QT is dangerous because it increases the chance of polymorphic tachycardia, or TdP, which leads to sudden cardiac death (SCD). Early after depolarization (EAD) oscillation is a common side effect of prolonged ventricular repolarization. An ectopic beat may occur if the EAD crosses a crucial threshold in a significant portion of the myocardium. This ectopic

pulse is often followed by a protracted pause, and the next sinus beat exhibits a noticeable QT prolongation. The ectopic beat can cause reentrant excitement and TdP when the myocardium's action potential duration is markedly heterogeneous [(48)]. This short-long-short cycle onset pattern is typical of drug-induced TdP, also known as pause-dependent TdP [(49)]. Contrary to the congenital type, where TdP frequently follows an abrupt adrenergic spike like exercise or alertness. Regardless of the method, TdP often doesn't last very long until it spontaneously ends. However, if it persists, it may progress to SCD and ventricular fibrillation [(50)].

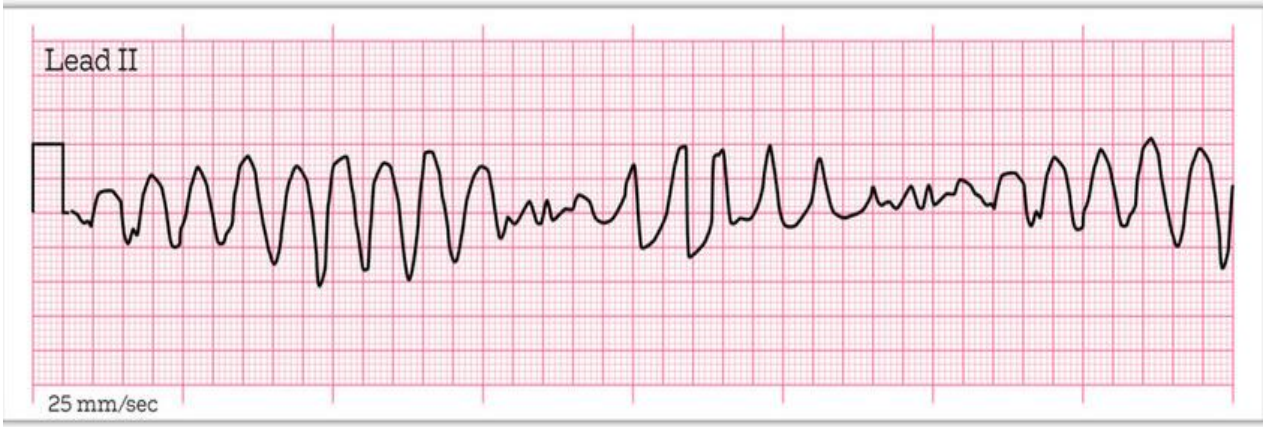


Figure 3. Ventricular premature contractions (VPCs) occurring in a patient with heart block and prolonged QT. The timing of the second VPC (arrow) is such that it occurs on the T-wave of a preceding T-wave, instigating an episode of Torsades de Pointes (TdP).

Concurrent risk factors for LQTS- patients

In any given person, drug-induced LQTS is unpredictable. The projected clinical impact of a medicine and its molecular action are not always in line with one another (51,52)]. Amiodarone, for instance, lengthens the QT interval in patients with a normal baseline QT but seldom ever results in TdP [(53)]. Terfenadine, a powerful IKr blocker, on the other hand, causes relatively little QT prolongation but was frequently linked to TdP, leading to its removal from the market [(54)]. Therefore, it would seem that some additional risk variables significantly contribute to drug-induced TdP (Box). Female sex poses the most frequent danger [(55)]. Women have a longer baseline QT interval than men do during puberty, and thus react differently to IKr blocking medications. It has been demonstrated that androgens enhance IKs and IKr channels and consequently shorten AP length, even if the mechanism behind the gender difference in

repolarization is not well known [(56,57)]. Extracellular potassium levels and heart pace also have a big impact. Bradycardia shortens the QT interval by reducing potassium outflow during phase 3 repolarization. Conversely, hypokalemia increases the drug's ability to block IKr channels [(58)].

K channel expression is downregulated while Ca channel expression is upregulated in heart failure and LVH [(59,60)]. TdP and QT prolongation risk are subsequently increased by this. If the concurrent medications have an additive or potentiating effect, such as a combination of antiarrhythmic medications, this might result in a longer QT interval. Pharmacokinetic interactions can happen when two medications compete for the same hepatic enzyme or when one drug lowers the clearance of the other [(61)]. Combining a medicine with cytochrome CYP3A4 inhibitors, such as '-azoles', '-mycins', or grapefruit juice, will raise its level [(62)].

Genetic susceptibility to LQTS

According to Paulussen et al. (2004), 5-20% of individuals with drug-induced TdP have gene alterations that result in LQTS. These individuals often have baseline QTc intervals that are normal to borderline, but when exposed to certain medicines, they become more sensitive to QT prolongation and TdP [(63,64)].

In addition, CYP2D6 dependent medications may not be properly metabolized due to polymorphism in the genes that code for the CYP2D6 enzyme. Approximately 5–10% of White patients are thought to be poor metabolizers and are at risk for QT prolongation and TdP, particularly if the parent medication has a propensity to do so [(65)].

The ratio EPTC/IC50, also known as the therapeutic/toxic ratio (EPTC: effective plasma therapeutic concentration; IC50: in vitro concentration that blocks 50% of hERG), can be used to assess the level of hERG blockage and the risk for TdP.

As the ratio rises, there is an increased chance of getting TdP. Drugs with a ratio larger than 1 include cisapride, sparfloxacin, quinidine, ibutilide, and thioridazine [(66)].

Increased risk of LQTS with specific drugs

It is mainly unknown how often drug-induced TdP is in the general population. Depending on the demographic being examined and the medications being utilized, it varies. 3.1% of patients

using noncardiac medicines in one observational trial developed TdP. Box has a list of various medications that may extend QT intervals.

Antiarrhythmic agents

The most common cause of drug-induced TdP is antiarrhythmic medication. Both Na and K channels are blocked by Class IA drugs (quinidine, procainamide, and disopyramide), and TdP can happen at therapeutic or subtherapeutic dosages [(67)]. Within a week of starting treatment, quinidine typically lengthens the QT interval by 10% to 15% and has a 1.5% chance of causing TdP [(5)]. When taking antiarrhythmic medication, hypokalemia or hypomagnesaemia frequently cause TdP. When used in atrial fibrillation for rhythm maintenance, the mortality increases by twofold [(68)]. Procainamide's major Na blocking action makes it less likely to cause TdP. However, through its active metabolite, N-acetyl procainamide (NAPA), which has a strong K blocking action, it can cause TdP in individuals with renal impairment or in quick acetylators [(69)]. Disopyramide has also been linked to TdP and should not be used in individuals who have hepatic, renal, or cardiac failure [(70)]. IKr blockers of class III drugs have strong inhibitory effects and extend QT intervals in a dose-dependent manner.

Due to the reverse usage dependence characteristic, the potassium blocking action is most at low heart rates [(71)]. Amiodarone carries the lowest risk for TdP, followed by dofetilide, ibutilide, and sotalol. According to Lehmann et al. (1996), sotalol induces TdP in 2-4% of patients, with women being more susceptible. According to Torp-Pedersen et al. (1999), the incidence of TdP caused by dofetilide is 2.1% with a higher risk in patients with renal failure. According to Stambler et al. (1996), intravenous ibutilide carries a TdP risk of 1% to 3%, with a greater incidence in patients with structural heart disease, heart failure, and electrolyte imbalance. Despite its ability to prolong the QT, amiodarone seldom results in TdP. It can be explained by a few of its distinctive characteristics, including a lack of dependence on reverse usage, a reduction in QT dispersion across the ventricular myocardium, effects on L-type calcium channels and blocking actions.

