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# Emetine dihydrochloride hydrate showed potent antimalarial properties on the multidrug resistant strain (K1) of *Plasmodium falciparum:* A step forward towards drug repurposing

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**Abstract**

**Malaria is a life threatening infectious disease that is characterized by recurrent high fevers and, neurological impairments such as brain damage and coma, in the case of cerebral malaria. Pregnant women and children are most at risk with 85% of deaths occurring in children under five years of age. Due to lack of a licensed malaria vaccine and resistance to available antimalarials including the frontline artemisinin combination therapies (ACTs), malaria imposes a heavy social burden that has delayed economic development in regions where it is endemic. Traditional drug discovery process is both cost and time consuming. Drug repositioning or the re-profiling of existing drugs for diseases other than those they were originally meant for, offers the opportunity to explore the existing knowledge on drugs, diseases and targets and helps us to find a new use of already available compound or drug. In addition, drug repurposing shortens the traditional drug discovery pipeline. SYBR Green flow cytometer method and microscopy were used to determine the *in vitro* antimalarial effect of the anti-protozoal drug emetine dihydrochloride hydrate namely on the multidrug resistant strain (K1) of *Plasmodium falciparum.* Emetine showed potent antimalarial activity on the multidrug resistant strain (K1) of *P. falciparum,* with about 50% parasite clearance between 12.5 and 25 nM, and an estimated 90% parasite clearance at 50 nM.The finding validates previous results of other research and contributes additional evidence suggesting the potent antimalarial effect of emetine on the K1 strain of *P. falciparum.***

**Keywords:** Malaria, Emetine dihydrochloride hydrate, SYBR green flow cytometry, Microscopy.

## INTRODUCTION

Drug discovery has always been an interesting domain and, platform for exploration. A typical drug discovery process would take around 8-15 years of time before the introduction of drug into the market, and approximately 1 billion dollars is expended on drug discovery. However, the downstreamsuccesses of the drugs developed are not guaranteed. It is estimated that 90% of all drugs entering various clinical trials are discontinued, due largely to problems related to efficacy and safety (Tartaglia, 2006). Because traditional drug discovery process is both costly, time consuming and, resistance to all available antimalarials, including the leading artemisinins is evident, it is important that faster drug discovery process are developed to avoid the impending possibility of drug failure, whose consequences could be disastrous (Matthews et al., 2013).

Taking the very fact into consideration, the re-profiling of already existing drugs for other diseases other than the diseases they were formerly made for is essential. Drug repositioning or drug repurposing offers the opportunity to

explore the existing knowledge on drugs, diseases and targets and may help us to find a novel use of already available compounds or drugs. Moreover, sincedrug repurposing offers a relatively low-risk, regaining losses, saves both costs and time, most research and pharmaceutical companies are now shifting towards re-profiling of existing drugs.
Starting with a molecule that has already undergone clinical trials in another indication provides several potential advantages. The clinical safety profile would have been understood, and safe therapeutic doses established. Importantly, human pharmacokinetic data already exist and provide some indication of whether therapeutic concentrations in a new indication can be safely achieved and maintained in patients. In addition, there are regulatory fast track processes, such as the US Food and Drug Administration (FDA) 505 (b) (2) process, where the applicant can rely on data from the studies done by others (with or without the right to reference them) to progress the compound for a new indication that has acted as a spur to finding new applications for old molecules (Lotharius et al., 2014). Because of the amount of both cost and time spent on traditional drug discovery process, repurposing of existing drugs meant for other diseases for use in malaria offers partner drugs for combination with artemisinins and also shorten the drug discovery pipeline (Matthews et al., 2013).

Malaria is a protozoan disease and is caused by unicellular eukaryotic parasites of the genus *Plasmodium.* The disease istransmitted to humans by the bites of infected female *Anopheles* mosquitoes. Four main species of *Plasmodium* cause disease in humans: *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae.* In addition, a simian parasite, *P. knowlesi*, was recently reported occasionally to infect humans (Sabbatani et al., 2010). *P. falciparum* is the most virulent and causes most of the deaths, however, *P. vivax* is the most widespread (Flannery et al., 2013).
Malaria is an infectious disease that is characterized by recurrent high fevers and, neurological impairments including brain damage and coma, in the case of cerebral malaria. Pregnant women and children are at high risk with 85% of deaths occurring in children under five years of age (WHO 2014).While antenatal women and children are predominantly vulnerable to the threat of malaria, severe disease is also a threat for immuno-compromised individuals such as HIV/AIDS patients, and non-immune travellers to regions where malaria transmission is endemic (Andrews et al., 2014).

While the World Health Organisation (WHO) reveal that peopleliving in the poorest countries of the world are the most vulnerable to malaria, records show an estimated 3.4 billion individuals, almost half of the world's population – at risk of getting infected with the disease (Tekwaniand Walker 2005; WHO 2014). In 2012, an estimated 207 million cases and roughly 627,000-deaths due to malaria were recorded (Noubiap, 2014). However, there are indications that malaria mortality had reduced by 42%, due to an increase in prevention and control measures globally, and by 49%, in the WHO African region since 2000 (WHO 2014). Despite these reductions, 90% of all malaria mortality occurred typically amongst children below the age of five in the WHO African Region, (WHO 2014).It is, therefore, thought thatmalaria imposes a heavy social burden that has delayed economic development in regions where it is endemic (WHO , 2010; Murray et al., 2012).

