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Evaluation of the *Ex vivo* Antimalarial Activity of Organotin (IV) Ethylphenyldithiocarbamate on Erythrocytes Infected With *Plasmodium berghei* Nk 65

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Abstract: Malaria is the most destructive and dangerous parasitic disease. The commonness of this disease is getting worse mainly due to the increasing resistance of *Plasmodium falciparum* against antimalarial drugs. Therefore, the search for new antimalarial drug is urgently needed. This study was carried out to evaluate the effects of dibutyltin (IV) ethylphenyldithiocarbamate (DBEP), diphenyltin(IV) ethylphenyldithiocarbamate (DPEP) and triphenyltin (IV) ethylphenyldithiocarbamate (TPEP) compounds as antimalarial agents. These compounds were evaluated against erythrocytes infected with *Plasmodium berghei* NK65 via *ex vivo*. Organotin (IV) ethylphenyldithiocarbamate, $[R_nSn(C_9H_{10}NS_2)_{4-n}]$ with R = C_4H_9 and C_6H_5 for n = 2; R = C_6H_5 for n = 3 is chemically synthesised for its potential activities. pLDH assay was employed for determination of the concentration that inhibited 50% of the *Plasmodium*'s activity (IC_{50}) after 24 h treatment at concentration range of 10-0.0000001 mg mL^{-1}. *Plasmodium berghei* NK65 was cultured *in vitro* to determine the different morphology of trophozoite and schizont. Only DPEP and TPEP compounds have antimalarial activity towards *P. berghei* NK65 at IC_{50} 0.094±0.011 and 0.892±0.088 mg mL^{-1}, respectively. The IC_{50} of DPEP and TPEP were lowest at 30% parasitemia with IC_{50} 0.001±0.00009 and 0.0009±0.0001 mg mL^{-1}, respectively. *In vitro* culture showed that TPEP was effective towards *P. berghei* NK65 in trophozoite and schizont morphology with IC_{50} 0.0001±0.00005 and 0.00009±0.00003 μg mL^{-1}, respectively. In conclusion, DPEP and TPEP have antimalarial effect on erythrocytes infected with *P. berghei* NK65 and have potential as antimalarial and schizonticidal agents.

Key words: Organotin (IV) ethylphenyldithiocarbamate, antimalaria, pLDH assay, *Plasmodium berghei*, triphenyltin (IV) ethylphenyldithiocarbamate

INTRODUCTION

Malaria is recognised as one of the most dangerous infectious parasitic disease associated to human in developing countries, especially in tropical and subtropical areas (Batista *et al*., 2009). About 300 to 500 million clinical cases have been reported each year with more than 1.2 to 2.7 million deaths of world's population due to the disease (WHO, 2005). According to WHO (2005), the highest number of cases of malaria reported in Malaysia is 59, 208 cases in 1995. However, the number of reported malarial cases in Malaysia have decreased to 3134 cases in 2011 whereby the *Plasmodium falciparum* and *Plasmodium vivax* being the major *plasmodium* species with 30 and 70% cases, respectively (WHO, 2012).

Resistance of *Plasmodium falciparum* towards antimalarial drugs such as chloroquine and more recently sulfadoxine-pyrimethamine (White, 2004) has caused this disease to be difficult to control especially in endemic areas (Soni *et al*., 2005). Due to that and other factors limiting the efficiency of malaria treatment, many research efforts have been carried out to identify and develop new compounds that have the potential to be an alternative treatment for malaria.

New perspectives in the treatment of malaria require unique strategy through the development of metal-based drugs as antimalarial agents (Bharti and Singh, 2009). Synthetic organometallic complexes are among the compounds that have the potential as antimalarial drugs and that have promising treatment towards malarial infection. *In vitro* study of organometallic complexes and

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metaloporfirin demonstrates the effectiveness against the parasite *Plasmodium falciparum* (Pradines *et al*., 2005). Tin (IV) dithiocarbamate complexes are also gaining attention because of their wide applications as antimalarial and schizonticidal agents (Pellei *et al*., 2006).

The main objective of this study is to evaluate the antimalarial activity of organotin (IV) ethylphenyldithiocarbamate against *Plasmodium berghei* NK65-infected erythrocytes via *ex vivo*. This study is expected to contribute to the finding of new antimalarial compound that can control the emergence of resistance of *Plasmodium* to chloroquine.

