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DETERMINE THE EFFICACY OF DEVELOPED ARTEMETHER-LUMEFANTRINE NANOLIPOSOMES

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Abstract

While its half-life in the circulation is rather brief, the antimalarial medication artemether (ATM) is quite effective. In addition to being related with pro-arrhythmogenic hazards, ATM is also cardiotoxic. Our goal was to create a delivery mechanism that would allow for a sustained release of ATM into the circulation with little cardiotoxicity. Two anti-malarial medicines, artemisinin-based combination therapy (ART) and lumefantrine (LUM), have had their stability assays developed and confirmed using high performance thin layer chromatography (HPTLC). The PCL-NCs had the slowest release rate and the greatest proportion of ATM loading. Atomic force microscopy revealed nanoscale, spherical particles with a narrow size distribution. We used PCL NCs containing ATM for intravenous injection in biological tests.

Keywords: HPTLC, Artemether, Lumefantrine, Nanoliposome, cardiotoxicity; artemether; malaria; self-assembled polymers;

INTRODUCTION

Once thought to be a problem just for poor nations, malaria is now widespread across both the developed and the developing world. Rapid medication resistance, malaria's pervasiveness, and the absence of incentive for the commercial sector to become involved are the biggest problems the health community has in its battle against the disease. It also takes a long time and a lot of money to get a new medicine to market (Aditya et al., 2012). The most important step toward solving this issue is improving the therapeutic efficacy of the medicines that are already available. Two antimalarial medicines, each with a different site of action or mode of action, are often used in artemisinin-based combination treatment. In theory, artemisinin or a derivative of it would lower the parasite load quickly, and the second medicine would eliminate any leftover parasites from the blood or the parasite reservoir to prevent a recurrence.

The ratio of artemether to lumefantrine in this treatment plan is 1:6. Lumefantrine works to kill any residual parasites or parasites that have emerged from hibernation (mainly merozoites) after artemether has already reduced the parasite load. Oral pills containing both artemether and lumefantrine may now be purchased. An alternative, injectable version of artemether with lumefantrine, is proposed as a solution to these issues. Standard injectable formulation is insufficient for effective disease therapy due to pharmacokinetic mismatch and the high hydrophobicity of the medicines involved.

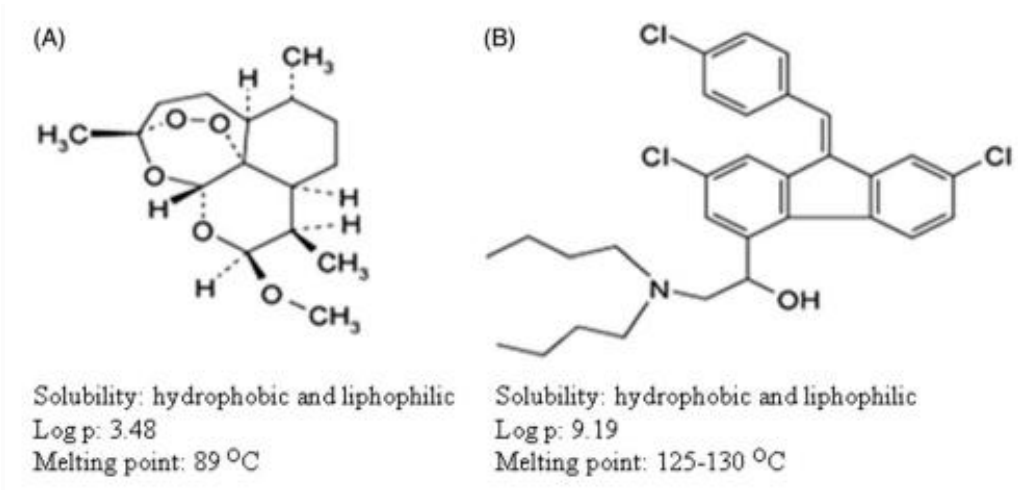

Figure 1: Chemical structure and technical information of artemether (A) and lumefantrine (B).

