Original Research Article

In-vivo antiplasmodial effect of dihydroartemisinin-piperaquine-nitrofurantoin on *Plasmodium berghei*- infected mice

ABSTRACT

Nitrofurantoin (NT) indicated for the treatment of urinary tract infection has potential *in-vitro* antiplasmodial activity. Dihydroartemisinin-piperaquine (DP) is an artemisinin based combination therapy used for the treatment of malaria. Drug repurposing has been used as an economic method for the discovery of antimalarial drugs.This study evaluated the antiplasmodial activity of dihydroartemisinin-piperaquine-nitrofurantoin (DP-NT) on mice infected with *Plasmodium berghei.* Adult Swiss albino mice (30-35g) of both sexes were used. The mice were randomly grouped, inoculated with *Plasmodium berghei*, and treated orally with DP (1.7/13.7mg/kg), NT (5.7mg/kg) and DP-NT (1.7/13.7/5.7 mg/kg), respectively using curative, prophylactic and suppressive tests. The negative control was orally treated with normal saline (0.3mL), while the positive control was orally treated with chloroquine CQ (10mg/kg). After treatment, blood samples were collected and evaluated for percentage parasitemia and hematological parameters. Liver samples were evaluated for histology. The mice were also observed for mean survival time (MST). Treatment with DP-NT decreased parasitemia levels when compared to individual doses of DP, and NT with significant difference observed at $p<0.05$. DP-NT prolonged MST when compared to individual doses of DP and NT with significant difference observed at $p<0.05$. The decreases in packed cell volume, red blood cells, hemoglobin and increases in white blood cells in parasitized mice were significantly restored by DP-NT when compared to individual doses

of DP and NT with difference observed at $p<0.05$. DP-NT eradicated liver *Plasmodium* parasites. DP-NT produced remarkable antiplasmodial activity. It may be used for the treatment of malaria.

Keywords: Nitrofurantoin, dihydroartemisinin, piperaquine, *Plasmodium*, mice.

INTRODUCTION

Malaria continues to be a major health challenge in tropical Africa despite the various anti-malarial programmes available. Malaria is a protozoan blood infection caused by mosquito borne apicomplexan parasite transmitted mostly by female anopheles mosquito species [1]. The incidence of malaria echoes globally with a burden of about 229 million cases in 2019 out of which 409, 000 deaths were recorded [2]. About 67% of malaria deaths in the world occurs in children under 5 years which forms the most vulnerable population. African continent has the highest amount of global malaria burden with 94% cases of malaria and deaths [3]. Malaria negative impact the economy of most African countries.The economic impact of malaria in the world was estimated to be 3 billion US Dollar in 2019 [2].

Artemisinin-based combination therapies (ACTs) are the mainstay for malaria treatment. The use of ACTs, which combined artemisinin derivatives with partner drugs has largely caused significant reduction in malaria-related mortality in endemic regions. Dihydroartemisinin–piperaquine (DP), one of the few ACTs still effective against *Plasmodium* falciparum was adopted as the first-line antimalarial treatment in Cambodia in 2008 [4,5]. ACTs including DP have also played a remarkable role in the 18% reduction in the incidence of reported cases of malaria between 2010 and 2016 [6]. However, the remarkable progress achieved with the use of D-P and other ACTs is seriously haunted by their decreased efficacies characterized by delayed parasite clearance and high recrudescence rates as reported in

Western Cambodia [7]. *Plasmodium* parasites resistance to ACTs has also spread to some countries in Southeast Asia. A primary concern is the spread of *Plasmodium* parasites resistance to Sub-Saharan Africa a malaria endemic region [8].

Plasmodium parasites resistance to ACTs can be curtailed by the use triple antimalarial regimen [9, 10]. Existing drugs such as antibiotics with potential antiplasmodium activity can be repurposed as partner drugs with artemisinin derivatives forming triple regimen [11]. Nitrofurantoin (NT) is a synthetic antibiotic derivative of imidazodinedione. It is a broad spectrum antibiotics used for treatment of urinary tract infection. It inhibits both gram-positive and gram-negative bacteria. It acts by inhibiting bacteria DNA, RNA and cell wall protein syntheses. NT is converted by the enzyme bacterial reductases to more electrophillic residues which are irreversible inhibitors of citric acid cycle, DNA, RNA and protein synthesis [12]. It also acts by activating bacterial flavour proteins to intermediates which inhibits bacterial ribosomal proteins [13]. Interestingly, in addition to the antibiotic effect of NT, it has potential antiplasmodial activity. NT and some nitoaromatic compounds are speculated to exhibit antiplasmodial activity, by inhibiting glutathione reductase and electron transport chain in *Plasmodium* parasites [14-16]. Glutathione reductase plays a significant role in the antioxidant defense of *Plasmodium* parasites [17]. This study therefore assessed if NT can augment the antiplasmodial activity of DP in *Plasmodium berghei* –infected mice