**Emetine dihydrochloride hydrate**

Emetine is a natural alkaloid of *Uragogaipecacuanha.* It is typically employed asdihydrochloride, although other forms such as dihydroiodide, dinitrate, sulphate and acertasone salt have been recognized (Frayha et al., 1997). The drug is previously described as an antiprotozoal compound used in the treatment of invasive intestinal amoebiasis and amoebic liver abscess (Matthews et al., 2013). In *Entamoebahistolytica,* emetineis effective against the tissue offensive trophozoites by causing degeneration of the nucleus and reticulation of the cytoplasm, thus interfering with multiplication. Furthermore, emetine was thought to inhibit protein synthesis in *E. histolytica* and other protozoans as well as mammals primarily by blocking the movement of mRNA along the ribosome and secondarily blocking DNA synthesis (Frayha et al., 1997). However, the use of emetine for the treatment of invasive intestinal amoebiasis has been discouraged. This may not be unconnected to its side effects, including cardiomyopathy, nausea and vomiting, induced by irritation around the intestinal mucosa, following oral ingestion (Emmanuel and Oladapo, 2011).
The public health challenge posed by malaria is heaviest in the Afican regions where an estimated 90% of all malaria death occurs. The numbers of low-density sub patent infections across Africa are considerably higher (WHO 2014). Therefore monitoring of particularly drug-resistant malaria has prompted us to screen the potential anti-malarial activity of Emetine dihydrocholoride to treat the disease, or to explore the underlying mechanisms found to circumvent the phenomenon of drug resistance.

**MATERIALS AND METHODS**

The K1 strain of *P. falciparum* were maintained in complete Rosewell Park Memorial Institute (RPMI 1640) (Gibco) medium as described by (Matthews et al., 2013). To prepare a complete medium for parasite culture, 10 mg of

hypoxanthine (Sigma, UK) was dissolved in 10 ml phosphate buffered saline (PBS) and stored at 20$^{o}$C, 2.5 ml aliquot of hypoxanthine was added to 2.5 ml of 40% glucose (Dextrose Anhydrous, Fischer Scientific, UK) in a sterile 50 ml centrifuge tube and 0.5 ml of gentamycin (Sigma, UK) were added, then 2.5g albumax (Sigma, UK), was finally added and completely dissolved in 20 ml RPMI 1640 and filtered using a 0.22 µM Millipore swinnex filter unit and a 20 ml syringe. The filtrate was then added to the 500 ml RPMI 1640 media to make it a complete mediaand stored at 4$^{o}$ C in a refrigerator, until needed.

Blood (group O+) collected from healthy donors was provided by the UK health services. Whole blood was transferred into a 50 ml tube and an equal volume of RPMI 1640 was added and centrifuged at 3000g for 5 min, after which the supernatant and buffy coat were removed. The packed cells were re-suspended in RPMI 1640 and further centrifuged at 3000 g two to three times for 15 min each, to ensure that the white cells are completely removed. The exact volume of packed cells was determined, and an equal volume of RPMI 1640 was added to yield a 50% haematocrit. Prepared blood was stored at 4$^{o}$C until required.

*P. falciparum* parasites (strain K1) were cultured in 12.5 cm$^{3}$ flasks in final culture volumes of 10 ml and maintained at 5% final haematocrit. Parasitaemia was determined by transferring a drop of parasitized blood onto a clean glass slide and a thin blood film for giemsa staining was prepared. Dilution factor of parasitized blood with fresh blood was calculated to give approximately 0.5-1% parasitaemia. 10 ml of complete media was transferred into a 12.5 cm$^{3}$ culture flask, and 1 ml of the previously stored 50% haematocrit was added to give a final culture volume of 5% haematocrit. Flasks were incubated at 37$^{o}$ C for 30 to 45 minutes to warm the medium. The flask were then taken and shaken gently ensuring that blood cells did not form dry film at the bottom and avoided shaking toward the mouth to prevent contamination. The calculated volume of parasitized blood was transferred into the freshly prepared flask.Flasks were gassed with air mixture (BOC Limited, UK) containing 90% Nitrogen gas, 5% Carbon dioxide and 5% Oxygen, and incubated at 37$^{o}$C in the dark.

**Checking Parasitaemia**

In order to determine the level of parasitaemia in culture, a Giemsa solution was first prepared by using the standard procedure. Briefly, one buffer tablet (pH 6.4) for preparing buffer solution for staining blood smears (BDH Laboratories, UK) was dissolved in one litre of water to make a Stock Sorensen buffer. 35 ml of Sorensen buffer was then transferred to a fresh beaker and 3.5 ml of giemsa stain improved R66 solution (BDH Laboratories UK) was added and diluted.

A drop of parasitized blood from the culture was transferred onto a clean microscope glass slide and smeared to form thin blood film. This was allowed to air dry and later fixed by covering with 100% methanol and allowed to air dry. Slides were then immersed in Giemsa stain solution and allowed for approximately 20 minutes to stain, after which slides were then removed and gently rinsed in tap water and allowed to dry.