MATERIALS AND METHODS

Parasites: *Plasmodium berghei* NK65 (provided by Faculty of Health Sciences, University Kebangsaan Malaysia). *Plasmodium berghei* strains were used in this study. All strains were maintained in mice ICR. Viability and parasitemia in mice blood were calculated by light microscopy analysis of blood smear using Field's stain method. After the level of parasitemia reached 5, 10 and 30%. The antimalarial compounds were then treated against erythrocytes infected with *P. berghei* NK65 via *ex vivo*. *Plasmodium berghei* was also cultured *in vitro* to determine the different morphology of trophozoite and schizont. Blood filtration was performed by cellulose fibre 11 (CF11). CF11 cellulose filters are an inexpensive method for the removal of leukocytes and platelets.

Drugs: The series of organotin (IV) ethylphenyldithiocarbamate [$R_nSn\ (C_9H_{10}NS_2)_{4-n}$] with R = C_4H_9 and C_6H_5 for n = 2; R = C_6H_5 for n = 3 was synthesised chemically like that of Awang *et al*. (2010). The compounds used in this study were dibutyltin (IV) ethylphenyldithiocarbamate (DBEP), diphenyltin (IV) ethylphenyldithiocarbamate (DPEP) and triphenyltin (IV) ethylphenyldithiocarbamate (TPEP). The effects of antimalarial activity of these compounds against *Plasmodium* were evaluated. Chloroquine was used as the gold standard in this study as *P. berghei* was non-resistant and susceptible to this antimalarial drug especially *P. berghei* NK65. Chloroquine was obtained from Sigma USA suppliers.

pLDH assay: This technique was used to measure antimalarial activity of compounds. pLDH assay is based on the efficiency of pLDH enzyme to reduce nitro blue tetrazolium salt (NBT) to formazan. Antimalarial activity was performed in microtiter plate wells. The wells containing 90 μL of drug were mixed with 10 μL of *P. Berghei*-parasitised erythrocytes (5% parasitemia and 10% hematocrit). Different controls were included in each microplate: nonparasitised mice erythrocytes (negative control), *P. Berghei*-parasitised mice erythrocytes with drug and *P. Berghei*-parasitised mice erythrocytes without drug in the presence of complete media. Inhibited pLDH enzyme is not able to reduce the NBT thus causing the reading of ELISA microplate reader to be lower.

Calculation of IC_{50}: IC_{50} of tested compounds was calculated based on the graph of pLDH activity after non-linear regression transformation and its fitness to a generalised sigmoidal function (Nagard *et al*., 2010).

RESULTS AND DISCUSSION

Level of parasitemia: Level of parasitemia was measured quantitatively based on the number of erythrocytes infected with *Plasmodium berghei* NK65. Figure 1 shows the image of a thin blood film under light microscope with magnification 1000× after Field's stain. Figure 1 A shows the image of non-infected erythrocytes from normal mice. Figure 1 B, C and D show infected erythrocytes at 5, 10 and 30% parasitemia, respectively, from the infected mice.

In this study, the level of parasitemia was used as a parameter associated with the morphological development of *P. berghei* NK65. Based on the observation from the thin blood film slides in Fig. 1, the 5% parasitemia shows high presence of young trophozoite (rings). The presence of mature trophozoite is very high in 10% parasitemia. The 30% parasitemia, which is late asexual stage of *Plasmodium berghei*, shows high presence of schizont.

Early screening of antimalarial activity: Early screening of antimalarial activity through pLDH assay was carried out using mice blood infected with *P. berghei* NK65 at 10% parasitemia. Figure 2 shows the result obtained from the calculation of percentage of pLDH activity after treatment with dibutyltin (IV) ethylphenyldithiocarbamate (DBEP), diphenyltin (IV) ethylphenyldithiocarbamate (DPEP) and triphenyltin (IV) ethylphenyldithiocarbamate (TPEP) compounds at different concentrations for 24 h compared to the gold standard, chloroquine. The 50% inhibitory concentration (IC_{50}) of DBEP compound could not be obtained from the graph. This result did not necessarily reflect that antimalarial activity was not present in DBEP but this compound should be tested against other *Plasmodium* species to determine the existence of antimalarial activity.

