

Comparative Study of Diuretics & Antimalarial Drugs on QT - Interval Prolongation in Rat

Bhavesh Barot

Kasi Sarva vidyalay University, K B Inst of Pharmaceutical Education and Research, Kadi Kampus, Gh-6, Sector-23, Gandhinagar, Ahmedabad, Gujarat 382023, India
bhavesh.barot[at]zyduscadila.com

Abstract: *Recently, numerous cases of electrocardiographic QT- interval prolongation and subsequently fatal polymorphic ventricular tachycardia termed Torsed de Pointes related to treatment with cardiac and non-cardiac drugs. The drugs are known to belong to different chemical class that may induce long QT syndrome via inhibition of a rapid component of delayed rectifier K⁺ channels (I_{Kr}) encoded by the human ether-a-go-go related gene encoding mutation often underlies such preclinical and clinical finding. Some drugs like diuretics (Hydrochlorothiazide and Furosemide) and antimalarial (Mefloquine, Artemether and Lumefantrine) has also been reported to be preclinically and clinically induce long QT Syndrome and further torsade de point and leads to sudden death.*

Keywords: LQTS: Long QT Syndrome, IKr: Rapid component of delayed rectified current, QTc: Corrected QT interval, APD: Action potential duration, ICH: International Conference on Harmonization, ECG: Electrocardiograph, IKs: Slow Rectified Potassium Current, INa: Rectified Sodium Current, ICa: Rectified calcium current.

1. Introduction

In the past decade, the single most common cause of the withdrawal or restriction of the use of drugs that have already been marketed has been the prolongation myocardial repolarization, an abnormally long QT interval in surface (ECG). Drugs that produce acquired long QT syndrome associated with polymorphic ventricular tachycardia, or torsade de pointes, which can be fatal. The prevalence of LQTS is difficult to estimate, but, based on the currently increasing frequency of diagnosis, LQTS is expected to occur in 1 in 1,000-2,000 individuals.^{[1]-[3]}

Drug induced LQTS is characterized by prolonged ventricular action potentials duration (APD) due to Mutation occurs in Human Ether a go go related genes that can inhibit or decrease the repolarization of potassium Current (IKr) and cause QT prolongation. It is responsible to susceptibility of potentially life-threatening arrhythmias known as Torsade de pointes (TdP). Several experimental and clinical observations using monophasic action potential suggest a significant role for early afterdepolarization (EAD)-induced triggered activity in the genesis of TdP. These EADs can induce reentry and TdP if there is dispersion of repolarization across the wall of the heart. EADs may also be induced either by interventions that decrease the repolarizing K⁺ currents or increase the inward currents INa or ICa. Torsade de pointes (TdP) is a characteristic polymorphic ventricular arrhythmia associated with delayed ventricular repolarization as evidenced on the surface electrocardiogram by QT interval prolongation. It typically occurs in self-limiting bursts, causing dizziness and syncope, but may occasionally progress to ventricular fibrillation and sudden death. Acquired long QT syndromes are mainly caused by cardiac disease, electrolyte abnormalities or exposure to drugs that block rectifying potassium channels, especially IKr.^{[4]-[10]}

The ICH E14 established on Oct 2005 for provides recommendations to sponsors concerning the design,

conduct, analysis and interpretation of clinical studies to assess the potential of a drug to delay cardiac repolarization. Specifically, it calls for a clinical 'thorough QT/QTc study' (typically conducted in healthy volunteers), which is intended to determine whether a drug has a threshold pharmacological effect on cardiac repolarization, as detected by QT/QTc interval prolongation. The E14 recommendations are generally applicable not only to new drugs that have systemic bioavailability but also to approved drugs when a new dose, route of administration or target population that may result in an increased risk is explored.^[11]

According to ICH recommendation, there are many antihypertensive drugs like Hydrochlorothiazide, Furosemide and antimalarial drugs like Artemether, Lumefantrine and Mefloquine available in market, which are used since long years that cause QT prolongation risk. Long term use of these drugs alone or in combination with other QT prolonging drugs may cause fatal condition in patient due to Torsade de point.^{[12]-[20]} currently there is little study data available which indicate the safer therapeutic use of combination of this class of drugs.