A drop of oil immersion was applied to the slides and viewed under 100× magnification in a light microscope (Leica Microsystems, Germany).

Parasitaemia was estimated by counting the number of red blood cells in microscope field and the number of parasite-infected red blood cells and, percentage of parasite-infected RBCs per 100 RBC counted was determined.

**Drug treatment of K1 strain of *P. falciparum.***

In this assay, to determine parasite density in drug treated samples in a 12.5 cm$^{3}$ culture flask, continuous parasite culture was centrifuged and the spent media removed. Percentage parasitaemia was determined by smear as described earlier. The exact volume of parasitized blood was determined, after which the parasitized blood was diluted to approximately 0.5% parasitaemia. 5 ml of diluted parasite culture was transferred into a 12.5 cm$^{3}$ culture flask at 5% haematocrit. 5 mM emetine dihydrochloride hydrate was prepared in DMSO, from which 5 µl was dissolved in 5ml RPMI 1640 (1:1000 dilution) to make a 5 µM stock required for the experiment. 1 ml of the stock (500nM) was added to the culture flask to account for resistance of the K1 strain of *P. falciparum* as previously described by Matthews et al., (2013).

A non-treated control flask was set up and both flasks were gassed as described previously and incubated at 37$^{o}$C in the dark for 24 hours and analysed using SYBR Green flow cytometer method.

**Staining Flask Sample with SYBR Green 1 for Flow Cytometry.**

From the previously incubated (drug treated and non-treated control) cultures, 500 µl was transferred into a 1.5 ml

eppendorf tube. 50 µl was taken from each tube and transferred to different eppendorf tubes (duplicate) and the remaining 400 µl were centrifuged at 13000g for 90 seconds.

To each of the duplicate samples (control, drug treated and non-infected blood), 1 ml of PBS was added and centrifuged at 13000 g for 90 seconds to wash off the media, after which the supernatant was discarded. 1 ml 5× SYBR Green was added to each sample, mixed and incubated in the dark for 20 minutes at room temperature. The 5× SYBR Green was prepared by transferring 10 ml PBS to 50 ml tube and adding 5 µl of SYBR Green nucleic acid gel stain (10,000 ×, Sigma, UK) and diluted. After staining, the samples were centrifuged at 14000g for 1 minute. Subsequently, a fixation solution was prepared by diluting 20 µl of formaldehyde solution 36.5% (Sigma-Aldrich) in 3 ml PBS (final concentration of 0.37%). An aliquot, 250 µl of the fixative was added to the samples and incubated for 15 minutes at $4^{o}$C. Samples were later centrifuged at 13000g for 90 seconds and the supernatant was discarded. 1 ml of PBS was added and washing was repeated twice at 13000g for 90 seconds. Samples were stored at $4^{o}$C and later analysed using BDFACs Verse flow cytometer.

**SYBR Green Flow Cytometer Method to Analyse the Samples**

To optimize the SYBR Green micro titre plate assay, continuous culture was treated as described above, and parasitaemia was determined by blood smear. The infected blood (50% haematocrit) was diluted with complete media to 1% parasitaemia (5% haematocrit). 18 wells in a 96 well plate were used for 5 treatments and one control (triplicate wells). To the first triplicate wells, 84 µl of complete media was transferred and 100 µl of the 5% haematocrit was added. To the remaining 15 wells, 100 µl of complete media was added. 16 µl (200 nM) of 5 mM emetine stock solution was added (highest dose) to the first triplicate wells, making a total volume of 200 µl. Two fold serial dilution was performed up to the $5^{th}$ well with 100 µl of the 5% haematocrit, leaving a final volume of 100 µl per well (2.5% haematocrit).
Controls included non-drug treated, infected blood. Additional controls included wells containing 100 µl of non-infected blood. Plates were gassed as described above and incubated at 37 $^{o}$C for 72 hours.

**Staining plate Sample with SYBR Green 1 for flow cytometry**

In order to analyse the efficacy of different doses of emetine on plate treated samples, a SYBR green flow cytometry was performed. For each dose and the non-drug treated controls, a thin smear was also performed for microscopic analysis. 12 different 1.5 ml eppendorf tubes were labelled each (duplicate) with the different doses (200 nM, 100 nM, 50 nM, 25 nM, 12.5 nM and Controls), to which 1 ml PBS was transferred and 100 µl of samples from each treatment (well) was added. After a single washing step in PBS (centrifugation 13000 g for 90 seconds), each pellet was resuspended in 1 ml of 2.5 × SYBR green and incubated in the dark for 20 minutes at room temperature.
Later, the samples were centrifuged (13000 g, 90 seconds) and dissolved in 250 µl of 0.37% formaldehyde solution to fix as described above. Following fixation, samples were washed three times in PBS and resuspended in 1 ml PBS. Samples were analysed using BDFACs Verse flow cytometer and 50,000 events were recorded for each sample.
To determine the $\mathbf{IC_{50}}$, data obtained from SYBR green flow cytometry assay were compared and processed using Microsoft excel. The percentage of parasite in infected red blood cells from SYBR green flow cytometry assay was plotted against the drug dosage (nM) used and the $\mathbf{IC_{50}}$ value was then estimated**.** All other data were analysed using Microsoft excel.