The first fixed-dose artemisinin-based combination therapy, artemether (ART) and lumefantrine (LUM) have been used successfully to treat uncomplicated malaria due to their synergistic impact and reduced side effects. The chemical formula for ART is [(3R, 5aS)-6R-8S-9S-10R-12R-12R].It is a semisynthetic polyoxygenated amorphene with the chemical formula [10-methoxy-3,6,9-trimethyl-3,12-epoxy-12H-pyrano(4,3-j)-1,2-benzodioxepin]. (Figure 1a) exhibit potent antimalarial activities due to a peroxide bridge. Proven effective against falciparum malaria, including chloroquine-resistant strains, falciparum malaria with sequelae, and cerebral malaria, this drug acts rapidly as a potent blood schizonticide. Drugs that are chemically activated inside the feeding vacuole of the parasite during its intraerythrocytic stage are thought to be effective against malaria.

LITERATURE REVIEW

Gubae, K., Mohammed, H., Sime, H. *et al.* (2023), Shecha Health Centre in Arba Minch town, Southern Ethiopia, performed a prospective, one-arm, 28-day study of the clinical and parasitological response to AL. Patients were given a three-day, six-dose course of AL and then evaluated clinically and in the lab again 28 days later. The selection of participants and the classification of results followed the same criteria as those established by the World Health Organization in 2009 for tracking the efficacy of anti-malarial medications. Eighty-eight people were recruited for the trial, and 69 of them showed a satisfactory clinical and parasitological response. Cure rates calculated using PCR were 98.6% (95% CI 92.3-100). The parasite and fever were quickly cleared in AL, with no parasitaemia on day 2 and no febrile patients on day 3. The removal of gametes was completed on day 3. Throughout the 28-day follow-up period, no major adverse events occurred. The research showed that AL has a strong therapeutic effectiveness and a low safety profile. This data provides further support for AL's continued use as the first medication of choice for treating uncomplicated P. falciparum malaria in Ethiopia.

Ali, S., Imam, S.S., Rahman, M.A., Jain, G., & Ahmad, F.J. Shakeel, K., Raisuddin, S. (2019). Artemether and lumefantrine (ART/LUM) combination therapy is widely used to treat uncomplicated malaria across the globe. Both medications were encapsulated in nanoliposomes (NLs) and then freeze-dried. A 30-hour in vitro release investigation revealed a burst effect at first, followed by a persistent release pattern. Histopathological analysis and liver and kidney function testing were used to evaluate toxicity in in vivo research. When each component was analyzed on its own, nanoliposomes had the lowest hemolytic potential (10%) of the bunch. Tissue analysis showed that RES organs, especially the liver and spleen, had a relatively significant absorption of ART + LUM-NLs. Hepato- and nephrotoxicity testing verified the proposed formulation was biocompatible by revealing no fibrosis,

fatty infiltration, centrilobular necrosis, or lymphocyte infiltration.

Erasmus Kamugisha, Reginald A. Kavishe, and Abdunoor M. Kabanywanyi (2019), Patients who met the inclusion criteria were recruited at each of the four locations, given normal dosages of AL, and followed for 28 days with clinical and laboratory evaluations. Using Plasmodium falciparum kelch 13 (Pfk13) and P. falciparum multi-drug resistance 1 (Pfmdr1) genes as surrogates for resistance to artemisinin and lumefantrine, we surveyed the frequency of single nucleotide polymorphisms in these genes. There were 344 patients initially recruited but only 335 (97.4%) were included in the analysis due to withdrawals and patients who were lost to follow-up. A clinical and parasitological response of > 98% was achieved, despite treatment failure in two individuals that were subjected to PCR correction. Rates of positive on day 3 varied from zero to 5.7 percent. Coughing, stomach discomfort, vomiting, and diarrhea were among the most frequently reported side effects. All 344 day 0 samples were sequenced successfully for the Pfk13 gene, while all 344 day 0 samples were sequenced successfully for the Pfmdr1 gene. Six of the isolates showed non-synonymous alterations in Pfk13, although this mutation type has not been associated with artemisinin resistance in the past. The study's findings of AL's effectiveness and safety were in line with prior research.