The data obtained from the study will help the physicians in choosing right antimalarial drugs for those patients who are already on diuretics.

2. Materials and Methods

Experimental Animal

Healthy adult Sprague dawley rats of male sex weighing 200-220 gm were selected and divided into group, each group containing six animals, were acclimatized to standard condition of temperature (20-220 °C), Relative humidity (30-70%). Standard pellet diet were used. The experimental protocol approved by the institutional animal ethics committee, (CPCSEA) of K.B.Institute of Pharmaceutical Education and Research, Gandhinagar. (Reg. No. KBIPER/15/542)

Drugs

The following chemical agents were used: Hydrochlorothiazide gifted from Suvik Hi Tech. Pharmaceuticals, India. Furosemide, Artemether, Lumefantrine and Mefloquine drugs gifted by the Intas pharmaceutical ltd, India.

Study design

Rats of male sex divided as per below groups in tables. Doses was prepared in 0.5% Carboxy Methyl Cellulose (CMC) Solution. Doses were calculated based on standard oral doses of human.

Table 1: Individual doses per Groups

Group No.	Drugs	Dose
I	Hydrochlorothiazide	9 mg/10 ml daily.p.o.
II	Furosemide	14.4 mg/10 ml daily.p.o.
III	Artemether	3.6 mg/10 ml daily.p.o.
IV	Lumefantrine	21.6 mg/10 ml daily.p.o.
V	mefloquine	6.42 mg/10 ml daily.p.o.

Table 2: Two Drugdoses combination per Groups

Group No.	Drug	Dose
I	A+C	9mg/3.6mg/10 ml daily.p.o.
II	A+D	9mg/21.6mg/10 ml daily.p.o.
III	A+E	9mg/6.42mg/10 ml daily.p.o.
IV	B+C	14.4mg/3.6mg/10 ml daily.p.o.
V	B+D	14.4mg/21.6mg/10 ml daily.p.o.
VI	B+E	14.4mg/6.42mg/10 ml daily.p.o.

(A: Hydrochlorothiazide, B: Furosemide, C: Artemether, D: Lumefantrine, E: Mefloquine)

Table 3: Triple Drug doses combination per Groups

Group No.	Drug	Dose
I	A+C+D	9 mg/3.6 mg/21.6 mg/10ml daily.p.o.
II	A+C+E	9 mg/3.6 mg/6.42 mg/10ml daily.p.o.
III	A+D+E	9 mg/21.6 mg/6.42 mg daily.p.o.
IV	B+C+D	14.4 mg/3.6mg/21.6mg/daily.p.o.
V	B+C+E	14.4mg/3.6mg/6.42 mg/daily.p.o.
VI	B+D+E	14.4mg/21.6 mg/6.42 mg/daily.p.o.

(A: Hydrochlorothiazide, B: Furosemide, C: Artemether, D: Lumefantrine, E: Mefloquine)

Instrument

Power lab (AD instrument)

Limb lead II use for ECG measurement

3. Study Procedure

a) Dosing

Each group contain 6 animal. Each group were treated with diuretics and antimalarial drugs individually as shown as table-1 up to the 14 days. After sometimes rehabilitation

animals were reuse for combination study of diuretics and antimalarial drugs shown as table-2 and table-3 up to the 14 days. After a wash out period of 14 days of animals reused for combination study of diuretics and antimalarial drugs. The drug dosing was continued for 14 days and ECG recording was done a day prior to dosing and then on 7th and 14th days.

b) Surface electrocardiogram (ECG) recording in anesthetic rats with used of Power lab instrument:

Surface ECG recording is the most commonly used technique in anesthetized Rats. Rats were anesthetized using Ketamine (10mg.kg⁻¹). ECG was recorded by using Power lab for ECG measurement. In which bipolar limb lead II was used that contain three electrodes.^[21] First the Positive electrode was placed on left limb in subcutaneous layer, Second negative electrode was placed on right arm in subcutaneously and the third electrode was referential which was placed on left arm subcutaneously. On day 0 the ECG was recorded 20 seconds for all animals that will be considered as basal reading. Then the ECG recorded at 7th and 14th days for 20 second at every 3hr for 3 time. The Power lab Instrument was set for auto calculation of QT and RR interval from ECG recording.