MATERIALS AND METHODS

Drugs and experimental animal

Dihydroartemisinin-piperaquine phosphate (D-P) (Bliss GVS pharma Ltd), nitrofurantoin (NT) (De-shawn Pharm. Lab. Ltd) and chloroquine phosphate (CQ) (Emzor Ltd) were used. Adult Swiss albino mice (30-35g) purchased from the animal house of the department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, Rivers State Nigeria were used. The mice were kept in cages under normal environmental conditions and were acclimated for 2 weeks with free access to feeds and water. The following doses of drugs were used: CQ (10 mg/kg) [18], NT (5.7mg/kg) [19] and DP (1.71/13.7mg/kg) [20].

Parasite inoculation

Chloroquine-sensitive *Plasmodium berghei* (*P. berghei*) (NK65) in donor mice obtained from National Institute for Malaria Research, Yaba Lagos, Nigeria were used. The parasites were maintained weekly by blood transfer from the *P. berghei*-infected mice to healthy mice through intraperitoneal (i.p) route.

Evaluation of antiplasmodial activity

Curative antiplasmodial activity

It was evaluated as described by Ryley and Peters (1970) [21]. Adult Swiss albino mice inoculated with *P. berghei* ($1x10^7$) (i.p) were randomly grouped into 5 of 5 mice each. After 3 days, the mice were orally treated daily for 4 days as follows: The negative and positive controls were treated with normal saline (0.3 mL) and CQ (10 mg/kg), respectively. Other groups were treated with NT (5.7mg/kg), DP (1.71/13.7mg/kg), and DP-NT (5.7/1.71/13.7mg/kg), respectively. On day 5, tail blood samples were collected, thin blood

films were prepared on slides. The slides were fixed with methanol and stained with Geimsa stain and examined using a light microscope. Percentage parasitemia and inhibitions were calculated using the formula below

$$\% \text{ Parasitemia} = \frac{\text{Number of parasitized red blood cells (RBC)}}{\text{Total number of RBC count}} \times 100\%$$

$$\% \text{ Inhibition} = \frac{(\% \text{ Parasitemia of negative control} - \% \text{ Parasitemia of treated group})}{\% \text{ Parasitemia of negative control}}$$

Suppressive antiplasmodial activity

It was evaluated using the procedure reported by Knight and Peters (1980) [22]. Adult Swiss albino mice were inoculated with *P. berghei* ($1x10^7$) and randomized into 5 groups of 5 mice/group. After 3 hours, the mice were orally treated daily for 4 days as follows: The negative and positive controls were treated with normal saline (0.3 mL) and CQ (10 mg/kg), respectively. Other groups were treated with NT (5.7mg/kg), DP (1.71/13.7mg/kg), and DP-NT (5.7/1.71/13.7mg/kg), respectively. The day 5, tail blood samples were collected and thin blood films were prepared on slides. Percentage parasitemia and inhibitions were calculated using the formula above.

Prophylactic antiplasmodial activity

It was evaluated using the method explained by Peters (1967) [23]. Adult Swiss albino mice were randomized into 5 groups of 5 mice/group. The mice were orally treated daily for 4 days as follows: The negative and positive controls were treated with normal saline (0.2mL) and CQ (10 mg/kg), respectively. Other groups were treated with NT (5.7mg/kg), DP (1.71/13.7mg/kg), and DP-NT (5.7/1.71/13.7mg/kg), respectively. On day 5, the mice were inoculated with *P. berghei* ($1x10^7$) (i.p). After 24 hours, tail blood samples were collected

and thin blood films were prepared on slides. Percentage parasitemia and inhibitions were calculated using the formula above.