Deepa Parashar, Aditya N. P. & Murthy R. S. R. (2016), In this investigation, we concentrate on nanostructured lipid carriers (NLCs) that may be injected and that carry the antimalarials artemether and lumefantrine. Homogenization and ultrasonication were used to create NLCs containing artemether and lumefantrine. We evaluated the synthesized NLCs' physicochemical qualities, and we used an in vivo animal model to examine the formulation's potential usefulness in treating malaria. The hydrodynamic diameter of artemether and lumefantrine NLCs was 145 nm, and their surface charge was 66 mV. Artemether (84%) and lumefantrine (79%) are both lipophilic drugs with a high encapsulation efficiency in both single-drug-loaded and co-loaded NLCs. Biphasic drug release was seen in an in vitro investigation, with 63% artemether released after 24 hours and 45% lumefantrine released after 30 hours.

Rita Naresh Wadetwar, Pranita Sunil Kanojiya, and Yogita Manohar Charde (2018), The antimalarial medication artemether (ART) has limited solubility and bioavailability. Therefore, the rapid dissolving tablet includes a designed solid dispersion of the medication utilizing Soluplus (SOL). The rotary evaporator solvent evaporation technique was used to make the solid dispersion (SD). The improved SD was tested, and subsequently included to the tablet. According to the results of the solubility tests, the solubility and dissolving rate of ART SD A3 with a ratio of 1:3 (ART: SOP) were substantially greater than those of plain ART. The medication and its hydrophilic carrier were shown to be compatible, according to FTIR analysis. The drug's crystalline state changed to an amorphous one, as shown by DSC and XRD analyses. Scanning electron microscopy analyses showed that ART had been deposited on the hydrophilic carrier. The SD formulation increased the ART's in-vitro antimalarial activity. ART SD A3 fast dissolving tablets were made using immediately compressible excipients like Ludiflash and Ludipress. A tablet containing ludiflash disintegrated rapidly and released its contents in high concentrations. The mice pharmacokinetic research demonstrated a 1.88- and 3.19-fold increase in Cmax and AUC0-24, respectively, compared to the plain medication. In conclusion, SOP-prepared SDs enhanced the solubility and bioavailability of ART, making tablet formulation more practical.

Analysis of ART and LUM in developed nanoliposome

Our lab-made nanoliposomes include ART and LUM, and this experiment aims to determine how much of each is there. After 20 minutes of sonication in 100 ml of methanol, the final volume was 100 ml, with the formulation comprising 20 mg ART and 120 mg LUM. After centrifuging the solution at 5000 rpm for 10 minutes, the concentration of the drug could be seen. A 1 L concentration of the filtered

solution was applied to a TLC plate, which was then developed and scanned in the same way as described before. The experiment was performed three times. Analysis interference by excipients was investigated.

Stability studies

The new HPTLC technique was put through forced degradation trials in compliance with ICH recommendations to assess its stability suggesting features. Studies of the conventional medications' degradation by acids, bases, oxidation, moist heat, dry heat, and light were conducted.

Acid- and base-induced degradation study

Five milliliters of ART and LUM stock solutions in methanol were combined with five milliliters of HCl and five milliliters of NaOH (0.001 M) in 25 milliliter volumetric flasks. For 1 hour, the mixtures were subjected to a 60°C reflux. The forced deterioration was carried out without any ambient light present so as to rule out any potential light-induced degradation. TLC plates were used to transfer the resulting solutions, and chromatograms were taken at the concentrations indicated above.

Photo-degradation study

The standard powder medications were exposed to UV light in a photo-stability chamber for 24 hours for the photo-degradation investigation. In order to optimize the chromatographic conditions, suitable dilutions were made in methanol.

Study of acid-induced degradation kinetics

At predetermined time intervals, a microsyringe was used to quantitatively transfer the contents of the flask (100 l) into 10 ml volumetric flasks. Next, 1 l was spotted onto a TLC plate, and the concentration was determined using the proposed technique and an external standard. There were three separate trials of the experiment. For each combination of temperature and time, the concentration of unused medication was determined. Degradation kinetics constants were determined after further data processing.