**RESULTS**

**Microscopic Analysis of Samples**

The K1 strain of *P. falciparum* cultured for analysis of the antimalarial effects of emetine were either incubated at $37^{o}$ C for 24 hours in a 12.5 $cm^3$ culture flask or 72 hours in a micro titre plate as explained in the methods. Samples treated with different doses of emetine dihydrochloride hydrate were analysed microscopically by blood smear. Additionally analysis of parasitaemia by microscopy and SYBR green flow cytometer method were done in parallel on the same sample. The slides were examined at 100× magnification in oil immersion under the microscope. Results obtained indicate mixed stages of parasite load (different stages i.e. rings, trophozoites and schizonts) in the controls (non-drug treated) shown by arrows. The level of parasitaemia in the 24 hour emetine treated samples in culture flasks (Figure 1**B**) was similar to the controls (Figure 1**A**) despite the high dose treatment (500 nM). Although samples treated in micro-plates were incubated for 72 hours, there is high parasitaemia in the control and 12.5 nM emetine treated sample

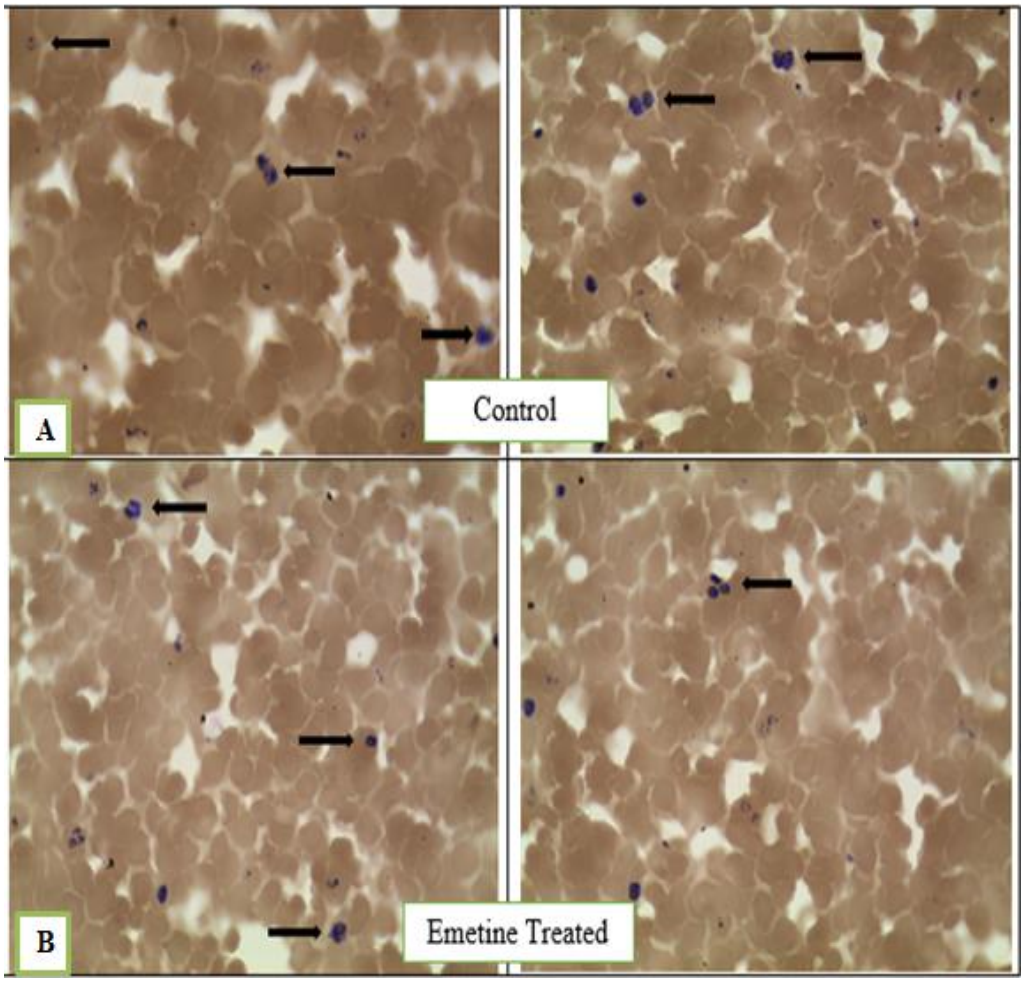


**Figure 1.** *P. falciparum* (strain K1) treated with emetine dihydrochloride hydrate, incubated for 24 hours and examined in oil immersion (100× magnification) under Leica microscope after staining smear with Giemsa.

(Figure 2, A and B). Despite the presence of some parasites in the 25 nM treated sample (Figure 2C), there is an indication of the parasites dying at this dose. Approximately, three different field views per slide were analysed in the 50 nM, 100 nM and 200 nMemetine doses, virtually no parasite was found per field view, indicating a parasite clearance effect of emetine from 50 nM dose.