Antihistamines

Antihistamines without sedation are often administered, although few medications have been linked to considerable arrhythmogenicity. Astemizole and terfenadine have been linked to TdP because they have strong IKr inhibiting effects even at low dosages. Both have been taken off the market. The cytochrome P450 enzyme CYP3A4 is responsible for the majority of antihistamines' metabolism. Higher drug levels may occur in patients with liver disease or when

CYP3A4 inhibitors are co-administered with other medications or foods. The prevalence of pro-arrhythmia is uncertain with the more recent non-sedating antihistamines; however, they appear to be less dangerous than earlier formulations.

Antipsychotic

Antipsychotic drugs are widely recognized to produce TdP and to lengthen QT interval in a dose-dependent manner.

The butyrophenone haloperidol is frequently used to treat severe agitation and schizophrenia. It is a strong IKr channel blocker and lengthens the QT interval by 15 to 30 ms ((72)). When risk variables are present, the impact is enhanced (**Table 1**). The Food and Drug Administration (FDA) of the United States issued a warning in 2007 recommending ECG monitoring while using it intravenously. However, based on the information at hand, it is believed to be safe to provide IV haloperidol to patients who do not have risk factors up to a cumulative dosage of 2 mg without ECG monitoring [(73)]. Haloperidol and droperidol have comparable effects, and individuals with risk factors should get close monitoring.

Chlorpromazine, for example, has an antipsychotic and antiemetic action. Due to their K blocking effects, thioridazine and chlorpromazine have both been linked to QT prolongation and TdP.

Atypical antipsychotics

The majority of atypical antipsychotics have varying potencies and can lengthen the QT interval in a dose-dependent manner. Ziprasidone has the largest QT prolongation while olanzapine has the lowest [(74)]. Sertindole was taken off the market in 1998 because it carried a TdP and sudden death risk. In hazardous levels, citalopram and zimelidine have been linked to TdP [(75,76)].

Antidepressants

In comparison to selective serotonin-reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs) are more frequently linked to a lengthening of the QTc interval [(77)].

TCAs primarily block the Na channel to extend the QTc. If a potassium channel blocking drug is also delivered, the impact is more apparent. According to Casazza et al. (1986), amitriptyline, desipramine, and imipramine have all been linked to TdP. TCA at toxic dosages can alter the

EKG in a number of ways, including expanding QRS complexes, QT prolongation, and TdP. By blocking the IKr channel, SSRIs lengthen the QT interval. Escitalopram and citalopram are linked to QT prolongation as well.

Antibiotics

Fluoroquinolones can affect the QTc interval differently, and TdP is a relatively unusual side effect. With ciprofloxacin and ofloxacin having the least impact on the IKr channel, grepafloxacin and sparfloxacin significantly delay repolarization compared to gatifloxacin, levofloxacin, and moxifloxacin [(40)]. Grepafloxacin and sparfloxacin were both abandoned during the early stages of medication development. The remaining fluoroquinolones are generally safe, although caution should be used if any risk factors exist or while co-administering QT-prolonging medications [(78)].

Clarithromycin and erythromycin are examples of macrolides that have been linked to QT prolongation and TdP [(2,77,79)]. Erythromycin was discovered to exhibit effects in animal tests that were comparable to class III antiarrhythmic drugs, including extension of the QT interval, induction of EAD, and transmural dispersion. Both erythromycin and clarithromycin are CYP3A4 inhibitors, and using them together or with other CYP3A4 inhibitors or CYP3A4-metabolizing medications might have serious side effects. Azithromycin is regarded as being safe, however TdP has also been linked to its usage [(80)].

All around the world, antimalarial drugs are often prescribed. Due to underreporting, the real incidence of QT prolongation and TdP with their usage is mainly unclear. The optical isomer of quinidine known as quinine has a limited influence on QT interval and is infrequently linked to TdP [(81)].

Halofantrine and chloroquine have been associated to TdP and can lengthen the QT interval. According to Wesche et al. (2000), halofantrine is the most effective substance with repolarization capabilities comparable to those of quinidine and Class III antiarrhythmic drugs.

The antiprotozoal medication pentamidine is used to treat Pneumocystis carinii pneumonia.

Pentamidine's electrophysiologic characteristics are unclear, however its structural resemblance to procainamide is notable. While the inhaled version is thought to be harmless, intravenous usage can result in TdP [(82,83)According to Eisenhauer et al. (1994), the proarrhythmic risk of IV usage is more closely connected to an idiosyncratic reaction than a dosage effect.

Antifungal drugs from the systemic azole group may cause TdP due to their pharmacologic and pharmacokinetic properties [(84)]. Particularly in those who have underlying risk factors for QT prolongation, care should be used when using it.

Other agents

For gastroesophageal reflux disease (GERD) and delayed stomach emptying time, cisapride is a gastrointestinal promoter. It shares a structural resemblance with procainamide and blocks both IKr and IKs (IKr>IKs) [(85)]. According to Wysowski et al. (2001), cisapride use was linked to more TdP instances than antiarrhythmic medications, which led to its removal from the US market in July 2000.

The screening method for LQT

It is currently standard practice for biotechnology and pharmaceutical firms to test substances for hERG channel functionality early on during preclinical safety evaluation due to the significant potential for QT prolongation and TdP with various medicines.

The creation of the "thorough QT/QTc study," which was meant to confidently identify medications that could extend QT, was a focal point of this practice. This research has become an essential part of all research and development initiatives for novel molecular entities. It is a randomized, double-blinded study that includes a placebo and a positive control arm and is rigorously powered to rule out any impact on the QTc interval more than 10 ms. Once the drug's pharmacokinetics and tolerability have been established, it is undertaken on healthy volunteers. RR interval, cardiac rate, and QT interval adjusted using Bazetts' (QTcB = QT/RR0.5) and Fridericia's (QTcF = QT/RR0.33) corrections are all recorded during the experiments. ECG interval measuring techniques are either "fully manual" or "manually adjudicated" to some extent.

The purpose of the positive control is to show the assay's sensitivity to detect a tiny change in the QT interval, in this example a QT prolongation of little over 5 ms. A smaller QT impact for the test medication should suggest that the test drug does not considerably extend the QT interval if the research is able to identify such a little QT extension by the control.

Moxifloxacin, a fluoroquinoline antibiotic with a slight QT prolonging effect, has been employed in the great majority of TQT trials. The observed 7.5–12.5 ms increase in the mean

placebo– and baseline–corrected QTc interval for moxifloxacin 400 mg supports the efficacy of its usage as a positive control in TQT studies [Bloomfield et al. 2008].

There are typically three clinical outcomes from a QT study: the medicine investigated prolongs the mean QTc interval by around 10 ms and does not appear to induce TdP (or the elevated risk is too minor to be seen). The examined medication causes a mean QTc interval prolongation of >10 ms but 20 ms, resulting in a 'uncertain' probability of TdP induction. The investigated medicine increases the mean QT/QTc interval by more than 20 ms and may be highly likely to result in clinically significant arrhythmic events. Phase II-III of clinical drug testing would most likely be able to acquire standard ECG data in accordance with normal procedure if the result was "negative." Phase II and III studies must gather more ECG data to get thorough dose-response information in order to obtain a "nonnegative" outcome (QTc interval >10 ms).

Patients with additional risk factors for TdP, such as those with electrolyte abnormalities (such as hypokalemia), congestive heart failure, impaired drug metabolizing capacity or clearance (such as renal or hepatic impairment, drug interactions), female patients, and patients aged 16 and over 65 years, must be included in these analyses in order to fully evaluate the risk of QT prolongation.

Recent studies have shown that when tested on drugs with a known QT-prolonging effect, fully or partially automated methods of QT interval measurements using computer algorithms produce similar results to the labor-intensive manual methods [(86,87)]. It is anticipated that the adoption of automated QT algorithms will not only replace human QT measurements but will also enhance the identification of small T-wave variations in the assessment of novel drugs [(88)].