c) QTc Interval Measurement

One of the earliest efforts to acquire a standardized heart rate correction formula was made by Bazett in 1920 ($QTc = QT / \sqrt{RR/100}$ [Sec], where RR is determined in the preceding RR interval). This exponential method enabled the comparison of QT intervals at different heart rates. However, it works best between 60 and 100 beats per minute, while it may give erroneous results at both slower (overcorrection) and faster heart rates (under correction).^[22]

d) Statistical analysis

The various intervals here expressed as Mean +/- SEM. The Statistical significance was determined using two way analysis of variance followed by Bornferroni's test (Graph pad Prism 5). The results were considered statistically significant at $P < 0.05$.

4. Results

The QTc intervals before and after chronic administration of some antimalarial and diuretic drugs were determined in rats. Each drug was administered by the most commonly used route in humans for 14 days either singly or in combinations. The ECG lead II was taken on day 0, 7 and 14 and QTc intervals were calculated. The summary of results obtained are described in Table. 6.

Table 4: QTc intervals of rats before and 7 and 14 days after drug treatment

Treatment	QTc (Sec) Days		
	0th	7 th	14th
Hydrochlorothiazide	0.1222±0.0120	0.1432±0.0058	0.1669±0.0054***
Furosemide	0.1325±0.0064	0.1477±0.0080**	0.1834±0.0140****
Artemether	0.1288±0.0031	0.1493±0.0052	0.1576±0.0060*
Lumefantrine	0.1508±0.0130	0.1765±0.0045**	0.2107±0.0110****
Mefloquine	0.1414±0.0100	0.1572±0.0043	0.1720±0.0047*
H+A	0.1238±0.0100*	0.1545±0.0042**	0.1885±0.0061****
H+L	0.1548±0.0092	0.1790±0.0044*	0.2102±0.0068****

H+M	0.1499±0.0086	0.1740±0.0104	0.1980±0.0076****
F+A	0.1498±0.0053	0.1637±0.0057****	0.2134±0.0063****
F+L	0.1541±0.0073	0.1762±0.0090**	0.2124±0.0067****
F+M	0.1437±0.0069	0.1686±0.0098*	0.2004±0.0067****
H+A+L	0.1429±0.0064***	0.1885±0.0064****	0.2442±0.0098****
H+L+M	0.1361±0.0049****	0.1801±0.0053****	0.2411±0.0076****
H+M+A	0.1437±0.0042****	0.1882±0.0048****	0.2458±0.0099****
F+A+L	0.1331±0.0036****	0.1776±0.0043****	0.2316±0.0045****
F+L+M	0.1380±0.0056****	0.1960±0.0070****	0.2479±0.0086****
F+M+A	0.1367±0.0051****	0.1899±0.0053****	0.2448±0.0100****

All Value represent Mean±SEM, n-6; the (*) symbols represent the significantly different values from 0 day values. Level of significance was denoted by number of asterisk. (*P<0.05).