RESULTS AND DISCUSSION

With the goal of creating a stability indicating assay technique for concurrently quantifying ART and LUM, the TLC approach was refined. TLC plates were used to separate pure medicine from degraded products, and many solvent systems were attempted for this purpose. Toluene:ethylacetate:ammonia is a mobile phase in which the best resolution was attained with Rf values of 0.700.02 for ART and 0.520.02 for LUM, respectively. Clearer separation was seen between standard and depredate spots on TLC plates that had been pre-soaked in concentrated ammonia vapors for 20 minutes in the TLC chamber. Removing the plate's edges and ensuring uniform solvent evaporation across the plate are necessary precautions before developing, this was required, both of which may cause erratic behavior and, ultimately, poor repeatability in Rf values.

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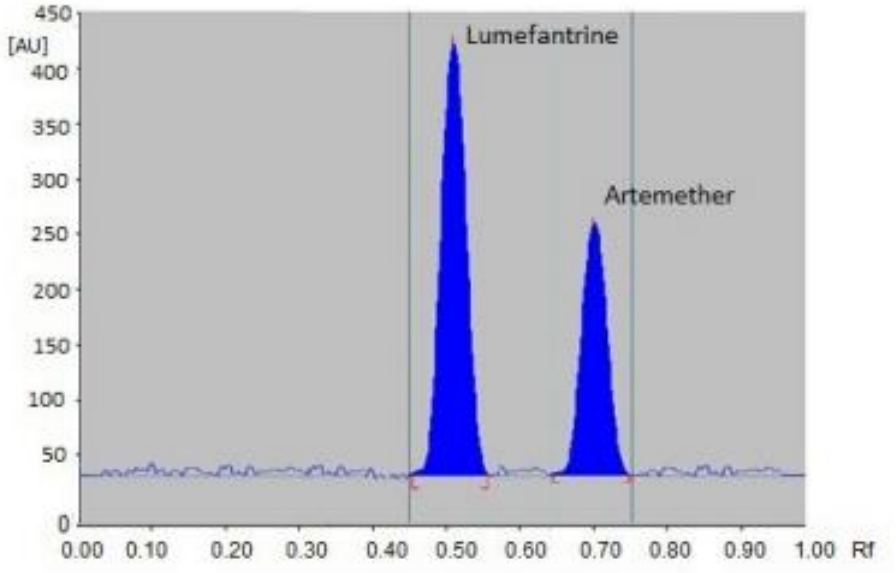

Figure 2: Chromatogram of standard

Method validation Linearity

A linear association between peak area and concentration was found to be more robust than that between peak height and concentration. The r2 value for ART was 0.997 while the r2 value for LUM was 0.998. The concentration ranges used to build the calibration curves for ART and LUM were 20-120 ng spot-1 and 100-600 ng spot-1, respectively. Table 1 displays the results of the regression analysis performed on the two medicines, including the correlation coefficients, y-intercepts, and slopes. There was no statistically significant variation in the slopes of the standard plots (analysis of variance, $P > 0.05$).

Precision and accuracy

Table 2 displays the coefficient of variation (%CV) and percentage recovery for ART and LUM at three different levels of quality control. Precisions of 0.99% and 0.84% for ART and LUM, respectively, within a single day ($n = 6$), and 1.12% and 0.62%, respectively, across days, demonstrate the high quality of the proposed method. Both ART and LUM had intraday accuracies of 99.2–99.8% and 99.6–99.8%, respectively, while their interday accuracies were 99.1–100.2% and 99.7-99.9%. These results are within the allowable margin of error, suggesting the procedure was successful.

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Table 1: Linear regression data

Parameters	ART	LUM
Linearity range	20-120	100-600
Linear regression equation	y=118.4x + 52.03	y=176.7x + 2183
Slope ± SD	118.4±0.252	176.7 ± 0.426
Intercept ± SD	52.03 ± 0.725	2183 ± 1.236
Correlation coefficient (r2)	0.997	0.998
Limit of detection (LOD)	1.73	5.19
Limit of quantification (LOQ)	5.24	15.72