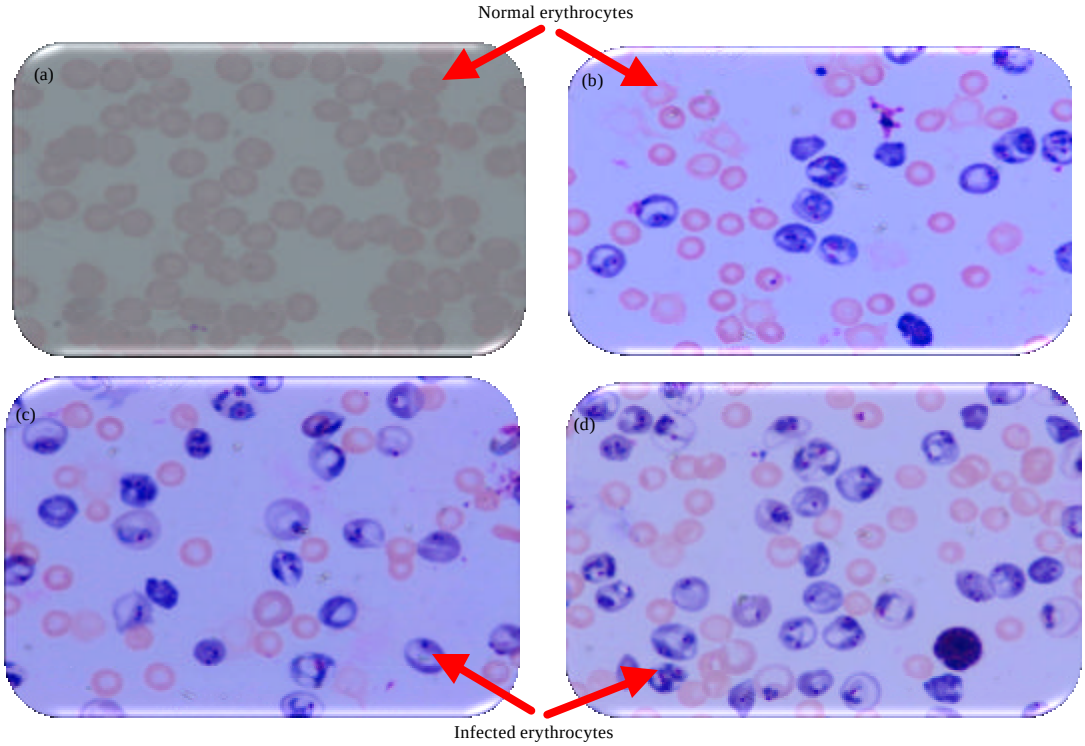

Fig. 1(a-d): (a) Thin blood film of normal erythrocytes, (b) Thin blood film of infected erythrocytes at 5% parasitemia, (c) Thin blood film of infected erythrocytes at 10% parasitemia and (d) Thin blood film of infected erythrocytes at 30% parasitemia under 1000× magnification by Field's stain

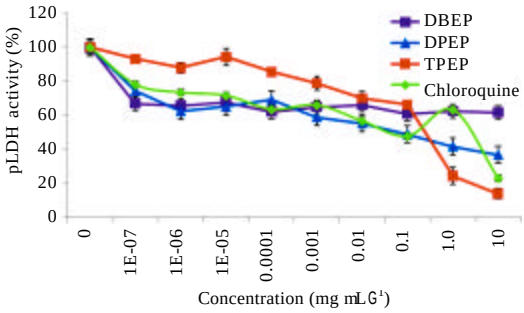

Fig. 2: The graph shows the percentage of pLDH activities against the treatment with DBEP, DPEP, TPEP and chloroquine at concentration range of 10-0.0000001 mg mL^{-1}. The IC_{50} of DPEP, TPEP and chloroquine were obtained based on the inhibitory sigmoid Emax model with an estimated IC_{50} value through non-linear regression

Based on the graphs in Fig. 3, the IC_{50} of DPEP and TPEP are 0.094±0.011 and 0.892±0088 mg mL^{-1}, respectively. The positive control, chloroquine shows IC_{50} of 0.093±0013 mg mL^{-1}. Although, both DBEP and DPEP are diorganotin (IV) derivative compounds, the DPEP shows better inhibitory effect on *P. berghei* NK65. The result of this study is supported by Gumy *et al.* (2008) who stated that diphenyltin compounds are more toxic compared to other diorganotin (IV) compounds.