The treatment of TdP

The hemodynamic stability affects the course of therapy. Immediate direct-current cardioversion should be carried out on individuals with TdP that does not end spontaneously or that develops into ventricular fibrillation. Stable individuals can be managed in the following ways:

i. Even in individuals with normal levels of magnesium, intravenous magnesium is the first line of treatment. In addition to being immediately effective, it also stops TdP and ectopic beats from happening again. It functions by reducing the calcium current's inflow and, as a result, the EAD's amplitude, which finally stops ectopic beats. The dosage is 2 g given over 1-2 minutes, followed by a 2-4 mg/min infusion. For recurrence, a repeat bolus of 2 g should be administered [(89)].

ii. Since intravenous potassium has been found to reduce QT anomalies in the acute phase (0.5 meq/kg to a mean of 40 meq), it should be taken into consideration even in normokalemic individuals.

iii. When a patient does not react to IV magnesium, overdrive transvenous pacing with a target heart rate of between 90 and 110 beats per minute should be taken into consideration. This reduces EAD and QT dispersion and shortens the QT interval [(90)].

iv. When a pacemaker is not accessible or as a temporary bridge to one, isoproterenol can be used to raise heart rate. However, people with congenital LQTS should not use it.

v. The medication list should be carefully examined for any potential offending medications. QT-prolonging medication information is excellently current on the website CredibleMeds.

vi. In individuals with persistent bradycardia, such as sick sinus syndrome or AV block, permanent pacemakers should be implanted. ICDs (implantable cardioverter-defibrillators) are recommended in situations where the offending agent cannot be avoided.

Prevention and monitoring of drug induced LQTS

In individuals with pre-existing cardiac illness, a history of ventricular arrhythmias, or with metabolic abnormalities such hypokalemia, QT-prolonging medications should be avoided. Compared to outpatients on the same QT prolonging medications, hospitalized patients had a higher chance of acquiring TdP. Patients who are hospitalized are frequently older persons with underlying cardiac disease who may also have renal or

hepatic failure, electrolyte problems, or bradycardia. These patients may require urgent intravenous medication administration. It is best to avoid concomitant use of medications that inhibit cytochrome P450, particularly imidazole antifungals, macrolide antibiotics, medications that might extend the QT interval, and medications that alter electrolytes. It is advised to undertake surveillance EKGs both before and after starting any QT-prolonging medications. It's also advised to regularly check electrolytes, especially potassium, in those on diuretics and QT-extension medications. For instance, if the dosage of methadone is greater than 100 mg per day in women, TdP has been linked to it [(91)]. Methadone is used by around a million Americans to treat either chronic pain or drug dependence [(92)]. Pretreatment ECG for QTc interval screening, a follow-up ECG within 30 days, and thereafter a yearly ECG are all advised by methadone recommendations [(92)].

In the hospital context, EKG monitoring of the QT interval is recommended for the following situations: starting a medication known to produce TdP; overdosing on potentially proarrhythmic substances; newly developing bradyarrhythmias; and severe hypokalemia or hypomagnesemia [(93)].

The patient's personal and family history should be carefully examined if drug-induced TdP has occurred to rule out the potential of congenital LQTS. All first-degree relatives should get a 12-lead ECG if there is a personal or family history of unexplained syncope or premature abrupt death, and clinically available genetic testing for congenital LQTS should be taken into account.

Material and methods

1. Drugs

The following chemical agents were used: clarithromycin injection (Klacid IV) from Abbott Healthcare Pvt.Ltd., India; furosemide injection (Lasix) from Sanofi India Limited; Rabeprazole Injection (Rabicip-I.V.) from Cipla Ltd, India; Ketamine hydrochloride Injection from Vulcan Laboratories, India; Xylazine Injection from Indian Immunological.

2. Chemicals

Magnesium sulphate, Potassium chloride

Magnesium assay kit, Sodium/Potassium/Chloride (ELYTE®) assay kit were manufactured by Coral Clinical System, India

3. Animal Husbandry and maintenance

Healthy adult male Wistar albino rats (8–9 weeks) weighing 140-180 gm were procured from West Bengal Livestock Development Corporation Limited. Buddha Park, Kalyani, Nadia- 741235. FSSAI REG NO – 10012031000104, and used for the study.

The animals were kept in polypropylene cages with well-aerated stainless-steel covers. All animals were kept in a departmental animal house. Animals were kept under controlled conditions of temperature (12-hour light and dark cycle, temperature of 25 ± 2°C and 50 ± 20 % relative humidity).

The study followed the Institutional Ethical Committee (Constituted under the Guidelines Committee for Control and Supervision of Experiments on Animals).

4. Experiment Protocols

4.1. Experiment-I

Animals were divided into four groups (n=6): Group I (Compromised group) received furosemide (FUR) intra-peritoneal (IP) injection at 1 mg./kg/day and clarithromycin (CLA) IP injection at 50 mg./kg/day; Group II (Compromised +Test drug OD) received FUR IP at 1 mg./kg/day, CLA injection at 50 mg./kg. day-1 and Rabeprazole IP 2 mg./kg/day; Group III (Compromised +Test drug BD) received FUR IP at 1 mg./kg/day, CLA injection at 50 mg./kg and Rabeprazole IP 2 mg./kg twice daily; and Group IV (Rabeprazole) received only Rabeprazole IP 2 mg./kg/day. After seventh days of above-mentioned treatment, blood sample were collected in heparinised tubes under anaesthesia. About 250µl of the collected blood was centrifuged at 850 xg for 5 min at 10°C for harvesting plasma and finally plasma was stored at -80°C until analysis.

4.2. Experiment-II

Animals were divided into four groups (n=6): Group I RAB IP at 2 mg/kg/day, Magnesium sulphate at 14.28mg/kg/day; Group II RAB IP at 2 mg/kg/day, Potassium chloride at 0.016mg/kg/day. After seventh days of above-mentioned treatment, blood sample were collected in heparinised tubes under anaesthesia. About 250µl of the collected blood was centrifuged at 850 xg for 5 min at 10°C for harvesting plasma and finally plasma was stored at -80°C until analysis.

5. Measurement of surface electrocardiogram (ECG) recording of anaesthetic rats

Rats were anesthetized using Ketamine (60mg./kg) and xylazine (10mg./kg) according to the method of ECG was recorded for 10 min, 2 h after medication using standard

lead II (metal ECG leads). The ECG signals were acquired and analysed by BIOPAC (Biosystems, USA) MP36. ECG tracing on zero day i.e., before administration of drug was considered as self-control while that of the seventh day was compared with the said control. Duration of QT was determined from the onset of QRS complex to the end of T wave. Measured QT was corrected using normalised Bazett's equation to obtain the corrected QT (QTc) interval

6. QT correction

QT interval is highly dependent on the heart rate, while that of rats it varies over a wide range. Accordingly, the measured QT was thus corrected using normalized Bazett's equation $QTc=QT/\sqrt{(RR/f)}$, where f is the normalization factor. In this present study, the normalization factor is the value of the average RR duration of each group.