Determination of mean survival time

The mice in the control group and treated groups were routinely observed for mortality and expressed in days. Mortality expressed as mean survival time (MST) was calculated as shown below

$$\text{MST:} \quad \frac{\text{Sum of survival time in days of all the mice in the group}}{\text{Total number of mice}}$$

Evaluation of hematological parameters

Blood specimen from the curative group were collected and evaluated for red blood cells (RBCs), packed cell volume (PCV), white blood cells (WBCs), and hemoglobin (Hb).

Histology of the liver

The liver tissues were sliced and fixed in 10% formalin for 24hours and dehydrated in alcohol of ascending concentrations. Liver tissues were embedded in paraffin and sectioned (3µg) using a microtome. Liver tissues were stained with Hematoxylin and Eosin on slides, examined using a light microscope and relevant sections photographed

Statistical Analysis

Results as mean $\pm$ standard error of mean (SEM). Variations between groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test. Significance was considered at $p < 0.05$.

RESULTS

Curative activity of dihydroarttemisinin-piperaquine-nitrofurantoin on *Plasmodium berghei*-infected mice.

Treatment with DP-NT significantly decreased percentage parasitemia when compared to treatment with individual doses of N and DTP at $p<0.05$. Treatment with NT, DP, and DP-NT produced 56.11%, 75.62%, and 85.13%, parasitemia inhibitions, respectively when compared to 78.41% produced by CQ (Table 1). MST was significantly prolonged by treatment with DP-NT when compared to treatment with individual doses of NT and DP at $p<0.05$ (Table 1).

Suppressive activity of dihydroartemisinin-piperaquine-nitrofurantion on *Plasmodium berghei*-infected mice.

Treatment with DP-NT produced significant decreases in percentage parasitemia when compared to treatment with individual doses of NT and DP at $p<0.05$. Treatment with NT, DP, and DP-NT produced the following inhibitions 66.11%, 80.47% and 93.93%, respectively while CQ produced 87.47% (Table 2). Treatment with DP-NT significantly prolonged MST when compared to treatment with individual doses of NT and DP at $p<0.05$ (Table 2).

Prophylactic activity of dihydroartemisinin-piperaquine-nitrofurantion on *Plasmodium berghei*-infected mice.

Percentage parasitemia was significantly decreased in mice treated with DP-NT when compared to treatment with individual doses of NT, and DP at $p<0.05$. NT, DP and DP-NT produced 78.21%, 91.76% and 93.42% parasitemia inhibitions, respectively whereas CQ produced 89.51% parasitemia inhibition (Table 3). Treatment with DP-NT significantly prolonged MST when compared individual doses of NT and DP at $p<0.05$ (Table 3).

Effect of dihdroartemisinin-piperaquine-nitrofurantoin on hematological parameters of *Plasmodium berghei*-infected mice.

Significant ($p<0.05$) decreases in RBCs, Hb, and PCV levels with significant ($p<0.05$) increases in WBCs levels occurred in *P. berghei* infected mice when compared to non-parasitized mice (MC) (Table 4). On the other hand, treatment with DP-NT significantly increased RBCs, Hb and PCV levels and significantly decreased WBCs levels at $p<0.05$ when compared to treatment with individual doses of NT and DP (Table 4).

Effect of dihydroartemisinin-piperaquine-nitrofurantoin on histopathology of the liver of *Plasmodium berghei*-infected mice.

The liver of the control mice showed normal hepatocytes, central vein, and sonusiods (Figure A). Liver of parasitized mice showed merozoites, congested central vein, steatosis and normal hepatocytes and sinusoids (Figure B). Liver of parasitized mice treated with CQ showed normal hepatocytes, central vein, and sonusiods (Figure C). Liver of parasitized mice treated with NT showed absence of merozoites, normal hepatocytes, sinusoids and congested central vein (Figure D). Liver of mice treated with individual doses of DP and DP-NT showed absence of merozoites, normal hepatocytes, and sinusoids (Figures E and F).

Table 1. Curative activity of dihydroartemisinin-piperaquine-nitrofurantoin on *Plasmodium berghei*-infected mice.