**Determination of Parasitaemia in 24 hour incubated samples using SYBR Green Flow Cytometery Method**

Samples cultured in 12.5 $cm^3$ flasks were incubated for 24 hours at 37$^oC$ as explained earlier. Non-infected RBCs, infected (emetine treated) samples and infected (non-drug treated) controls were analysed using the SYBR green flow cytometer method. For these analyses, the gating approach previously described by Matthews et al., (2013) was adapted. Prior to analysis, samples were stained with SYBR green and fixed with formaldehyde. In principle, SYBR green binds DNA, and because RBCs lack DNAs (due to lack of nucleus), parasitized RBC can be distinguished from non-parasitized ones when SYBR green binds to parasite DNA. A total of 50,000 events were acquired for each sample and accurate percentage parasitaemia was determined using the BDFACS Verse software programme. Scatterplots presented in Figure 3 shows that uninfected samples (Figure 3, a) have only 0.06% cells in the gates. Untreatedsamples (Figure 3, b) have about 1.00% parasite infected cells in the gates (possibly rings, trophozoites and schizonts) as identified by smear during microscopy because samples were not frequently synchronized. However, drug treated samples (Figure 3, c) also show parasitized cells in the gates. The parasite load only reduces by 0.16% between the untreated and treated samples

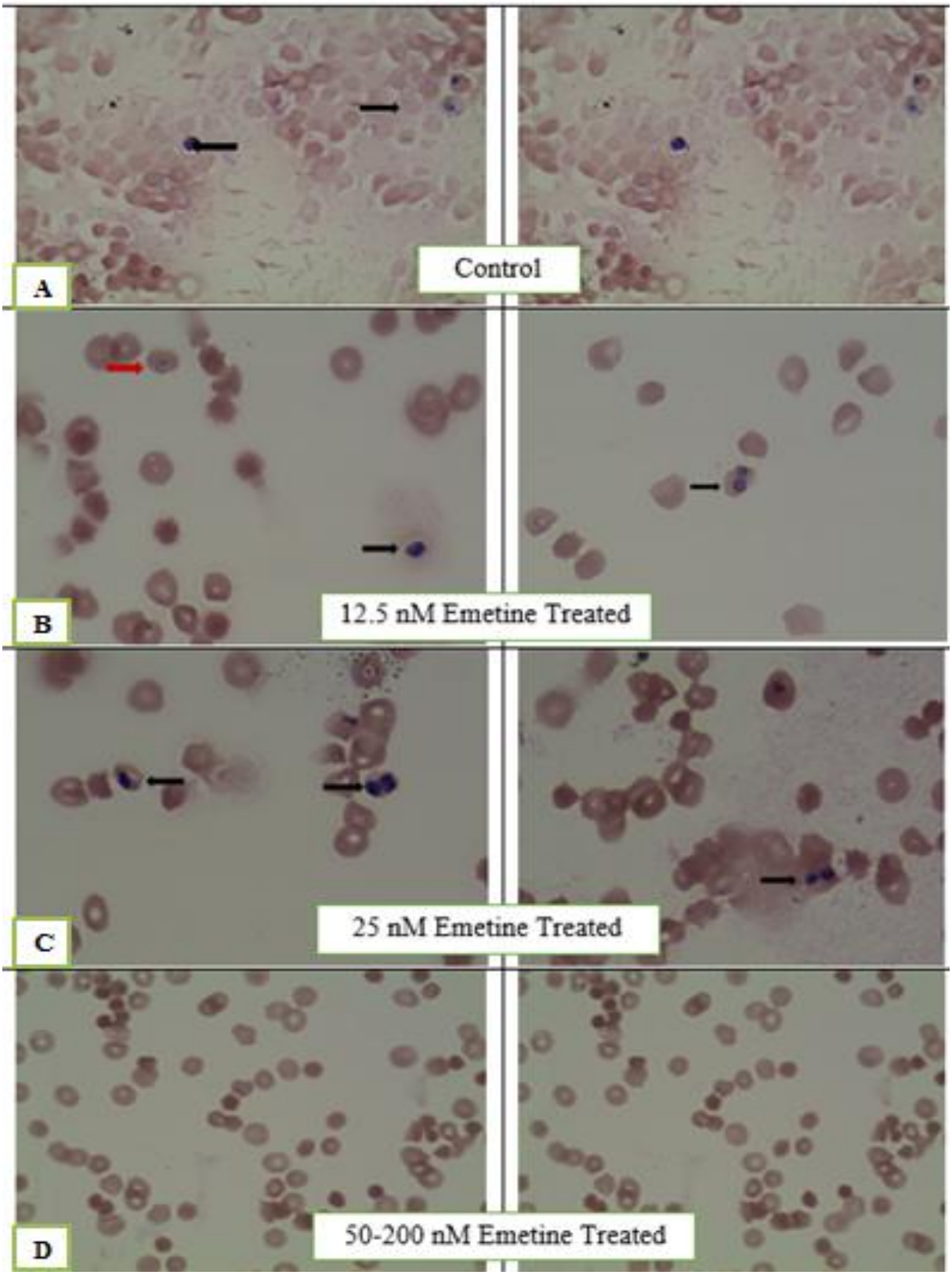


**Figure 2.** *P. falciparum* (strain K1) treated with different doses of emetine dihydrochloride hydrate incubated for 24 hours and examined in oil immersion (100× magnification) under Leica microscope after staining smear with Giemsa.