7. Analysis of serum electrolyte

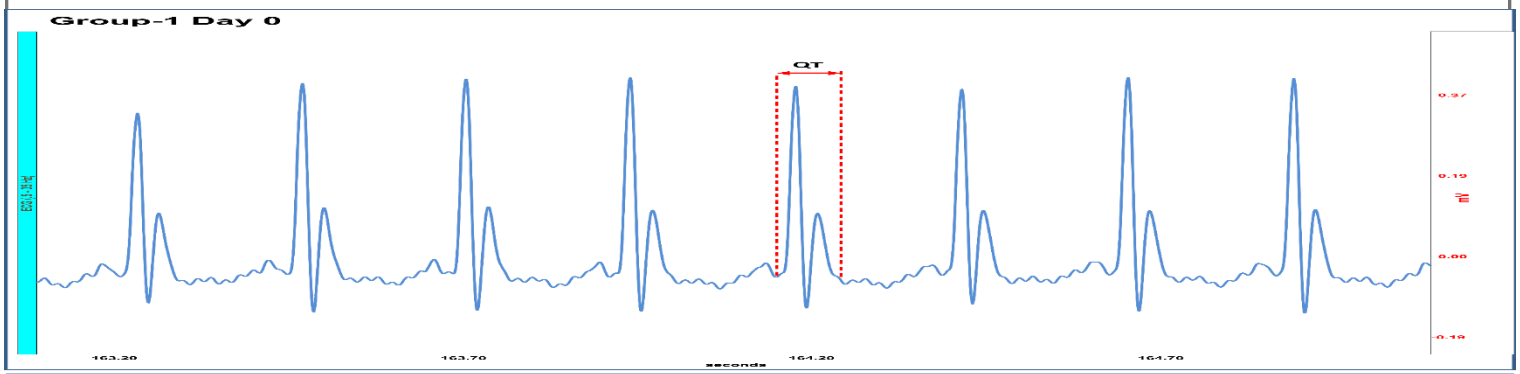
Rat blood samples on zero day i.e., before administration of drug was considered as self-control while that of the seventh day (2 hrs after CLA dose) was compared with the said control. The blood samples were then centrifuged at 2400g for 10 min and serum was separated and were analysed for electrolyte concentration. Serum potassium, magnesium, sodium concentrations were determined using kit.

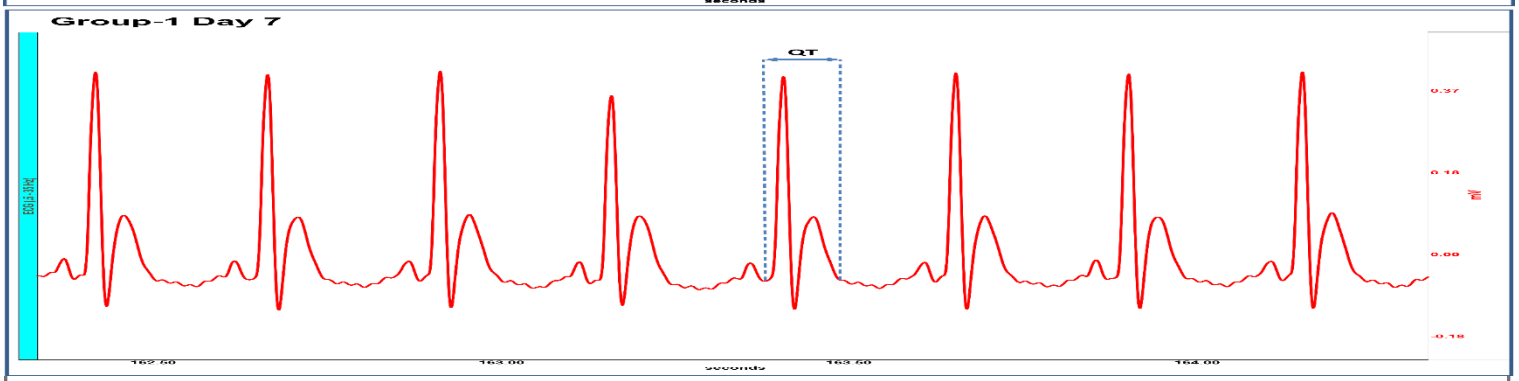
8. Statistical analysis

Values are expressed as Mean±SD (n=6). Statistical analysis was performed using Student's t-test (GraphPad Prism5, USA), *$p< 0.05$ was taken as the criterion of statistical significance.