Both DPEP and TPEP compounds have low inhibitory concentration during early screening. These findings showed that both compounds bound to the atoms of the phenyl group of tin(IV) had improved antimalarial activity on the erythrocytes infected with *P. berghei* NK65. Previous studies conducted by Delgado *et al.* (2009) showed that triphenyltin (IV) derivative compounds can act as antimalarial agents. A group of mice treated with triphenyltin (IV) hydroxide (TPTH) showed growth inhibitory effects of *Plasmodium yoelii* parasitemia via *in vivo* and reduced lymphatic enlargement that was due to malaria.

Only two compounds showed antimalarial activities towards *Plasmodium berghei* NK65, which are DPEP and TPEP. These compounds were selected to be tested for the antimalarial activity in the 5 and 30% parasitemia to determine whether the compounds acted effectively in the young trophozoite (rings) and schizont.

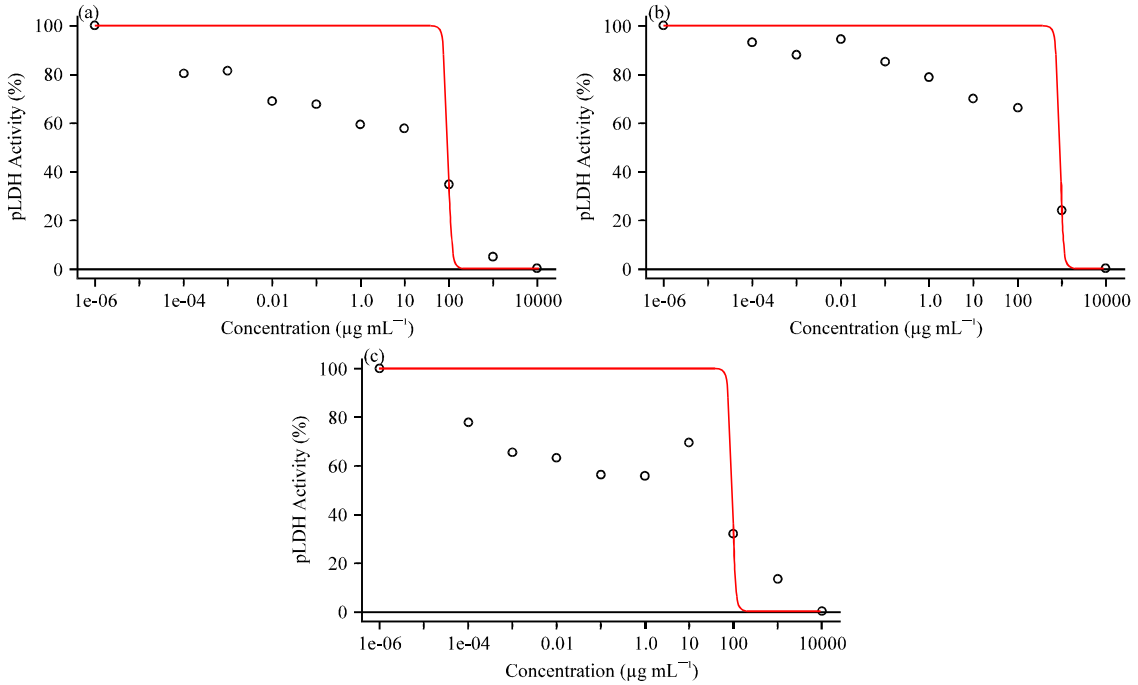

Fig. 3(a-c): (a) IC_{50} graph of DPEP, (b) IC_{50} graph of TPEP and (c) IC_{50} graph of positive control, chloroquine during early screening

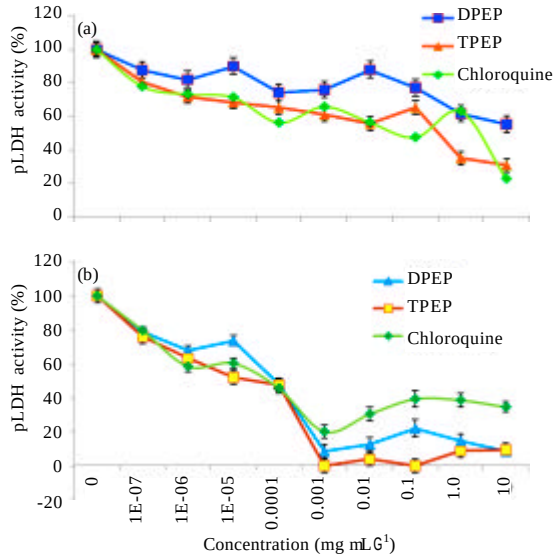

Fig. 4(a-b): Graphs show the percentage of pLDH activities against DPEP, TPEP and chloroquine at (a) 5% parasitemia and (b) 30% parasitemia. The IC_{50} of DPEP, TPEP and chloroquine at 5 and 30% parasitemia were obtained based on the inhibitory sigmoid Emax model with an estimated IC_{50} value through non-linear regression