Table 2: Precision and accuracy

Drug	Nominal Conc. (ng spot-1)	Conc. found (ng spot-1) ±SD	Precision (% CV)	Accuracy (%)	Conc. found (ng spot-1) ±SD	Precision (% CV)	Accuracy (%)
		Intra-day batch			Inter-day batch		
	20	19.84 ± 0.16	0.81	99.2	20.04 ± 0.13	0.65	100.2
ART	60	59.6 ± 0.43	0.72	99.3	59.48 ± 0.22	0.34	99.1
	120	119.75 ± 1.18	0.99	99.8	119.18 ± 1.34	1.12	99.3
	100	99.63 ± 0.84	0.84	99.6	99.83 ± 0.54	0.54	99.8
LUM	300	299.75 ± 2.12	0.71	99.9	299.17 ± 1.86	0.62	99.7
	600	598.94 ± 2.78	0.46	99.8	599.62 ± 2.75	0.46	99.9

Antimalarial Efficacy in P. berghei-Infected Mice

ATM-PCL-NCs had antimalarial effects similar to free-ATM in P. berghei-infected Swiss mice. Mice with low parasitemia were administered intravenously (IV) doses of 40 or 80 mg/kg four times daily. Even at these high dosages, infected mice showed no signs of systemic damage. Each approach was also applied to a control group that did not receive any treatment. Parasite loads remained high in the control groups. Between day 5 and day 10, all of the animals perished (Figure 3). When given intravenously

(IV) for four days in a row, either free ATM or ATM-PCL-NCs at 40 or 80 mg/kg/day decreased parasitemia to extremely low levels, prevented infection progression, and enhanced mouse survival (Figure 3). The therapeutic efficacy of ATM treatment for durations up to 10 days was confirmed across all study groups. After 60 days of therapy, there was no recurrence. Note that blank-PCL-NCs had no antimalarial effect, since their survival profile and parasitemia levels were identical to those of mice treated with glucose solution. Both dosages and formulations were equally effective in reducing parasitemia and increasing host survival after several administrations (Figure 3).