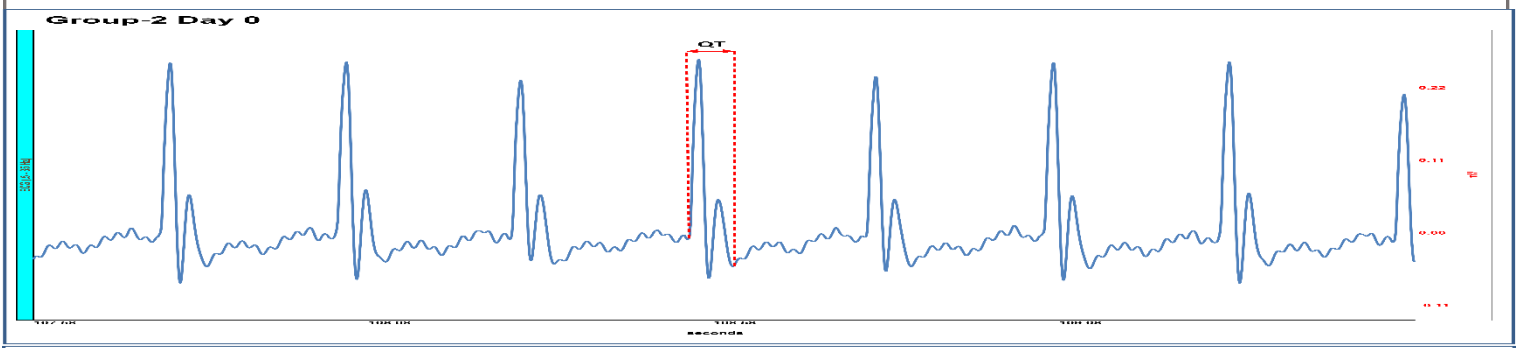
Results

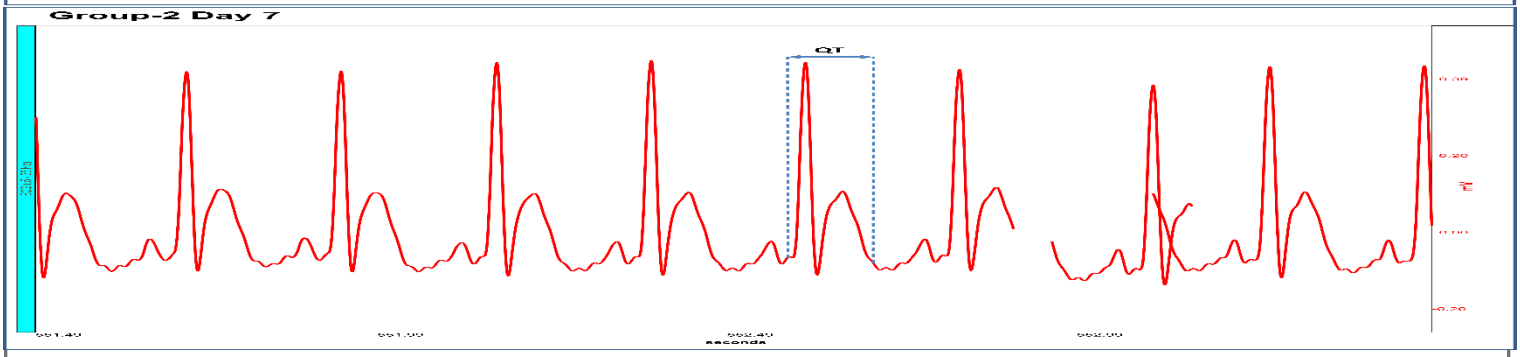
1. Electrocardiographic measurement



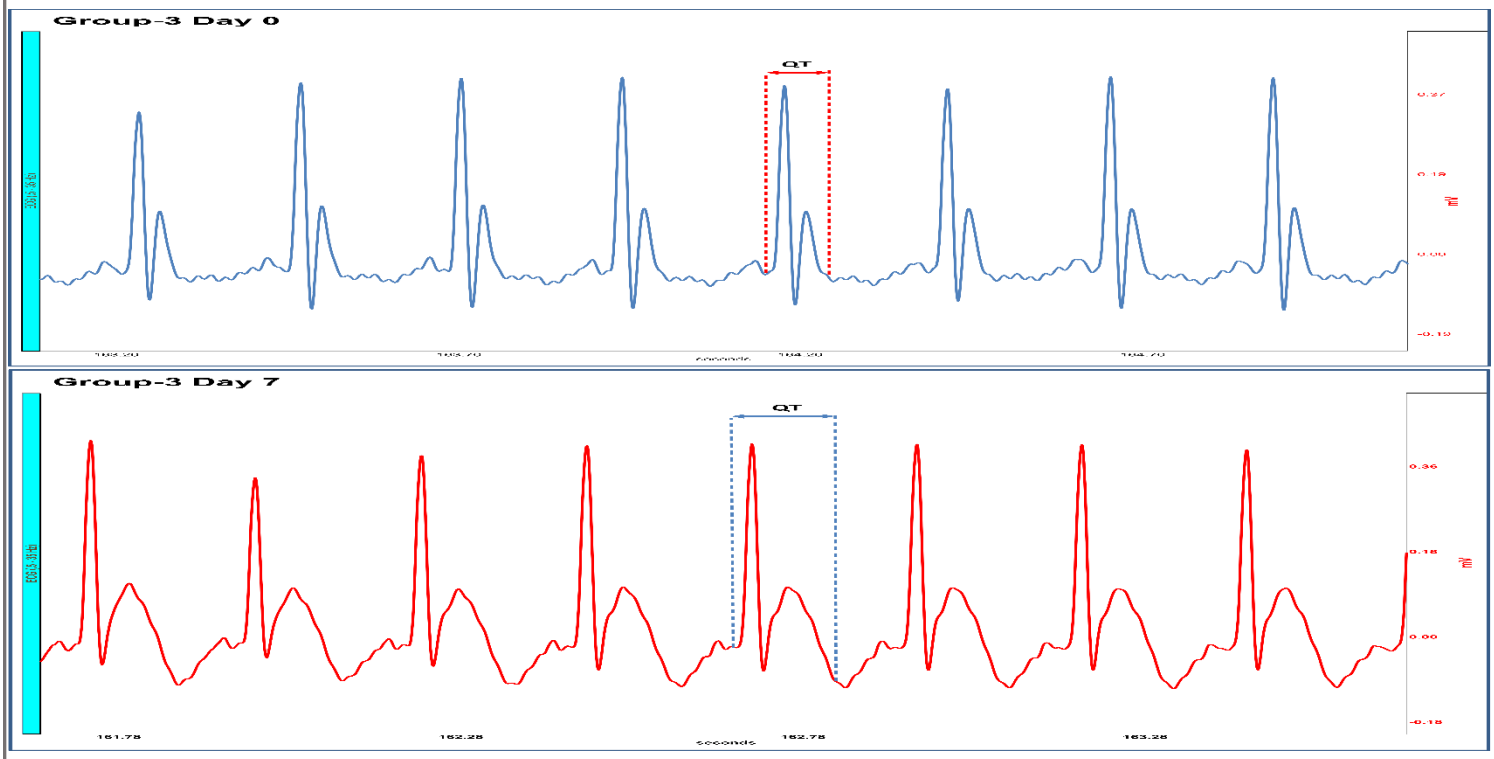


Group- I (FUR+ CLA)

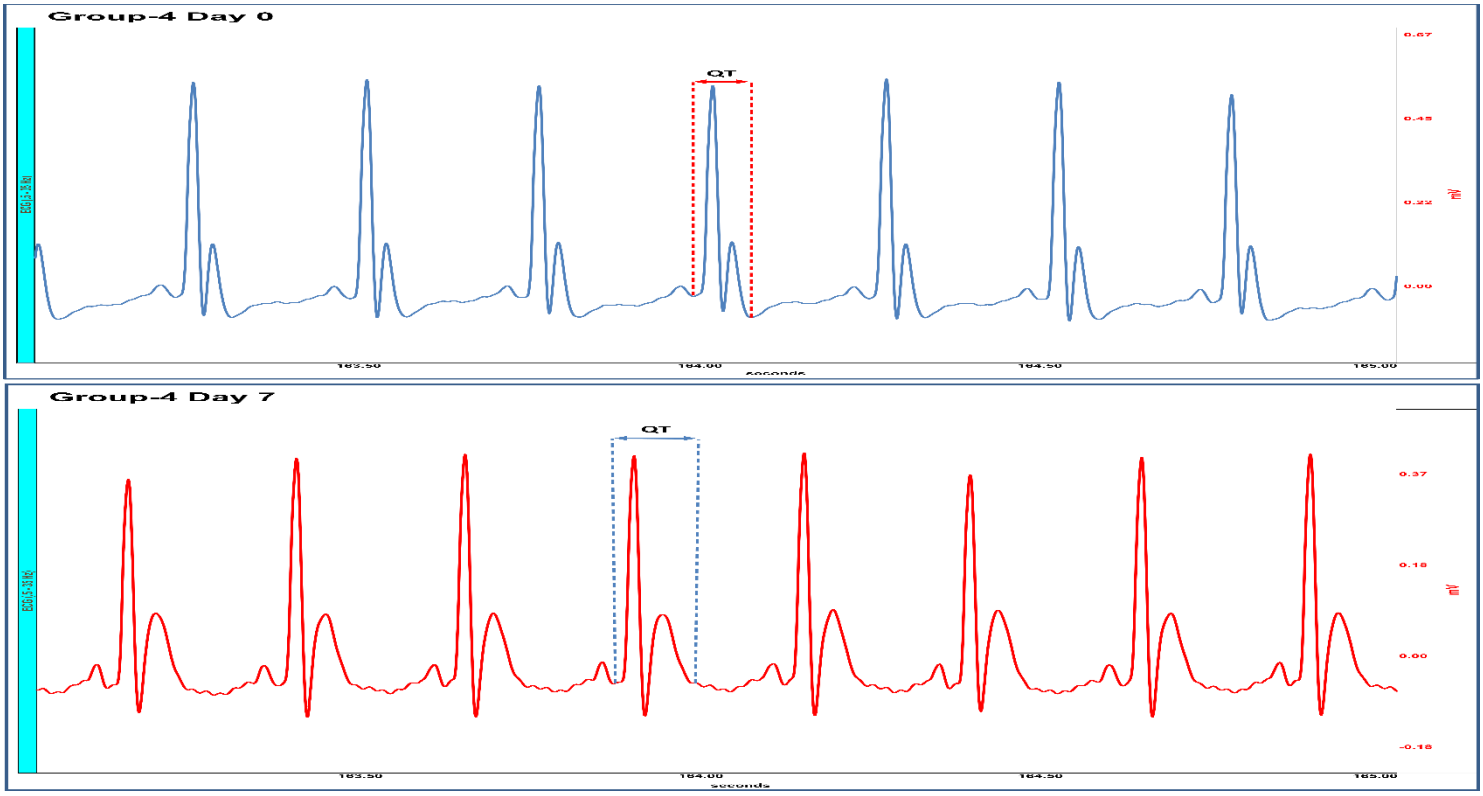




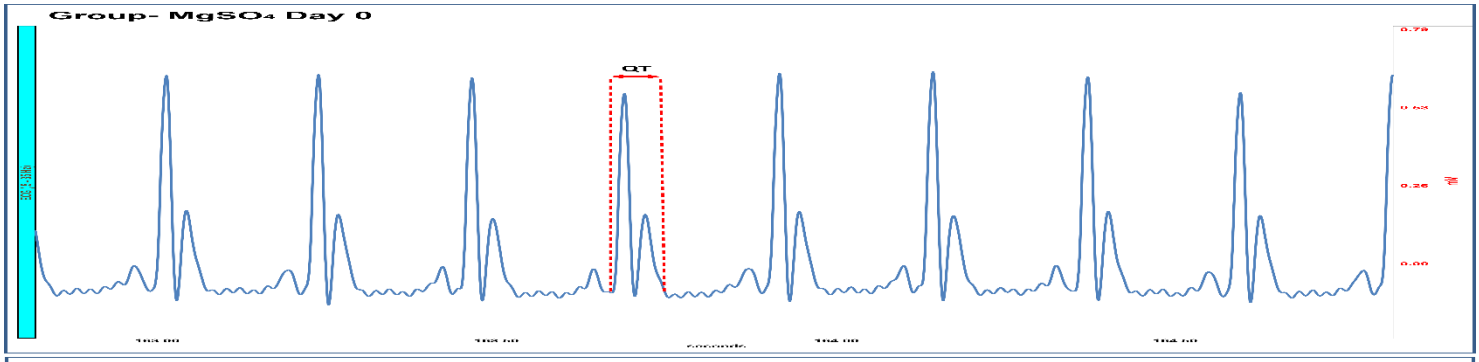
Group- II (FUR+ CLA+RAB-OD)