Antimalarial activity at 5 and 30% parasitemia: The evaluation of antimalarial activities by DPEP and TPEP on erythrocytes infected with *P. berghei* NK65 was conducted to compare the pLDH inhibitory effects at 5% and 30% parasitemia, which are the stages of young trophozoite (rings) and schizont, respectively.

Graphs presented in Fig. 4 show the result obtained from the calculation of percentage of pLDH activity after treatment with DPEP, TPEP and chloroquine at 5 and 30% parasitemia. The IC_{50} graphs of TPEP and chloroquine at 5 and 30% parasitemia and DPEP at 5% parasitemia were shown in Fig. 5. Based on graphs in Fig. 3, no IC_{50} of DPEP was obtained at 5% parasitemia but the compound showed IC_{50} at 30% parasitemia. Next, TPEP showed IC_{50} at both 5 and 30% parasitemia. Gessler *et al.* (1994) categorised the antiplasmodial activities of compounds based on their IC_{50}. High activity is characterised by IC_{50}<0.05 mg mL^{-1}, active activity is characterised by 0.01 to 0.05 mg mL^{-1} and low activity is characterised by >0.05 mg mL^{-1}. Table 1 shows the IC_{50} of the compounds at 5, 10 and 30% parasitemia. Based on results depicted in Table 1, DPEP shows low activity at 5% parasitemia, which is young trophozoite (rings) stage but high activity against schizont at 30% parasitemia with IC_{50} 0.001±0.00009 mg mL^{-1}. This is because of the high presence of young trophozoite or rings that are less sensitive to the antimalarial compounds tested thus