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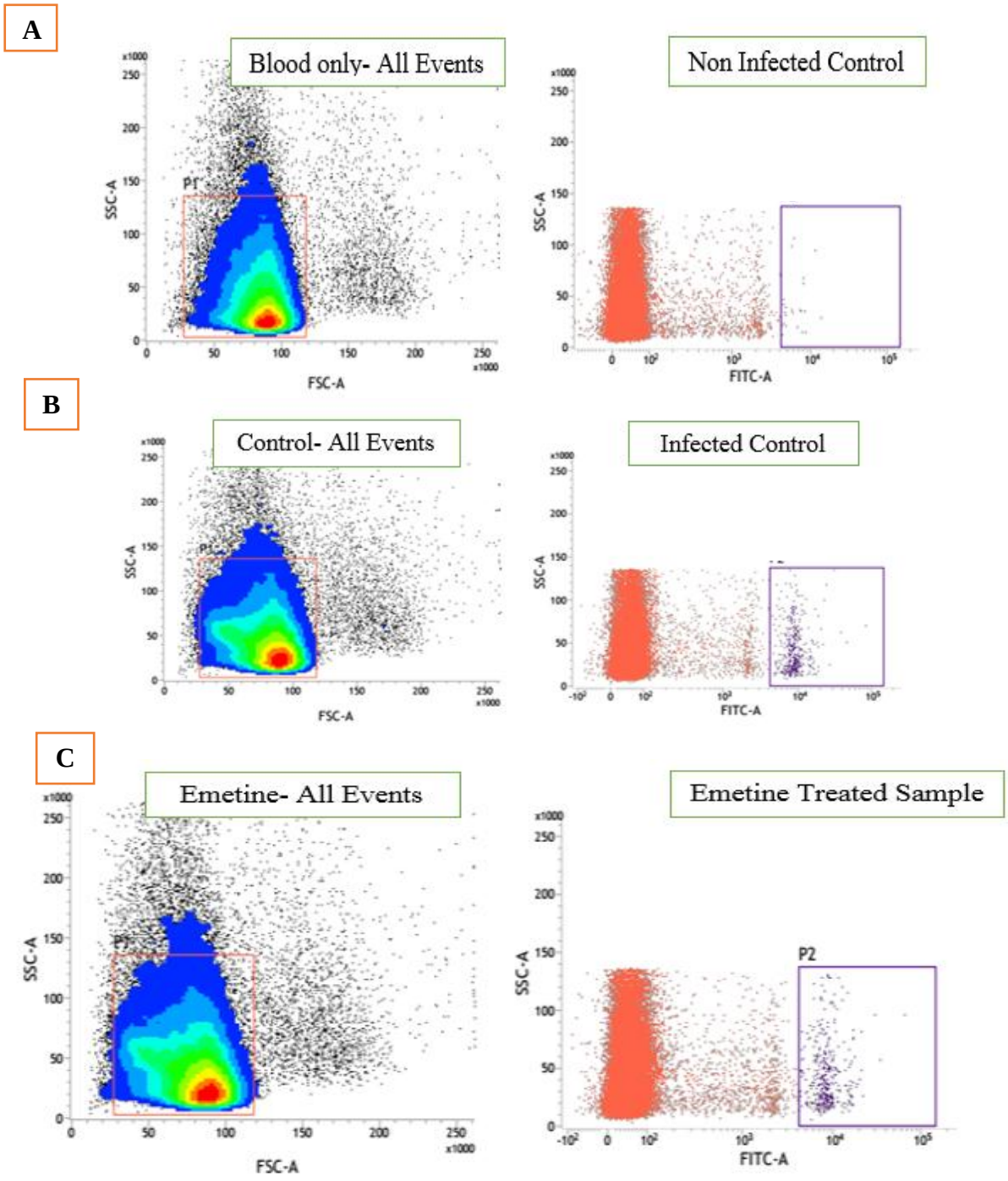


**Figure 3.** Comparison of uninfected and parasitized blood samples using the SYBR green flow cytometry method. Scatterplots from (a) uninfected blood (b) infected (untreated) and (c) infected (Emetine treated) samples 24 hours after treatment.

**Determination of Parasitaemia in 72 hour incubated samples using SYBR Green Flow Cytometery Method.**

As explained earlier, plate samples treated with emetine were incubated for 72 hours at 37$^{o}$C. Samples were stained with SYBR green and analysed as stated above. Two replicates were analysed for each sample except the uninfected control (blood only). Table 1 summarizes the percentage parasitaemia determined for each sample using the BDFACS Verse software programme.

**Table 1.** The percentage of parasite in infected red blood cells from flow cytometry analysis.

| Dose (nM) | Replica 1 (%) | Replica 2 (%) | Average |
|---|---|---|---|
| Blood | 0.06 | 0 | 0.03 |
| Control | 1.19 | 1.24 | 1.22 |
| 12.5 | 1.06 | 0.93 | 0.995 |
| 25 | 0.63 | 0.19 | 0.41 |
| 50 | 0.12 | 0.08 | 0.10 |
| 100 | 0.20 | 0.18 | 0.19 |
| 200 | 0.19 | 0.24 | 0.22 |