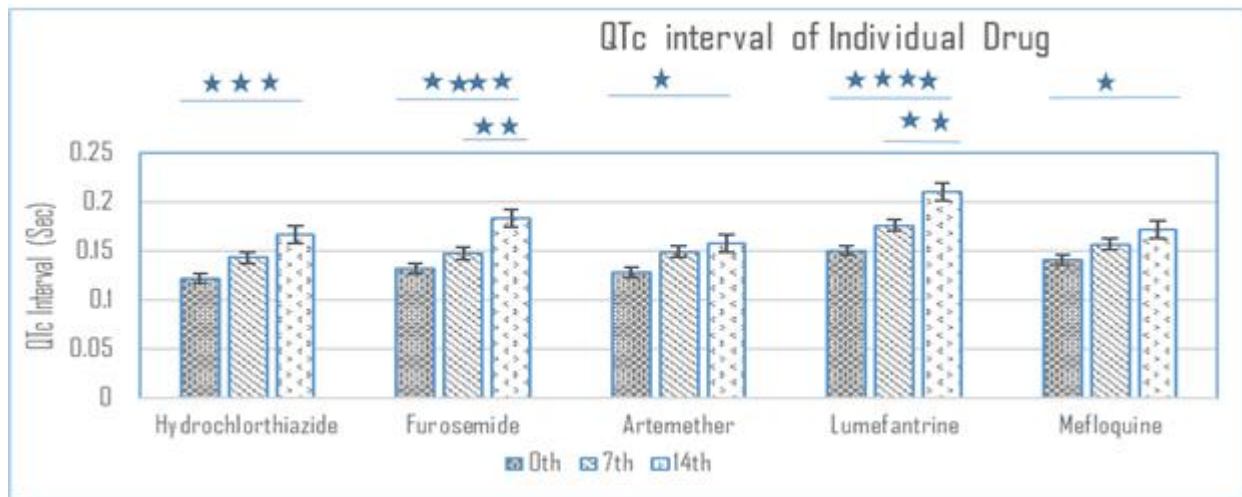


Figure (a): The effect of Individual drug treatment on QTc interval of Rat ECG

Each bar represents Mean±SEM of six animals measured on day 0, 7 and 14. The means of each groups were compared using Two way ANOVA followed by posthoc Bonferroni test at *P<0.05.

Effect of individually treated drug to each group on ECG parameter (QTc):

On day 7, the individual drugs were not shown significant effect on QTc interval. In Furosemide and Lumefantrine were increased significant effect after 7 days treatment on

QTc interval. Same in Hydrochlorothiazide, Artemether and Mefloquine were not shown significant effect after 7 days treatment. A significant increase in QTc interval from 0 day value was observed on day 14 for all the drugs

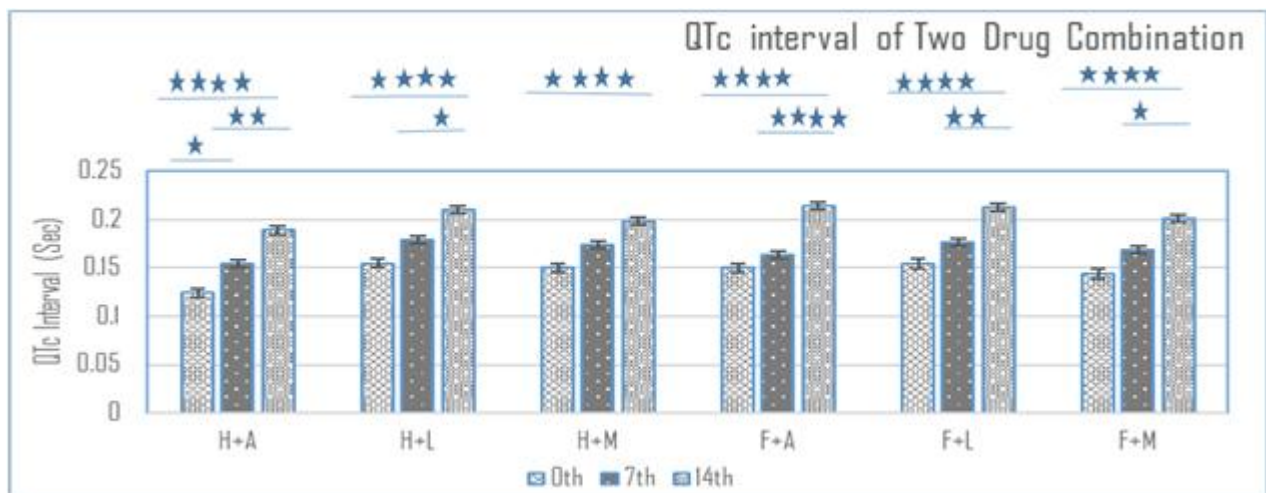


Figure (b): The effect of two drug combination drug treatment on QTc interval of Rat ECG.

Each bar represents Mean±SEM of six animals measured on day 0, 7 and 14. The means of each groups were compared using Two way ANOVA followed by posthoc Bonferroni test at *P<0.05.

Effect of two drug combination on ECG parameter (QTc):

The combination of two drugs, one from diuretics and other from antimalarial drugs were administered to same animal

after a washout of 14 days. The increase in QTc interval was still higher as compared to individual drugs. Thus combination has more impact on QTc interval than single agent.