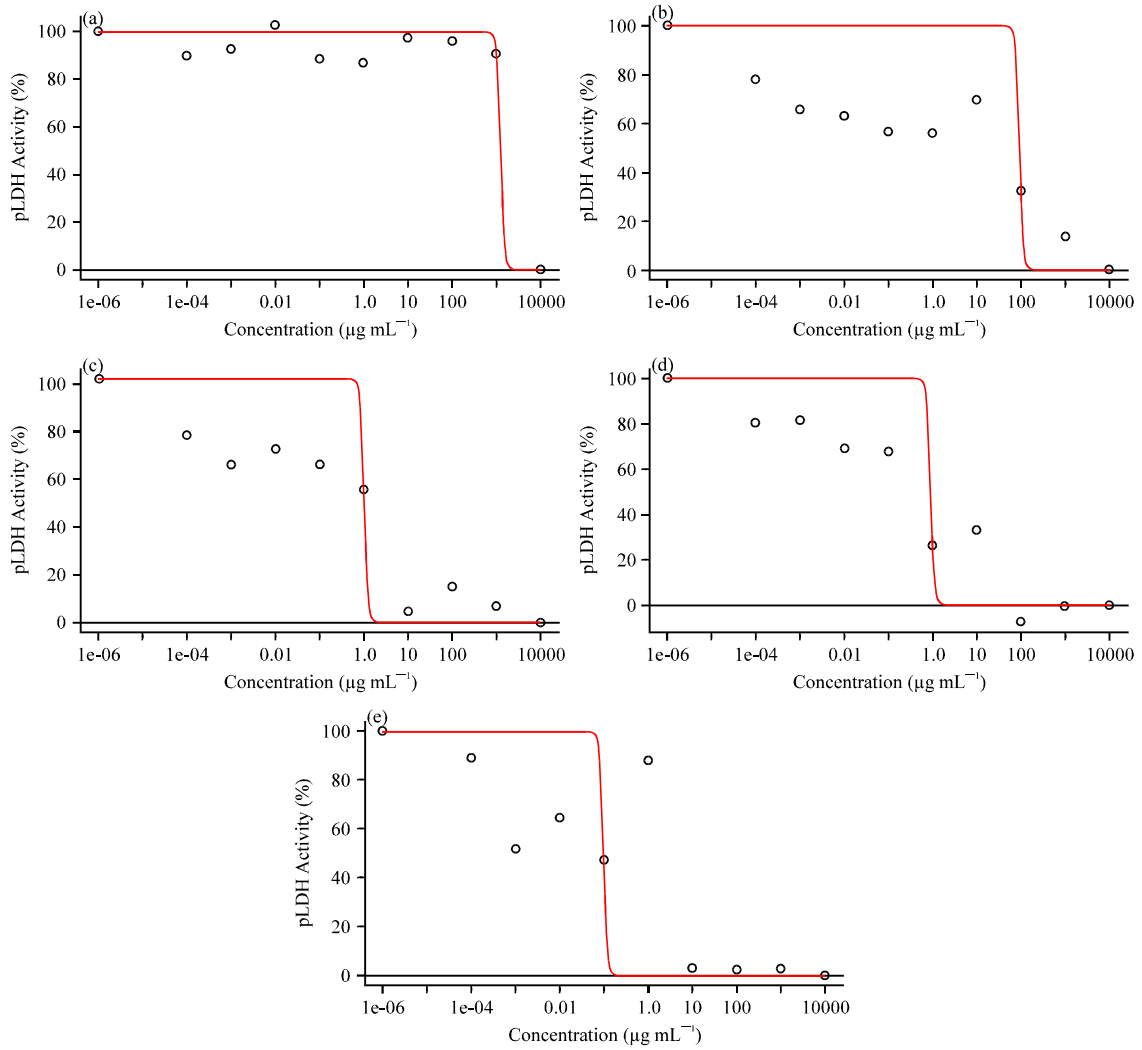

Fig. 5(a-e): IC_{50} graph of TPEP at (a) 5% parasitemia, IC_{50} graph of chloroquine at (b) 5% parasitemia, IC_{50} graph of DPEP at (c) 30% parasitemia, IC_{50} graph of TPEP at (d) 30% parasitemia and IC_{50} graph of positive control, chloroquine at (e) 30% parasitemia

Table 1: IC_{50} of diphenyltin (IV) ethylphenyldithiocarbamate, triphenyltin (IV) ethylphenyldithiocarbamate and chloroquine at 5, 10 and 30% parasitemia

Compound	Parasitemia (%)	IC_{50} (mg mL^{-1})
Diphenyltin(IV) ethylphenyldithiocarbamate	5	-
	10	0.094±0.011
	30	0.001±0.00009
Triphenyltin(IV) ethylphenyldithiocarbamate	5	1.255±0.11
	10	0.892±0.088
	30	0.0009±0.0001
Chloroquine	5	0.98±0.012
	10	0.093±0.013
	30	0.0001±0.00001

resulting in low or absent inhibition from the compounds. These factors are also influenced by the susceptibility of low-ring morphology compared to the later stages of *Plasmodium* asexual cycle (Balmer *et al*., 2000).

TPEP showed low activity at 5% parasitemia and high activity at 30% parasitemia with IC_{50} 1.255±0.11and 0.0009±0.0001, respectively. Therefore, the tested antimalarial compounds were in stage-specific action against *P. berghei* NK65 is more effective against schizont morphology. The number of schizont was highest on hyperparasitemia level. The shizonticidal activity demonstrated by organotin (IV) compounds against *Plasmodium* in our experiments was consistent with that reported by Wasi *et al*. (1987).

TPEP showed 50% inhibition concentration at 5, 10 and 30% parasitemia. This means that it has better antimalarial activity than DPEP. The lowest IC_{50} for TPEP was obtained at 30% parasitemia and not at 10% parasitemia. At 5% parasitemia, TPEP showed the highest

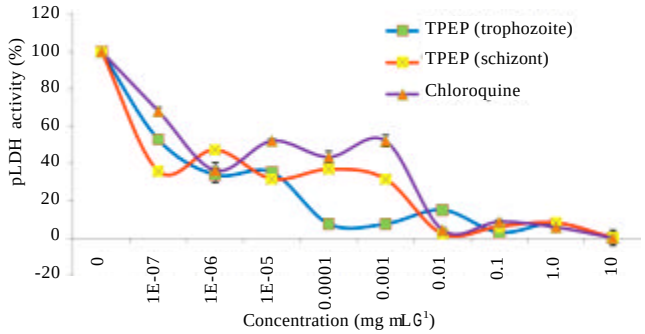

Fig. 6: Percentages of pLDH activities against TPEP and chloroquine in trophozoite and schizont