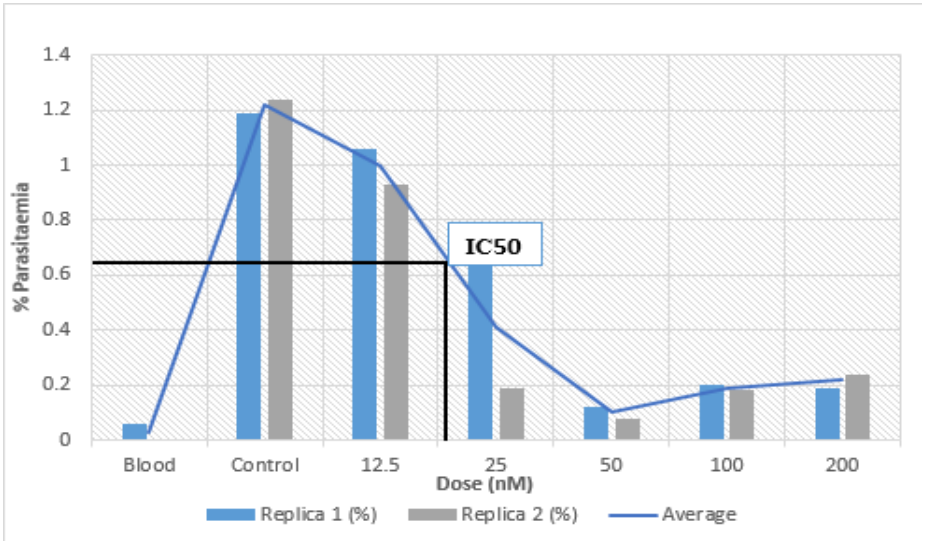


**Figure 4.** Dose response of emetine treated samples from SYBR Green flow cytometry assay, analysed 72 hours after treatment. The estimated $IC_{50}$ is between 12.5 and 25 nM.

As shown in Table 1, the untreated control samples have high parasitaemia when compared to the emetine treated samples. This is more clearly demonstrated in a bar chart (Figure 4) showing the level of parasitaemia in both the duplicate samples. The parasitaemia is gradually decreasing from the 12.5 nM emetine treated samples to the 50 nM treated sample where an estimated 90% ($IC_{90}$) of the parasites have died. Although, an increase in parasitaemia was seen from 50 nM to 100 and 200 nM respectively, this increase is expected to be due partly to background staining as data from microscopy does not show any parasite in the different field views between the 50 nM to 100 and 200 nM samples. Between 12.5 nM and 25 nM, an estimated 50% of the parasites were assumed to have died when compared with the control. This, thus, shows that the $IC_{50}$ value of the emetine falls between 12.5 nM and 25 nM respectively. At 50 nM, an estimated 90% of the parasites were believed to have died when compared with the control, suggesting an $IC_{90}$ value of 50 nM.

## DISCUSSION

In this study, the *in vitro* antimalarial efficacy of emetine dihydrochloride hydrate was investigated to determine the dose response of emetine on the multidrug resistant strain (K1) of *P. falciparum,* also validate previous finding by Matthews et al., (2013), which suggest that emetine has an antimalarial effect on the K1 strain of *P. falciparum*. However, the combination of emetine with dihydroartemisinin was previously reported to be effective, this study only uses emetine. Of note Emetine derived from the root of *Carapicheaipecacuanha*(Ipecac®) was previously used as an antiprotozoal drug

for the treatment of invasive intestinal amoebiasis and amoebic liver abscess (Matthews et al., 2013).
To determine the antimalarial effect of emetine dihydrochloride hydrate on the multidrug resistant strain (K1) of *P. falciparum*, samples treated with 500 nM emetine (10× the previously reported $IC_{50}$), incubated for 24 hours and analysed by the smear method, showed similar parasitaemia between the control (non-drug treated) and drug treated cultures (figure 1AandB). This similarity in parasitaemia is possibly because of the incubation period (24 hours). This indicates that, despite the high treatment dose used, emetine does not show parasite clearance effect 24 hours post incubation. Although, when these samples were analysed using SYBR green flow cytometry, the result showed a decrease (1% to 0.84%) in parasitaemia (table 1), which is an indication that at higher doses, parasitaemia is reduced after only 24 hours, but cannot be determined by conventional microscopy. However, since RBC lack DNA and SYBR green binds and stains parasite DNA, intra-erythrocytic parasite stages would be stained and the exact level of parasitaemia can be ascertained by SYBR green flow cytometry.

Further determination of the antimalarial effect of emetine was to monitor the dose response of the drug in cultures treated with different doses and incubated at 37$^{o}$C for 72 hours in a microplate. Result from smear (figure 2) show that control (non-drug treated) culture have similar parasite load with the lowest (12.5 nM) dose. This similarity might be due to the low drug concentration. However, result of the same sample from SYBR green flow cytometry (figure 4) show a decrease in parasitaemia by 0.44%. This is further an indication that SYBR green fluorescence-based assays enable accurate and reproducible estimation of the effects of drugs on intracellular parasite stages (Matthews et al., 2013). Similarly, increase in drug concentration result in decrease in parasitaemia as seen in result from smear (figure 2), indicating a dose response relationship. As stated in result part, three field views per slide were checked for the presence of parasites in the 50 nM, 100 nM and 200 nM emetine treated cultures, no parasite was identified for each field view per slide.