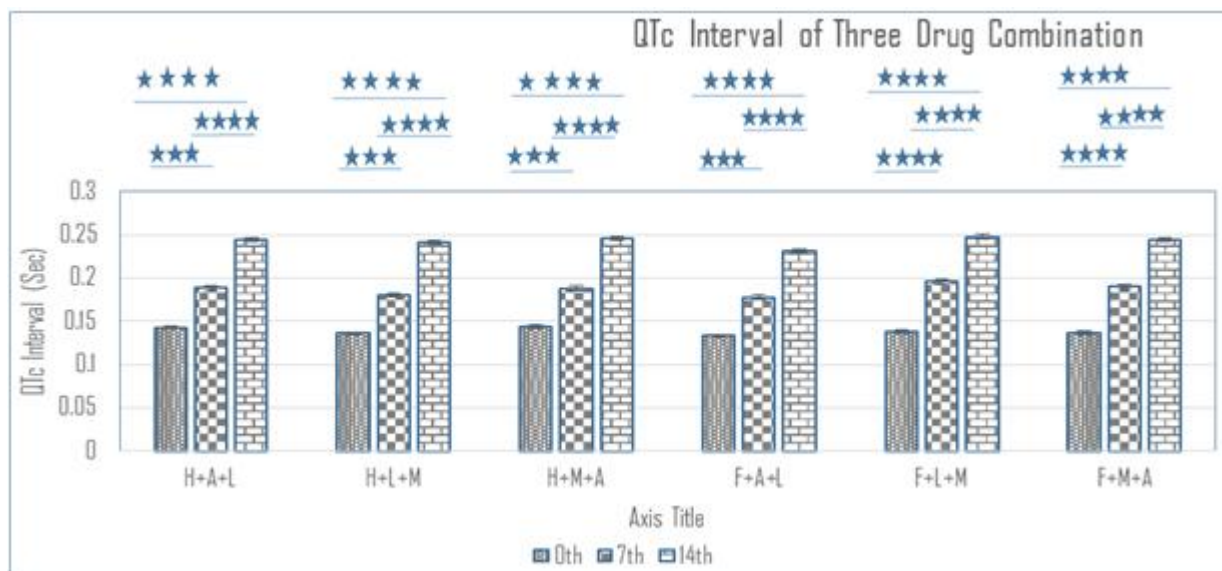


Figure (c): The effect of three drug combination drug treatment on QTc interval of Rat ECG.

Each bar represents Mean \pm SEM of six animals measured on day 0, 7 and 14. The means of each groups were compared using Two way ANOVA followed by posthoc Bonferroni test at * $P < 0.05$.

Effect of three drug combination on ECG parameter (QTc):

After another 14 days of washout period, the combination of three drugs was given to the same animals after recording the ECG. The three drugs combinations included one diuretics and two antimalarial drugs. The increase in QTc interval at 14th day as compared to basal value was still higher then observed for single drug or two drug combination. An additive effect is observed. The increase with all combinations was almost 2 folds.

5. Discussion

The present study was undertaken to evaluate pharmacodynamics interaction of diuretics and antimalarial drugs using experimental evaluation in rats. Some diuretics drugs like hydrochlorothiazide and furosemide and anti-malarial drugs like Artemether, lumefantrine and mefloquine that cause polymorphic ventricular tachycardia known as Torsade de Point.

Hydrochlorothiazide affects the renal tubular mechanisms of electrolyte reabsorption, directly increasing excretion of sodium and chloride in approximately equivalent amounts. Indirectly, the diuretic action of HCTZ reduces plasma volume, with consequent increase in urinary potassium loss, plasma renin activity, and aldosterone secretion, and decrease in serum potassium. It cause hypokalaemia that increases risk of cardiac arrhythmias.^[23]