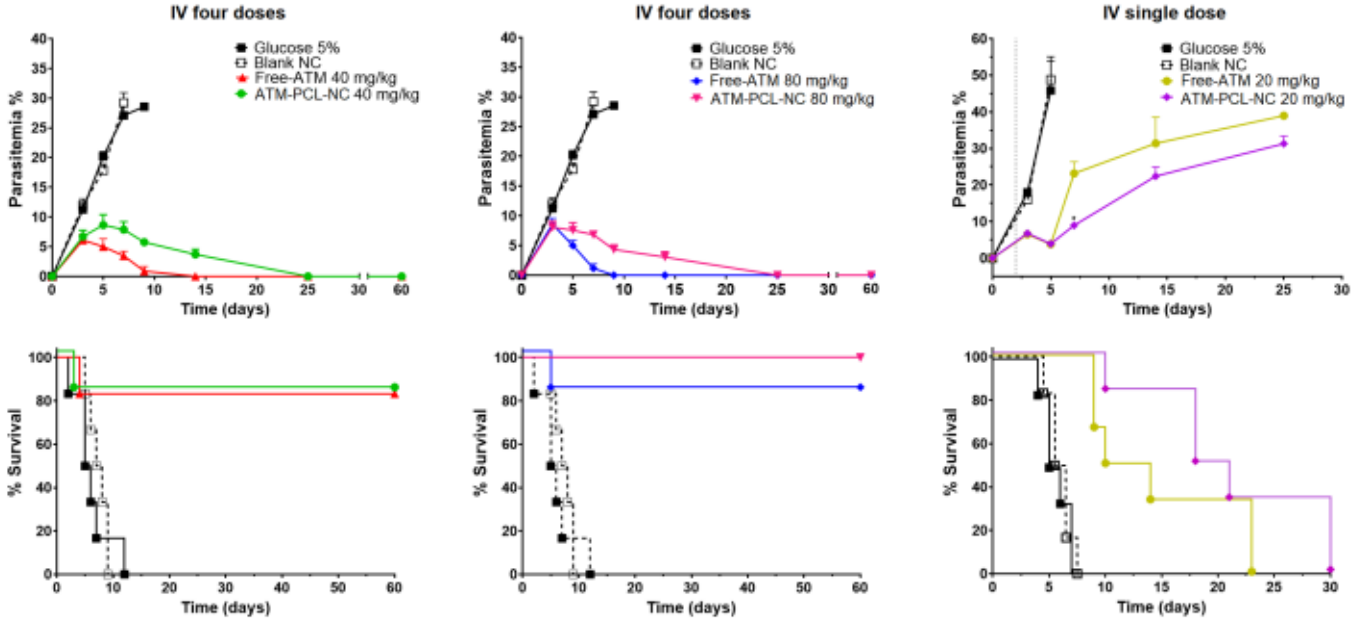

Figure 3. Efficacy of the artemether formulations represented in graphs of parasitemia (%) and survival (%) for both protocols (four-day-test) and single low dose (20 mg/kg) with established parasitemia (15%).

We used the second method for evaluating the efficacy of treatments to see how a single, modest dosage of ATM affected the mice with a persistent illness. ATM-PCL-NCs expedited increases in mouse survival and decreases in parasitemia compared to single doses of free-ATM solution.

Determination of Cardiovascular Effects of ATM

Electrocardiogram (ECG) examples before and after intravenous (IV) injection of blank NCs, free-ATM, and ATM-PCL-NCs in rats are shown in Figure 4. Rapidly (within one to twenty minutes after delivery), and persistently (throughout the trial), the QT and QTc intervals of the ECG were prolonged following intravenous injection of free-ATM (Figure 5). The intensity of the results depended on the amount consumed. Lessening of QT and QTc prolongation after administration of ATM-PCL-NCs was observed. When compared to the control group, the ECG's PR and QRS intervals were not affected by free-ATM or ATM-PCL-NCs. The free-ATM dose of 120 mg/kg was also investigated, however at such dose, most of the animals died from ECG abnormalities and arrhythmia.

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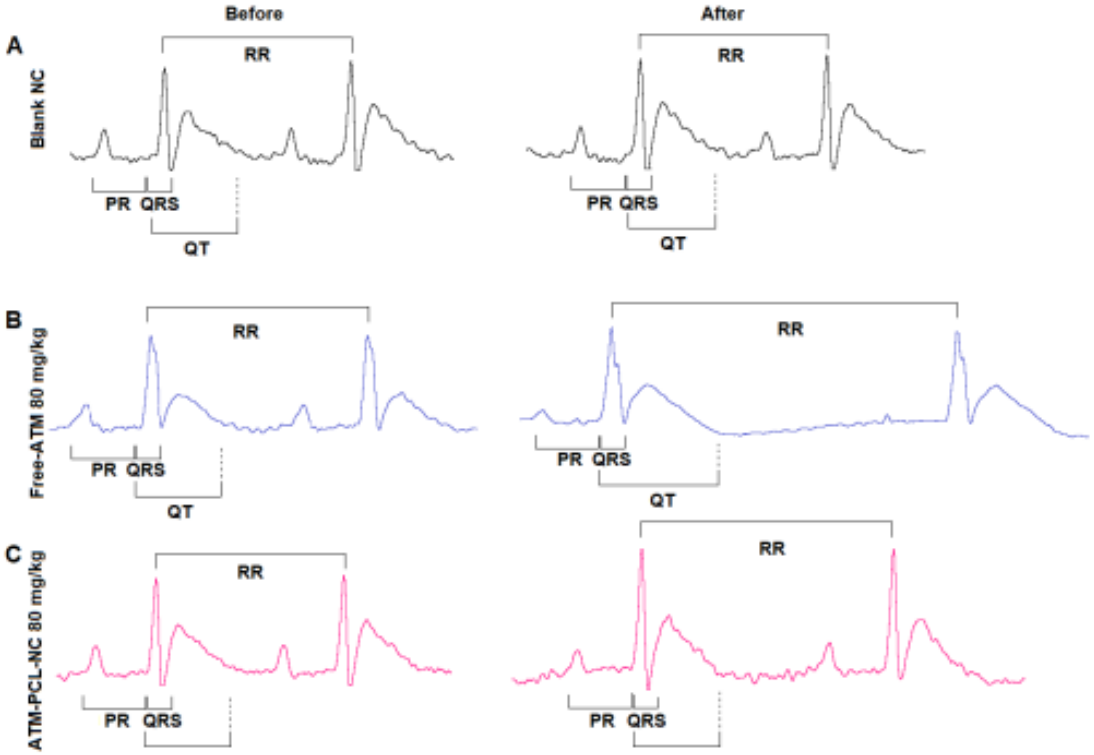
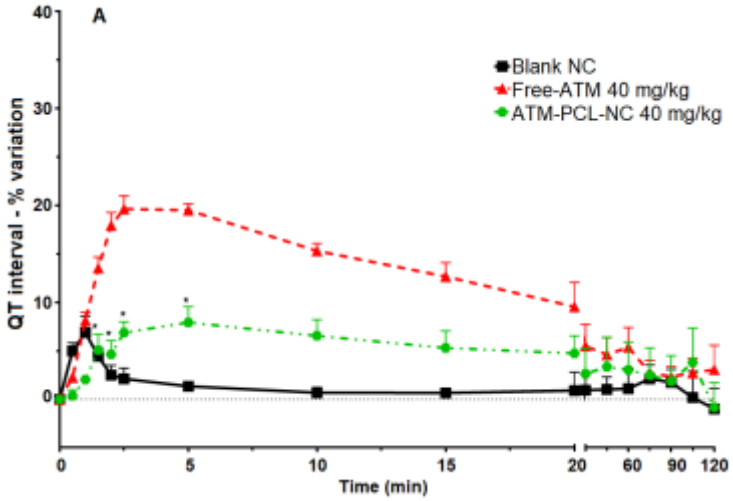