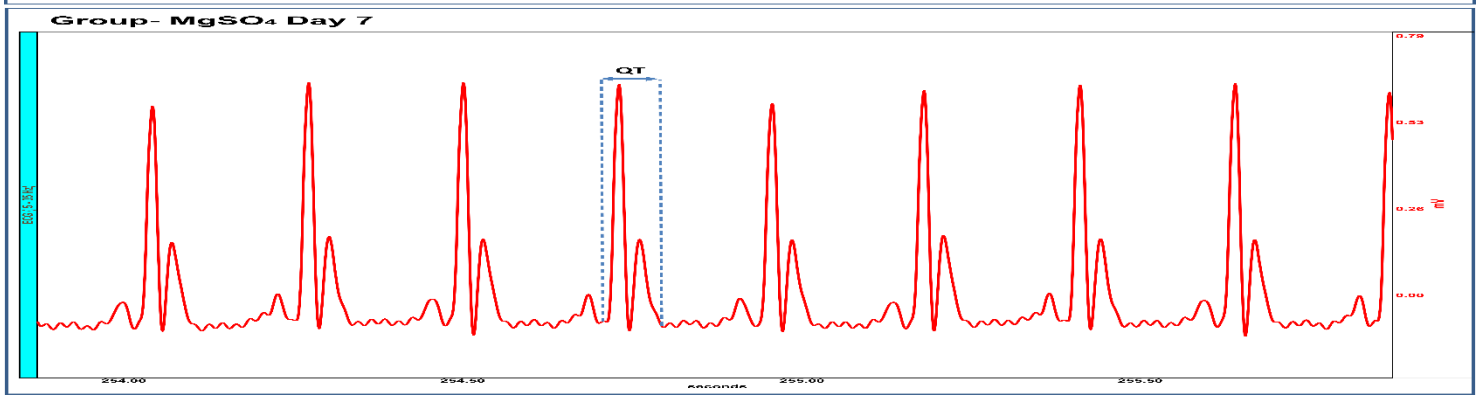


Group- III (FUR+ CLA+RAB-BD)

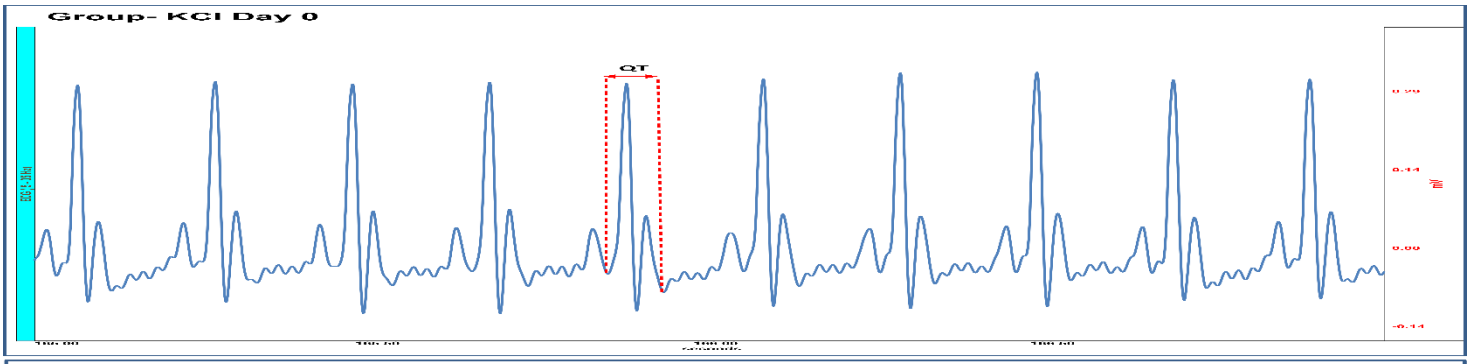


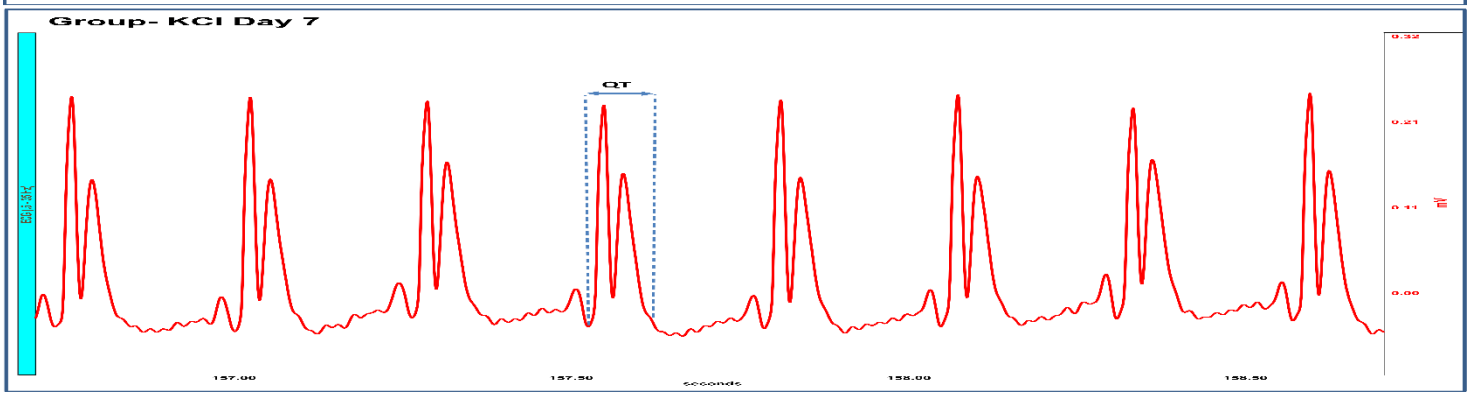
Group- IV (RAB)





Group- V ($MgSO_4$)





Group- VI (KCl)

ECG of all the animals of each group was recorded; the QT and R-R interval were used to calculate the QTc value. Due to inter strain variability and dearth of standard QT value in rats, self-control value was considered as normal; any higher value which showed significant increase compared to self-control was considered as prolongation in QT interval. Sample ECG tracing of each group after the seventh day of dosing and its corresponding self-control.