Furosemide induce highly severe hypokalaemia with a serum potassium level of 1.1 mmol/l, is rare and associated with QT prolongation. If given for longer time it can cause life-threatening cardiac arrhythmias and cardiac arrest. Typically, furosemide-induced hypokalaemia is characterized by hypernatremia, hypocalcaemia, hypomagnesemia, high urine calcium creatinine ratio, high urine sodium excretion and high urine calcium/creatinine ratio. Hypokalaemia is the one of the reason in which the QT prolongation by inhibition of I_{Kr} current to cause

blocking of hERG channel^{[24], [25]}

Marked drug induce QT prolongations were also observed for artemisinin-based combination therapies (Artemether-lumefantrine [A-L]; Artesunate + Mefloquine; Artesunate + Amodiaquine).^[26] In these studies, Artemether and Lumefantrine induce with either Furosemide or Hydrochlorothiazide as for a longer period produce high risk cardiac toxicity and may increase with increasing combination that reduce ventricular repolarization and cause QT prolongation or cardiac arrhythmia. The treatment effect on QT interval may have been under- or overestimated.

Mefloquine potentiates the effect of drug that are given in combination to cause risk of QT prolongation Mefloquine also cause adverse cardiac effects. In case of drug administered for longer time in combination that produce risks for cardiac toxicity as compared to individual drugs and cause torsade de points. The Mefloquine cause L-type Ca⁺ channel blocking effect so the effect produce is similar to the blocking of K⁺ channel. This study was determine whether QTc interval is increased determinentally when given in combination at therapeutic doses.^{[27], [28]} Mefloquine produce less risk of cardiac toxicity as compared to Artemether and Furosemide by taken in combination. But cardiac toxicity effect may also increasing with increasing drug combination.

It is a quite common scenario in India medical practice that a CCF or hypertension patient on either hydrochlorothiazide or furosemide may contract malaria and a concurrent anti-malarial treatment may be given which can continued more than 7 days treatment that cause worsen condition. Thus clinically such combined therapy is quite frequent. Also in most cases more than two antimalarial may be prescribed the commonly with diuretics and antimalarial drugs were selected for the study.

We observed that the antimalarial drugs which have good cardiac safety when after drugs are given, aggravates the

QTc prolongation produced by hydrochlorothiazide or furosemide. The increasing is almost two folds and could be quite determinant to patients if we extrapolate our animal data to intervals.

Thus the present study generates warnings against the combined use of diuretics and antimalarial and physicians should take due care while prescribing.

6. Conclusion

Hydrochlorothiazide and furosemide when given chronically increase QTc interval. This increase is further enhanced by adding antimalarial drugs combination (one diuretic and two antimalarial) produce more increase in QTc intervals.