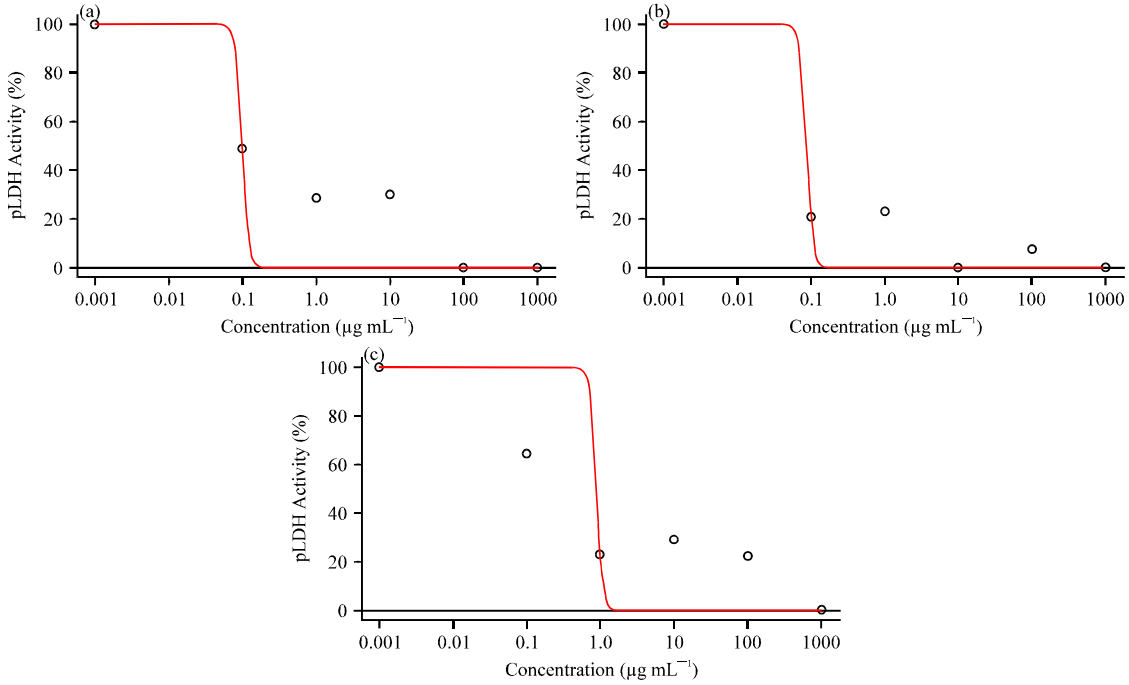

Fig. 7(a-c): (a) IC_{50} graph of TPEP at trophozoite morphology, (b) IC_{50} graph of TPEP at schizon morphology and (c) IC_{50} graph of chloroquine at schizont

IC_{50}. TPEP is less effective towards young trophozoite (rings) but very effective against schizont. These results were consistent with the gold standard, chloroquine.

The results of gold standard chloroquine in this study are consistent with previous findings obtained by Yayon *et al*. (1983). The trophozoite and schizont stages were more sensitive to the drug than ring-stage parasites. Chloroquine sensitivity decreased as nuclear division neared completion. The pilot test indicated that the TPEP compound in asynchronisation is effective against schizont. To confirm these results, further tests were done using the method of synchronisation.

Antimalarial activity of TPEP against erythrocytes infected with *P. berghei* NK65 was selected to be evaluated in the trophozoite and schizont via *in vitro* test. Figure 6 shows the inhibition of pLDH activity by TPEP against the erythrocytes infected with *P. berghei* NK65 in trophozoite and schizont and the antimalarial activity of positive control, chloroquine in schizont. Meanwhile, Fig. 7 shows the IC_{50} graphs of TPEP at trophozoite and schizont morphology.

The results obtained from this study showed that the inhibitory effects of TPEP were very effective against schizont with IC_{50} 0.00009±0.00003 µg mL^{-1}. At the trophozoite, the IC_{50} was slightly higher, 0.0001±0.00005 mg mL^{-1}. However, there was no significant difference between pLDH activity by TPEP at trophozoite and schizont.