Treatment	% Parasitemia	% Inhibition	MST (Days)
NC	36.22±2.11	0.00%	9.14±2.10
CQ	5.39±0.68 [a]	85.13%	26.56±3.24 [a]
NT	15.90±1.16 [b]	56.11%	20.19±3.21 [b]
DP	8.83±1.02 [a]	75.62%	25.87±3.07 [a]
DP-NT	3.11±0.37 [c]	91.41%	31.78±4.15 [c]

NC: Negative control, CQ: Chloroquine, NT: Nitrofurantoin, DP: Dihydroartemisinin-piperaquine, MST: Mean survival time; n= 5, Data expressed as mean+ SEM, SEM: Standard error of mean. Values with different superscript down the column differ at $p<0.05$.

Table 2. Suppressive activity of dihydroartemisinin-piperaquine-nitrofurantoin on *Plasmodium berghei*-infected mice.

Treatment	% Parasitemia	% Inhibition	MST (Days)
NC	30.22±1.20	0.00%	9.22±1.27
CQ	3.79±0.06 [a]	87.44%	28.1±1.44 [a]
NT	10.24±0.64[b]	66.11%	22.7±2.40 [b]
DP	2.40±0.83 [a]	80.47%	27.0±2.11 [a]
DP-NT	1.834±0.77 [c]	93.93%	33.0±3.03 [c]

NC: Negative control, CQ: Chloroquine, NT: Nitrofurantoin, DP: Dihydroartemisinin-piperaquine, MST: Mean survival time; n= 5, Data expressed as mean+ SEM, SEM: Standard error of mean. Values with different superscript down the column differ at $p<0.05$.

Table 3. Prophylactic activity of dihydroartemisinin-piperaquine-nitrofurantoin on *Plasmodium berghei*-infected mice.

Treatment	% Parasitemia	% Inhibition	MST (Days)
NC	20.01±2.20	0.00%	9.34±1.01
CQ	2.10±0.27 [a]	89.51%	30.52±2.12 [a]
NT	5.96±0.11 [b]	70.21.2%	23.84±3.21 [b]
DP	2.45±0.08 [a]	87.76%	28.54±2.10 [a]
DP-NT	0.52±0.16 [c]	97.42%	35.34±3.33 [c]

NC: Negative control, CQ: Chloroquine, NT: Nitrofurantoin, DP: Dihydroartemisinin-piperaquine, MST: Mean survival time; n= 5, Data as mean + SEM, SEM: Standard error of mean. Values with different superscript down the column differ at p<0.05.

Treatment	PCV %	HB g/dL	RBCs **x10^6**	WBCs g/dL
MC	57.50±6.50	16.01±0.01	6.49±0.13	4.27±0.01
NC	29.00±2.00	9.64±0.26	2.11±0.02	11.13±0.20
CQ	47.00±3.00 [a]	12.46±0.21[a]	5.22±0.15 [a]	6.56±0.05 [a]
NT	37.50±4.50 [b]	11.56±0.11[b]	3.29±0.02 [b]	8.00±0.30 [b]
DP	45.00±5.00 [a]	14.18±0.05[a]	5.10±0.09 [a]	6.98±0.12 [a]
DP-NT	55.50±6.50 [c]	13.83±0.05 [c]	6.30±0.05 [c]	4.76±0.10 [c]

Table 4. Effect of dihydroartemisinin-piperaquine-nitrofurantoin on hematological indices of *Plasmodium berghei*-infected mice.

MC: Normal Control, NC: Negative Control, CQ: Chloroquine, NT: Nitrofurartoin, DP: Dihydroartemisinin – piperaquine, RBCs: Red blood cells, WBCs: White blood cells, PCV: packed cell volume, Hb: Hemoglobin, n = 5, Data as mean + SEM, SEM: Standard error of mean. Values with different superscript down the column differ at p<0.05.

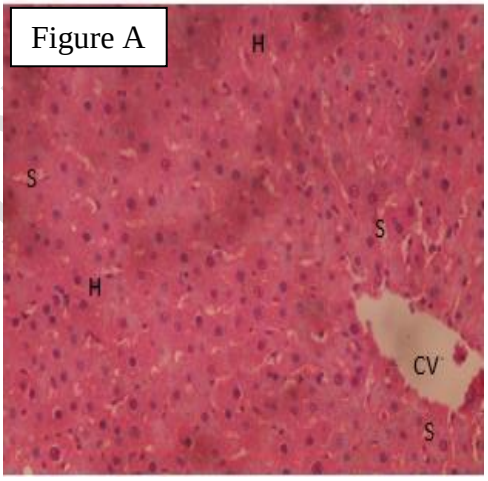

Figure A. Liver section of the control mice. CV: Central vein, H: Normal hepatocytes. S: Sinusoids with kupffer cells X400