References

- [1] Roden DM. Drug-Induced Prolongation of the QT Interval. *N Engl J Med* 2004; 350:1013-22.
- [2] Roden D M and Viswanathan PC. Genetics of acquired long QT syndrome. *J. Clin. Invest.* 115:2025–2032 (2005).
- [3] Koren et al. Animal models of long QT syndrome and uses thereof. 2006
- [4] Eleftherios M. Kallergis, Christos A. Goudis, Emmanuel N. Simantirakis, George E. Kochiadakis, and Panos E. Mechanisms, Risk Factors, and Management of Acquired Long QT Syndrome: A Comprehensive Review. *The Scientific World Journal*. 2012
- [5] Turker I, Ai T, Itoh H, & Horie M. Drug-induced Fatal Arrhythmias: Acquired long QT and Brugada Syndromes. *j.pharmthera*. 2017.05.001
- [6] Miranda D G, McMain C L and Smith A J, Medication-Induced QT-Interval Prolongation and Torsade de Pointes. *US Pharm.* 2011; 36(2): HS-2-HS-8.
- [7] Curran M E, Splawski I, Timothy K W, Vincent G M, Green E D and Keating M T, A Molecular Basis for Cardiac Arrhythmia: HERG Mutations Cause Long QT Syndrome, *Cell*, March 10, 1995, Vol. 80, 795-803
- [8] Yee GY, John CA. Drug induce QT prolongation and torsade de point. *Heart* 2003; 89:1363–1372
- [9] El-Sherif, N, Turitto, G, Boutjdir, M. Acquired long QT syndrome and torsade de pointes. *Pacing Clin Electrophysiol.* 2018; 41: 414– 421
- [10] Roden DM. Long QT syndrome: reduced repolarization reserve and the genetic link. *J Intern Med* 2006; 259:59–69.
- [11] Shah R R, Drugs, QTc Interval Prolongation and Final ICH E14 Guideline. *Drug Safety*, Nov. 2005, 28(11):1009-1028
- [12] Atsushi K, Harumi Y, Hajime K, Mikio K. Effects of Anti-Malarial Drugs on the Electrocardiographic QT Interval Model led In the Isolated Perfused Guinea Pig Heart System. *Malaria Journal* 2010;9:318
- [13] George OA, Collins OB, Et Al, Electrocardiographic Study In Ghanaian Children with Uncomplicated Malaria, Treated With Artesunate-Amodiaquine Or Artemether - Lumefantrine. *Malaria Journal* 2012; 11:420
- [14] Gilbert LV, Sornchai L, Et Al. A Clinical and Pharmacokinetic trial Of Six Doses of Artemether-Lumefantrine for multidrug-resistant Plasmodium Falciparum Malaria in Thailand. *Am. J. Trop. Med. Hyg.* 2001; 64(5, 6):247–256
- [15] Margaretha B, Gilbert LV Et Al. Comparison of the Cardiac Effects Of The antimalarials co-artemether And Halofantrine in Healthy Participants. *Am. J. Trop. Med. Hyg.* 2002;66(3):293–298
- [16] Maude RJ, Plewes K, Faiz MA, et al. Does Artesunate prolong the electrocardiograph QT interval in patients with severe malaria?. *Am J Trop Med Hyg?* 2009; 80(1):126-132.
- [17] Konstantinos & Efremidis Et. Al., Drug-induced long QT syndrome, *Hellenic J Cardiol*, 2007, 48: 296-299.
- [18] Fermini B and Fossa A A, The impact of drug-induced QT interval prolongation on drug discovery and development. *Nature Rev. Drug Discov.* 2, Jul 2003, 439-447
- [19] Manodeep C, Jagadish V k. Pharmacodynamics Interaction Of Green Tea Extract With Hydrochlorothiazide Against Isoproterenol Induced Myocardial Damage In Rats. *Int. Res. J. Pharm.* 2014; 5(4).
- [20] Sanmoy K, Asif A, Rammohan B, Hindol M and Tuhinadri S, QT prolongation by ranitidine in hypokalemia and arrhythmogen provoked rat heart, *Vascul Dis Ther*, 2017, Vol. 2(4): 3-5
- [21] Konopelski P, Ufnal M. Electrocardiography in rats: a comparison to human. *Physiol Res.* 2016 Nov 23;65(5):717-725
- [22] Postema P G and Arthur A M Wilde, the Measurement of the QT Interval, *Current Cardiology Reviews*, 2014, 10, 287-294
- [23] Asdaq SM, Inamdar MN. The potential for interaction of hydrochlorothiazide with garlic in rats. *Chem Biol Interact.* 2009 Oct 30;181(3):472-9
- [24] Wolfgang R, Claudia S, Josef F And Franz W. Furosemide-Induced Severe Hypokalemia With Rhabdomyolysis Without Cardiac Arrest, *Bmc Women's Health* 2013; 13:30
- [25] Megum A, Masayoshi K Et. Al., Ecg Changes During Furosemide-Induced Hypokalemia In The Rat, *Journal of Electrocardiology* 1998;31(1)
- [26] Funck-Brentano C, Bacchieri A, Valentini G, Pace S, Tommasini S, Voiriot P, Ubben D, Duparc S, Evène E, Felices M, Corsi M. Effects of Dihydroartemisinin-Piperazine Phosphate and Artemether-Lumefantrine on QTc Interval Prolongation. *Sci Rep.* 2019 Jan 28;9(1):777
- [27] Susan J C, Andrew J B Et. Al., Effects of mefloquine on cardiac contractility and electrical activity in vivo , in isolated cardiac preparations, and in single ventricular myocytes, *British Journal of Pharmacology*, 2000 Feb., 129(2):323-30
- [28] Lightbown ID, Lambert JP, Edwards G, Coker SJ. Potentiation of halofantrine-induced QTc prolongation by mefloquine: correlation with blood concentrations of halofantrine. *Br J Pharmacol.* 2001; 132(1):197-204. doi:10.1038/sj.bjp.0703823