Figure 4. Representative ECG signal (lead II) at basal (before treatment) and after treatment with



Blank-NC

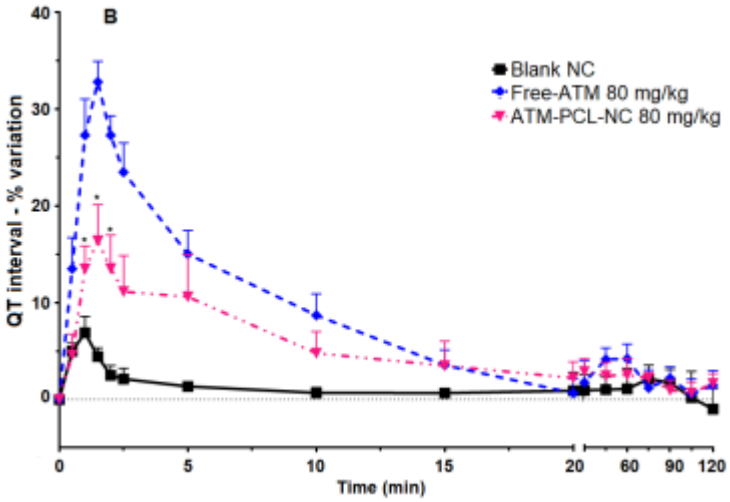


Figure 5. QT interval percentual variation

CONCLUSION

The new HPTLC method proved reliable and gave clear indications of stability. The study of data shows that the technique is appropriate for the simultaneous estimate of ART and LUM. This research is representative of the kind of work done to create a stability-indicating test in accordance with ICH criteria. One of the few studies that have tried forced decomposition under all of the recommended conditions and to have succeeded in resolving the degradation products, this is a unique and important contribution. The drug and degradation products may be identified in industrial stability samples with its help. It's also possible that the contaminant was introduced during the medication's manufacture or storage process as a result of hydrolysis or oxidation. The results presented above supported the proposed strategy for studying the dynamics of acid-induced deterioration of ART and LUM. Hence, combining the ATM with NCs is an attractive option for intravenous delivery of the ATM. It seems that additional lipophilic, pro-arrhythmogenic medicines might benefit from a similar method of modifying drug distribution using polymeric NCs. Important in vivo approach for assessing the cardiotoxicity of nanoparticulate drug carriers has been developed with help from this work. In conclusion, the usage of polymeric NCs is a potential technique for the development of less dangerous intravenous administrations of a wide variety of cardiotoxic medicines.

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