These results are also in agreement with that of Pellei *et al*. (2006), who stated that the tin (IV) dithiocarbamate complexes have a wide applications in the medical field including antimalarial agents and schizonticidal. Consistent with the results of the gold standard chloroquine, there was no significant difference between the inhibitory effects of TPEP and antimalarial compound chloroquine on schizont.

CONCLUSION

The study of antimalarial activity of organotin (IV) compounds showed that only DPEP and TPEP have the potential to be antimalarial agents towards *Plasmodium berghei* NK65, especially at schizont stage. These compounds have the potential to be antimalarial agents and schizonticide.

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REFERENCES

Awang, N., I. Baba, M.B. Yamin, and S.W. Ng, 2010. Di-n-butylbis (N-cyclohexyl-N-ethyldithiocarbamate-k2S,S')tin(IV). Acta Cryst., E66: m938-m938.

Balmer, P., H.M. Phillips, A.E. Maestre, F.A. McMonagle and R.S. Phillips, 2000. The effect of nitric oxide on the growth of *Plasmodium falciparum* P. chabaudi and P. berghei *in vitro*. Parasite Immunol., 22: 97-106.

Batista, R., A.D.J.S. Junior and A.B. de Oliveira, 2009. Plant-derived antimalarial agents: New leads and efficient phytomedicines. Part II. Non-Alkaloidal natural products. Molecules, 14: 3037-3072.

Bharti, S.K. and S.K. Singh, 2009. Metal based drugs: Current use and future potential. Der Pharm. Lett., 1: 39-51.

Delgado, I.F., V.G. Viana, M. Sarpa and F.J. Paumgartten, 2009. Postnatal development and resistance to *Plasmodium yoelii* infection of mice prenatally exposed to triphenyltin hydroxide. Environ. Toxicol., 24: 629-635.

Gessler, M.C., M.H.H. Nkunya, L.B. Mwasumbi, M. Heinrich and M. Tanner, 1994. Screening Tanzanian medicinal plants for antimalarial activity. Acta Trop., 56: 65-77.

Gumy, C., C. Chandsawangbhuwana, A.A. Dzyakanchuk, D.V. Kratschmar, M.E. Baker and A. Odermatt, 2008. Dibutyltin disrupts glucocorticoid receptor function and impairs glucocorticoid-induced suppression of cytokine production. PloS One, Vol. 3. 10.1371/journal.pone.0003545

Nagard, H.L., C. Vincent, F. Mentre and L.B. Jacques, 2010. Online analysis of *in vitro* resistance to antimalarial drugs through nonlinear regression. Comput. Methods Programs Biomed., 104: 10-18.

Pellei, M., G.G. Lobbia, M. Mancini, R. Spagna and C. Santini, 2006. Synthesis and characterization of new organotin (IV) complexes with polyfunctional ligands. J. Organometal. Chem., 691: 1615-1621.

Pradines, B., E. Orlandi-Pradines, M. Henry, H. Bogreau and T. Fusai *et al*., 2005. Metallocenes and malaria: A new therapeutic approach. Ann. Pharm. Francaises, 63: 284-294.

Soni, S., S. Dhawan, K.M. Rosen, M. Chafel, A.H. Chishti and M. Hanspal, 2005. Characterization of events preceding the release of malaria parasite from the host red blood cell. Blood Cells Mol. Dis., 35: 201-211.

WHO, 2005. Malaria vector control in development of tropical diseases. World Health Organization, Geneva, Switzerland.

WHO, 2012. World malaria report. World Health Organization, Geneva, Switzerland. http://www.who.int/malaria/publications/country-profiles/profile_mys_en.pdf

Wasi, N., H.B. Singh, A. Gajanana and A.N. Raichowdhary, 1987. Synthesis of metal complexes of antimalarial drugs and *in vitro* evaluation of their activity against *Plasmodium falciparum*. Inorg. Chim. Acta, 135: 133-137.

White, N.J., 2004. Antimalarial drug resistance. J. Clin. Invest., 113: 1084-1092.

Yayon, A., J.A. Vande Waa, M. Yayon, T.G. Geary and J.B. Jensen, 1983. Stage-dependent effects of chloroquine on *Plasmodium falciparum in vitro*. J. Protozool., 30: 642-647.