Figure B

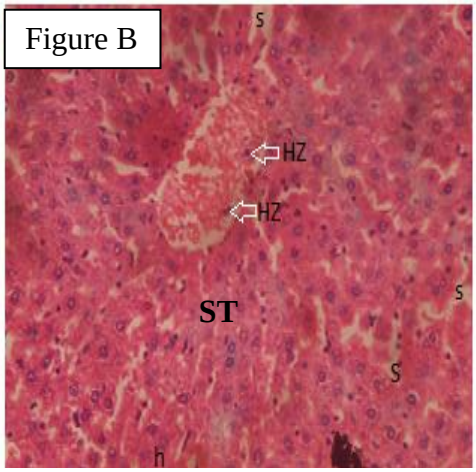

Figure B. Liver section of the parasitized mice. HZ: Congested central vein with merozoite (HZ), ST: Steatosis, H: Normal hepatocytes. S: Sinusoids containing kupffer cells X400

Figure C

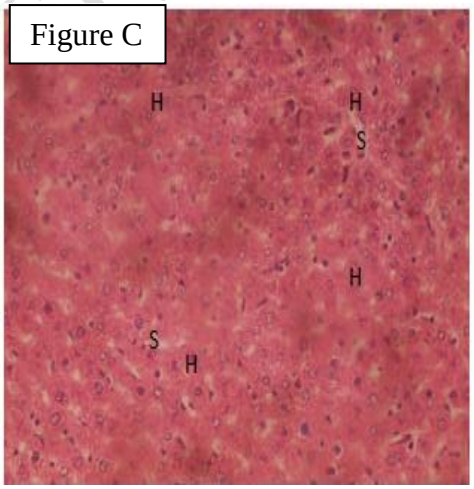

Figure C. Liver section of the parasitized mice treated with CQ (10mg/kg). H: Normal hepatocytes. S: Sinusoids containing kupffer cells X400

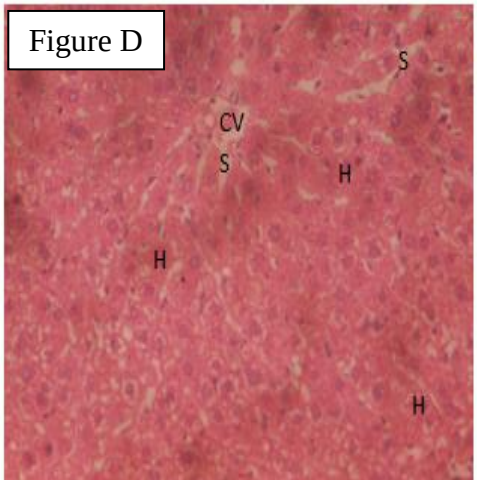

Figure D. Liver section of the parasitized mice treated with NT (5.7 mg/kg). CV: Congested central vein, H: Normal hepatocytes. S: Sinusoids containing kupffer cells X400

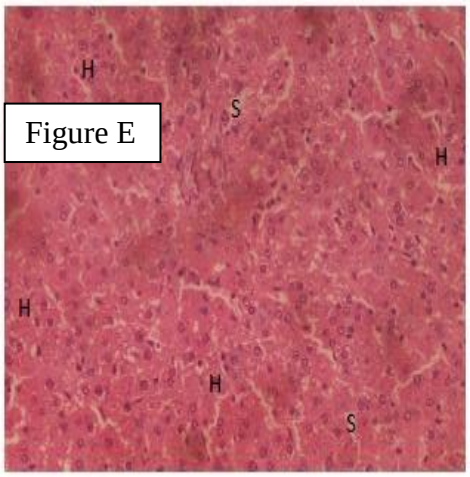

Figure E. Liver section of the parasitized mice treated with DP (1.71/13.7mg/kg). CV: Congested central vein, H: Normal hepatocytes. S: Sinusoids containing kupffer cells X400