TABLE 4. ECG Data (Mean±SEM)

Electrocardiogram Data				
Groups		**QT (msec)**	**RR (msec)**	**QTc (msec)**
Group-I (FUR+CLA)	Self-Control	0.069±0.001	0.246±0.002	0.140±0.002
	7th Day	0.089±0.001	0.253±0.001	0.173±0.002
Group-II (FUR+CLA+RB-OD)	Self-Control	0.066±0.001	0.255±0.003	0.122±0.001
	7th Day	0.139±0.001	0.244±0.004	0.287±0.002
Group-III (FUR+CLA+RAB-BD)	Self-Control	0.060±0.004	0.237±0.002	0.148±0.004
	7th Day	0.160±0.001	0.230±0.003	0.342±0.002
Group-IV (RAB)	Self-Control	0.072±0.002	0.196±0.001	0.183±0.005
	7th Day	0.100±0.001	0.191±0.001	0.271±0.002
Group-V ($MgSO_4$+RAB)	Self-Control	0.075±0.001	0.227±0.001	0.163±0.001
	7th Day	0.073±0.001	0.227±0.001	0.156±0.002
Group-VI (KCl+RAB)	Self-Control	0.079±0.001	0.212±0.001	0.186±0.002
	7th Day	0.094±0.004	0.271±0.001	0.174±0.001

1.1. Rabeprazole prolongs QT and QTc interval in hypokalaemic rat and also normal rat.

After 7day treatment of four groups (Group1 to 4) with previously mentioned doses, the QT, RR and QTc data were taken and compared to the self-control data. In all four groups QT and QTc were significantly prolonged ($p<0.001$ at CL 95%) where RR interval was not shown any significant changed. This indicate that rabeprazole has a potency to prolong QTc not only in compromised group but also in normal group.

1.2. Effects of $MgSO_4$ and KCl on rabeprazole induced QT prolongation.

Similarly, after 7day treatment of Group 5 and 6 with previously mentioned doses, the QT, RR and QTc data were taken and compared to the self-control data. In Groups-5 there is no significance change QT and QTc where in Group 6 QT and QTc were significantly prolonged ($p<0.001$ at CL 95%). However, there is no significant changed in RR interval in case of both groups. This indicate that $MgSO_4$ may have an ability to supress the rabeprazole induced QT prolongation toxicity but KCl may not have that type of activity.

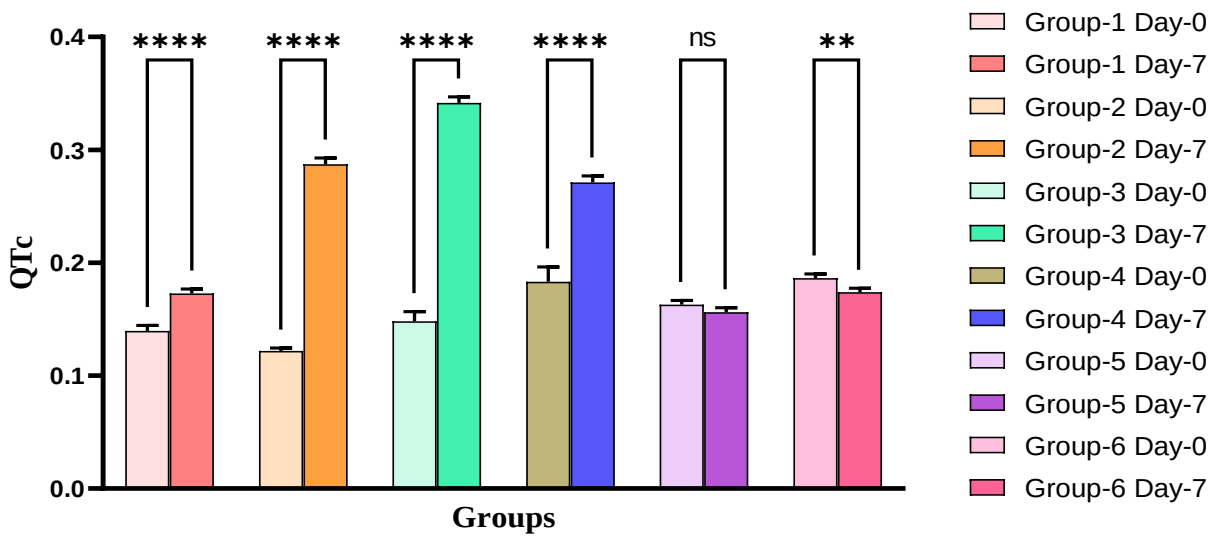


Figure 5a. QTc interval of groups with respect to their corresponding self-control. Values expressed as Mean ± SD. ****p<0.001, n=6.

TABLE 4. Serum electrolyte Data (Mean±SEM)

Serum Magnesium and Potassium Data			
Groups		**Mg^{2+} (mEq/L)**	**K^{+} (mEq/L)**
Group-I (FUR+CLA)	Self-Control	1.496±0.050	5.020±0.007
	7th Day	1.060±0.048	4.110±0.051
Group-II (FUR+CLA+RB-OD)	Self-Control	1.343±0.053	4.622±0.080
	7th Day	0.947±0.053	3.296±0.071
Group-III (FUR+CLA+RAB-BD)	Self-Control	1.214±0.045	3.975±0.072
	7th Day	0.781±0.044	2.223±0.000
Group-IV (RAB)	Self-Control	0.734±0.006	4.532±0.010
	7th Day	0.297±0.018	4.384±0.004
Group-V ($MgSO_4$+RAB)	Self-Control	1.252±0.015	4.524±0.030
	7th Day	1.136±0.032	4.008±0.001
Group-VI (KCl+RAB)	Self-Control	1.179±0.046	4.279±0.027
	7th Day	1.053±0.033	5.947±0.030

2. Serum electrolyte analysis

Serum potassium value of all the groups is tabulated in the table 4. A grouped serum magnesium and potassium data of the animals indicating their individual and group mean value is plotted in figure 5b and 5c. Seventh day serum magnesium data in Group 1, Group 2, Group 3, Group 4 is significantly lower than its self-control values ($p<0.001$ at CL 95%). The values observed are lower than the lower limits of magnesium required

in blood. Hence, a hypomagnesemia was established on comparison to their respective self-control values. In case of serum potassium level, Group 1-3 showed significantly low level ($p<0.001$ at CL 95%) where there was no significant change in Group 4 and in Group 6 serum potassium level significantly increased. So, it is established that in Group 1-3, hypokalaemic condition is appeared where in Group 6 hyperkaliaemic condition appeared.

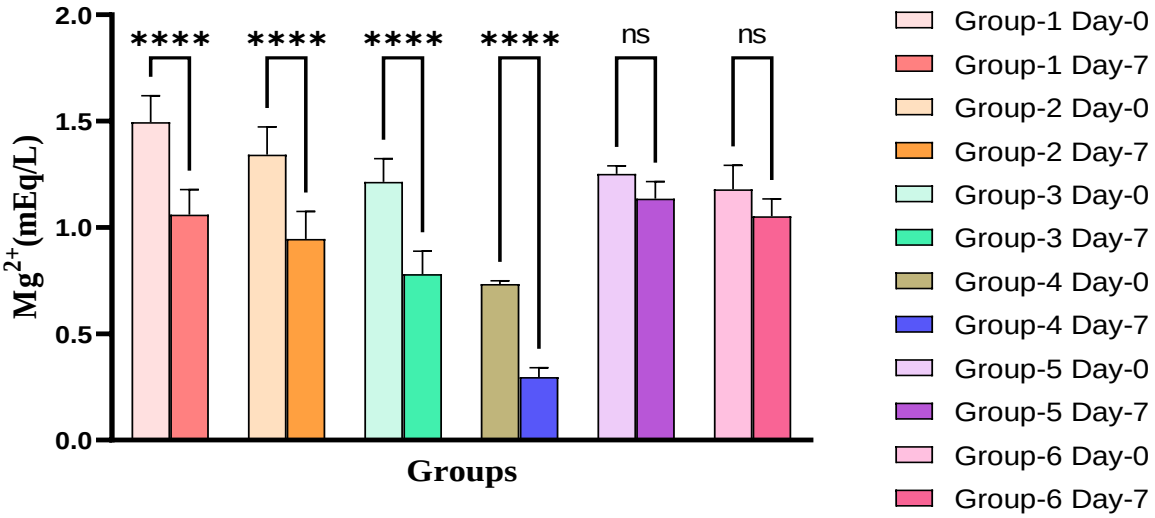


Figure 5b. Serum magnesium level of groups with respect to their corresponding self-control. Values expressed as Mean ± SD. ****p<0.001, n=6.