Analysis of the same sample by SYBR green flow cytometry (table 1) showed a decrease in parasitaemia in the 12.5 nM to 25 nM and 50 nM emetine treated samples that later increase in the 100 nM and 200 nM drug treated cultures. This fluctuation in parasitaemia from 50 nM to 100 nM and 200 nM might likely be due to background staining since smear results do not show parasite in the samples from 50 nM, 100 nM and 200 nM emetine treated cultures.

However, for emetine, an $IC_{50}$ value of 47 nM was established on the multidrug resistant stain (K1) of *P. falciparum,* while an $IC_{50}$ of 1 nM was established on the drug sensitive strain (3D7) Matthews et al., 2013). Although this study found an estimated $IC_{50}$ value between 1.25 nM and 25 nM on the multidrug resistant strain (K1), which is significantly lower than those reported by Matthews et al., (2013), 90% parasite clearance was seen (figure 4) at the 50 nM concentration, suggesting that 50 nM is the $IC_{90}$ value of emetine in this study.

Although, Matthews et al., (2013) reported that emetine presented strong antimalarial properties at nanomolar concentrations (~47 nM) on the multidrug resistant strain (K1) and (1 nM) in the drug sensitive strain (3D7) of *P. falciparum,* little is known about the mechanism of emetine action in the malaria parasite. However, more work is needed to determine the exact pathway on which emetine acts in the malaria parasite, *P. falciparum*.

## CONCLUSION

This study was designed to determine the antimalarial activity of emetine dihydrochloride hydrate on the multidrug resistant strain (K1) of *P. falciparum*. Results obtained validates previous findings which revealed that emetine showed strong antimalarial properties at nano molar concentrations (~47 nM) on the multidrug resistant strain (K1) and (1 nM) in the drug sensitive strain (3D7) of *P. falciparum.*

### Recommendation

Despite confirmation of the antimalarial activity of emetine dihydrochloride hydrate *in vitro,* more research is required to determine the exact pathway to which emetine act upon in the malaria parasite and, more broadly, further investigation into its antimalarial activities *in vivo* and the mechanism of emetine action in the malaria parasite is needed.

## REFERENCES

Andrews KT., Fisher G., and Skinner-Adams TS. (2014). Drug repurposing and human parasitic protozoan diseases.*Int J. Parasitol. Drugs and Drugs Resistance, 4*(2): 95-111.
Emmanuel AS., and Oladapo B. (2011). Biological Activities of Emetine. *The Open Natural Products J., 4*:8-15.
Flannery, EL., Chatterjee, AK., andWinzeler, EA (2013). Antimalarial Drug Discovery: Approaches and Progress towards

New Medicines. *Nature Reviews Microbiol.* 849-863.

Foley, M., and Tilley, L. (1998). Quinoline Antimalarials: Mechanisms of Action and Resistance and Prospects for New Agents. *Pharmacology and Therapeutics, 79*(1): 55-87.

Frayha GJ., Smyth, JD., Gobert GJ., andSavel, J (1997). The Mechanisms of Action of Antiprotozoal and Anthelminthic Drugs in Man. *General Pharmacology: The Vascular System, 28*(2), 273-299.

Lotharius J., Gamo-Benito, FJ., Angulo-Barturen I., Clark J., Connelly M., Ferrer-Bazaga, S., Wells T (2014). Repositioning: the fast track to new anti-malarial medicines?

Matthews H., Idris, MU., Khan F., Read, M., andNirmalan, N. (2013). Drug repositioning as a route to anti-malarial drug discovery: preliminary investigation of the in vitro anti-malarial efficacy of emetine dihydrochloride hydrate. *Malaria Journal*.doi:10.1186/1475-2875-12-359

Murray, C. J., Rosenfield, L. C., Lim, S. S., Andrews, K. G., Foreman, K. J., Haring, D., . . . Lopez, A. D. (2012). Global malaria mortality between 1980 and 2010: a systematic analysis. *Lancet, 379*(9814), 413-431.

Noubiap, JN. (2014). Shifting from quinine to artesunate as first-line treatment of severe malaria in children and adults: Saving more lives.*Journal of Infection and Public Health*. doi:10.1016/j.jiph.2014.04.007

Sabbatani, S., Fiorino, S., andManfredi, R. (2010).The emerging of the fifth malaria parasite (Plasmodium knowlesi).A public health concern?*Brazilian Journal of Infectious Diseases, 14*(3), 299-309.

Tartaglia, L. A. (2006). Complementary new approaches enable repositioning of failed drug candidates. *Expert Opinion on Inves. Drugs, 15*, 1295-1298.

Tekwani, B. L., and Walker, L. A. (2005).Targeting the Haemozoin Synthesis Pathway for New Antimalarial Drug Discovery: Technologies for In vitro β-Haematin Formation Assay.*Combinatorial Chemistry and High Throughput Screening, 8*, 63-79.

WHO . (2010).*Global Report on Antimalarial Drug Efficacy and Drug Resisatnce: 2000-1010.* Geneva: World Health Organisation.

WHO (2014, May 13). *10 Facts about Malaria*. Retrieved from World Health Organisation: http://www.who.int/features/factfiles/malaria/en/

WHO . (2014). *World Malaria Report.* Geneva: World Health Organization.

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