Figure F

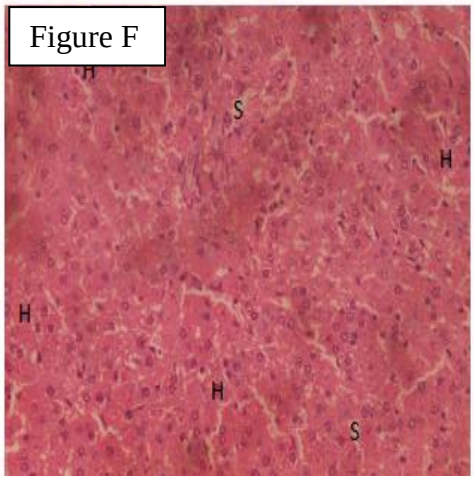

Figure F. Liver section of the parasitized mice treated with DP-NT (1.71/13.7/5.7 mg/kg). CV: Normal central vein, H: Normal hepatocytes. S: Sinusoids containing kupffer cells X400

DISCUSSION

The development of *Plasmodium* parasite resistance to antimalarial drugs is a major barrier to successful malaria treatment in malaria-endemic region. It has contributed to the resurgence of malaria infection and increase in malaria associated death in recent years [24]. Factors such as cost and length of clinical trials have slowed down new drug discovery and development process. This has encouraged the use of non-conventional approach including drug repurposing to fast track the discovery of new antimalarial drugs [25]. This study examined the antiplasmodial activity of NT in combination with DP in mice parasitized with

P. berghei. Curative, suppressive and curative tests which are used for the antiplasmodial assessments of candidate drugs were used for this study [26]. Mice model was used for the study because it allows for detailed assessment of multiple and specific pathophysiological processes caused by malaria infection, which is not possible in humans [27]. Due to the possible prodrug effect and involvement of the immune system in eradication of infection, an *in-vivo* model was used [28].

In this study, in the curative, suppressive and prophylactic tests, treatment with DP-NT decreased percentage parasitemia levels and increased percentage inhibitions. The assessment of MST is imperative in antiplasmodial studies. A drug candidate that significantly prolongs MST may be a potential antimalarial drug [26]. Treatment with DP-NT caused notable prolongation of MST in the curative, suppressive and prophylactic tests. Severe malarial anemia is a contributing factor to malarial morbidity in humans and is an important pathological feature of rodent model of malaria infections. During malaria infection, a number of factors including the destruction of RBCs due to parasite replication contributes to observed anemia [29]. A mice model of malaria induced anemia is often marked by decreased PCV, Hb and RBCs levels [26], which is consistent with the observation in the current study. It is interesting that treatment with DP-NT caused notable reduction in anemia marked by elevated serum PCV, RBCs and PCV levels with decreased WBCs levels.

The liver remains a significant hibernating ground for malaria parasites. The pathogenesis of liver impairment in malaria is complex and not well understood. Findings including vascular congestion, swollen hepatocytes, Kupffer cell hyperplasia, and steatosis were reported in malaria associated liver dysfunction [30]. In this study, the liver of parasitized mice showed vascular congestion, merozoites, and steatosis. However, the aforementioned liver changes were absent in the liver of DP-NT treated mice. This observation showed that DP-NT has potential to prevent recrudescent that can occur due to uneradicated *Plasmodium* parasites

hibernating in the liver. The antiplasmodial activity of DP-NT may be connected to the abilities of the constituent drugs to attack parasites at different sites. The D component of DP inhibits *Plasmodium* parasites through the cleavage of the end peroxide bridge and the generation of free radicals [31]. The P component of DP is said to have similar antiplasmodial mechanism with CQ. CQ forms CQ-haematin complex and hemoglobin in *Plasmodium* parasites food vacuole, which disrupt enzymatic processes [32]. The mechanisms of the antiplasmodial activity of NT are not well under stood. Its antibacterial activity involves the inhibition of bacterial DNA, RNA, and cell wall protein syntheses. It also acts by activating bacterial flavor proteins to intermediates which inhibit bacterial ribosomal proteins [33]. In *Plasmodium* parasites some studies speculated that NT produces free radicals [34] and inhibits glutathione reductase an antioxidant defense in *Plasmodium* parasites [35]. Also, it may act through redox cycling, oxidation of oxyhemoglobin, and the inhibition of electron transport chain [16].

Conclusion: In this study, DP-NT produced remarkable antiplasmodial activity in *P. berghei* infected mice therefore, it may serve as an effective antimalarial drug.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

.

REFERENCES

1. Carter R, Mendis KN. Evolutionary and historical aspects of the burden of malaria. Clinical microbiology reviews 202; 15(4):564-594.