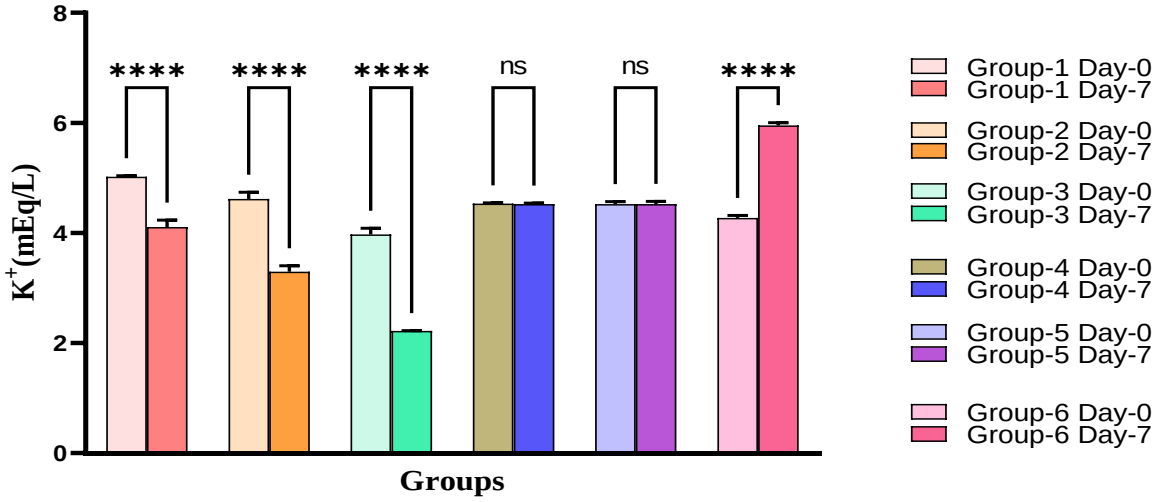


Figure 5a. Serum potassium level of groups with respect to their corresponding self-control. Values expressed as Mean ± SD. ****p<0.001, n=6.

Discussion

Involving rat for in-vivo model of QT prolongation studies is a highly debated idea, however the present instigation indicated some important findings which might be of value addition for this particular field of study. In our earlier research, we found that long-term administration of furosemide or clarithromycin did not significantly alter the QT/QTc interval; however, long-term (seven-day) co-administration of these medications (FUR+CLA; 10+50 mg.kg-1) resulted in a significantly prolonged QT/QTc interval.

Rabeprazole is a proton pump inhibitor which pharmacodynamic data show that it can achieve optimal acid suppression since the first administration and can maintain this advantage in the following days of therapy. Moreover, rabeprazole has the highest pKa (~ 5.0, the pH at which a drug becomes 50% protonated), and hence the molecule can be activated at higher pH levels much faster than other PPIs(94). It is believed to be safe with particular context of QT prolongation despite its major metabolism by CYP2C19 and CYP3A4(95,96) . PPIs affect the active transport route and not passive magnesium transport, was found to be somewhat linked with hypomagnesemia in some cases(97). It is still unclear how PPIs work to decrease intestinal magnesium absorption, and if certain PPI users are more likely to experience it than others or if it is a unique effect. PPIs may influence the active transport system's enzyme and/or channel activities directly or indirectly through changes in the pH of the intestine(98).

The important finding of this study is that in a state of artificially induced hypokalaemia co-administration of a specific dose of arrhythmogenic agent (clarithromycin) leads to attenuation of the safety margin of cardiac myocytes eventually leading to prolonged QT interval. Beyond which administration of ranitidine served as a provocation for further significant prolongation in the QT interval of rats. The heart has the exceptional capacity to adapt to stressful situations and continue to operate normally. However, it is evident that the heart may operate improperly and may exhibit an aberration in fundamental diagnostic indicators if its accommodating ability is depleted by any illness state or biological/chemical agent. One such substitute marker of the electrical function of the heart is the QT/QTc interval in the ECG. When the combination (FUR + CLA; 10+50 mg.kg-1) was delivered along with rabeprazole (OD and BD), a substantial QT interval lengthening was seen (Group II and Group III). However, in our experiment it was also observed that rabeprazole significantly the QT prolongation when concurrently used with Clarithromycin and Furosemide. However, rabeprazole induced QTc prolongation in normal

rat and significantly cause hypomagnesemia which can be attenuated by administration of Magnesium Sulphate impactfully than administration of KCl. It will be important to note that the extended QTc interval's measured duration exceeded that of the RR interval. Currently, cardiac arrhythmia is thought to be a signal of a cardiac emergency for this specific phenomenon.

This finding justifies that apart from the provocative group QT prolongation in normal group may cause due to lowering magnesium not lowering potassium. Mg^{2+} is commonly known as a natural Ca^{2+} channel blocker. Therefore, an intravenous injection of $MgSO_4$ causes a decrease of Ca^{2+} channel currents, especially the L-type Ca^{2+} current, and this should shorten the QT interval.(34) This probably indicate that lowering magnesium or chronic hypomagnesemia works in other ways to prolong QT which most likely be accompanied by an increase of L-type Ca^{2+} current.(38)Increase of $I_{ca}L$ should increase the intracellular Ca^{2+} concertation, activation of Na^{+}-Ca^{2+} exchanger current or transient inward current (I_{Ti}) would also be expected to prolong the QT interval. Mg^{2+} also cause transcriptional regulation of Kir 2.1-mRNA for Kir channel formation so loss of Mg^{2+} may also affect Kir transcription(38). The gene HERG(7q35-q36), which encodes a K1 channel that mediates IKr, was shown to be the cause of LQTS. When chromosome 7-associated familial LQTS was initially shown to be caused by the potassium ion channel HERG, it was also suggested that pharmacological blockage of the HERG channel would possibly cause acquired LQTS(98).

These facts refute the claim that rabeprazole is generally a cardiac-safe medication and point to the need for more research into rabeprazole safety issues. According to the hypothesis in the previous portion of this discussion, rabeprazole was the drug that stimulated the attenuated safety margin caused by the combination of FUR + CLA (10+50 mg.kg-1). Therefore, rabeprazole significantly prolonged QTc interval in our provocative model and also cause LQTS itself in normal group due to lowering of Magnesium levels. Insite of potassium level magnesium itself is an independent factor to prolong QT interval through another way and may precipitate arrythmia. This physiological condition may also be understood in actual clinical cases where hypokalaemia and hypomagnesemia brought on by medication, food, or herb use may increase the risk of cardiac arrest. Drugs like rabeprazole can be understood in terms of QT interval measurement.

Conclusion

Torsades de Pointes continues to be one of the most fatal types of cardiac arrhythmias in the world. Although the present advances of sciences have done a lot of work to minimize drug induced QT prolongation related deaths, many factors remain to be open in case of these incidents. The role of magnesium, and new pathways which are being discovered to prolong the action potential must be taken into consideration in the coming days while screening drugs for QT prolongation. Our work indicates the fact that common drugs which are widely used such as rabeprazole might precipitate arrhythmia and prolong QT intervals when used in combination with other QT prolonging drugs or when used in patients with genetic predisposition of LQT syndromes. Thus, a more stringent screening method keeping the factor of repolarisation reserve in mind and using modern personal medicine concepts where the patients' genome wide analysis will be done is needed to implement more safety measures in using the drugs. In the coming days, with approach of Systems Biology and Pharmacology we might understand the interaction of all these various channels which effect on the prolongation of action potential and finally a full proof screening method might come to screen the drugs.

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