2. World Health Organisation (2020). World Malaria Report 2020.Available online:https://www.who.int/teams/global-malaria-programme/report -2020. Last date accessed : June, 8th 2021.
3. World Health Organisation (2019). Rising to the challenges of malaria eradication. Available online: https://www.who.int/news-room/detail/23-08-2019-rising-to-the-challenge-of -malaria-eradication .Last date accessed: July 4th , 2021.
4. Karunajeewa H, Lim C, Hung TY, et al. Safety evaluation of fi xed combination piperaquine plus dihydroartemisinin (Artekin) in Cambodian children and adults with malaria. Br J Clin Pharmacol 2004; 57: 93–99
5. Denis MB, Davis TM, Hewitt S, et al. Efficacy and safety of dihydroartemisinin-piperaquine (Artekin) in Cambodian children and adults with uncomplicated falciparum malaria. Clin Infect Dis 2002; 35: 1469–76
6. Ouji M, Augereau J-M, Paloque L, Benoit-Vical F. Plasmodium falciparum resistance to artemisininbased combination therapies: A sword of Damocles in the path toward malaria elimination. Parasite 2018, 25, 24;1-11
7. Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM. 2008. Evidence of artemisinin-resistant malaria in Western Cambodia. New England Journal of Medicine, 359, 2619–2620
8. Mita T, Tanabe K, Kita K. Spread and evolution of Plasmodium falciparum drug resistance. Parasitology International, 209, 58, 201–209
9. Krishna, S. Triple artemisinin-containing combination anti-malarial treatments should be implemented now to delay the emergence of resistance: the case against. *Malar J* **18,** 339 (2019). 1-3
10. Tindana, P., de Haan, F., Amaratunga, C. *et al.* Deploying triple artemisinin-based combination therapy (TACT) for malaria treatment in Africa: ethical and practical considerations. *Malar J* 2021, **20 (**119)1-7
11. Udeme U. Georgewill, Elias Adikwu. Repurposing dihydroartemisinin-piperaquine-doxycycline as an antimalarial drug: A study in Plasmodium berghei infected mice. Advance Pharm J, 2021a; 6(1):22-28
12. McOsker CC, Fitzpatrick PM. Nitrofurantoin: mechanism of action and implications for resistance development in common uropathogens. J Antimicrob Chemother. 1994 (:23-30) doi: 10.1093/jac/33.suppl_a.23
13. Bains A, Buna, D., Hoag NA. A retrospective review assessing the efficacy and safety of nitrofurantoin in renal impairement. 2009, 142(5): 248-252.
14. Bhatt, S.; Weiss, D.J.; Cameron, E. et al. The effect of malaria control on Plasmodium falciparum in Africa betwen 2000 and 2015.Nature 2015, 526, 207–211.
15. Wiesner, J.; Kettler, K.; Sakowski, J.; Ortmann, R.; Jomaa, H.; Schlitzer, M. Structure-activity relationships of novel anti-malarial agents: Part 5. N-(4-acylamino-3-benzoylphenyl)-[5-(4-nitrophenyl)-2-furyl]acrylic acid amides. Bioorg. Med. Chem. Lett. 2003, 13, 361–363.
16. Grellier P Sarlauskas J, Anusevicius Z Maroziene A, Houee-Levin C, Schrevel J,Cenas N.Antiplasmodial Activity of Nitroaromatic and Quinoidal Compounds: Redox Potential vs Inhibition of Erythrocyte Glutathione Reductase. Arch of Biochem and Biophy 2001; 393, (2) 199–206.
17. Tyagi, C.; Bathke, J.; Goyal, S.; Fischer, M.; Dahse, H.M.; Chacko, S.; Becker, K.; Grover, A. Targeting theintersubunit cavity of Plasmodium falciparum glutathione

reductase by a novel natural inhibitor: Computationaland experimental evidence. Int. J. Biochem. Cell Biol. 2015, 61, 72–80.

18. Udeme U. Georgewill, Elias Adikwu. In-vivoAntiplasmodial activity of sulfadoxine-pyrimethamine-doxycycline on *Plasmodium berghei* infected mice with Journal of Adv Biol and Biotech. 2021, 24 (5): 1-8

19. Hooton TM, Besser R, Foxman B, Fritsche TR, Nicolle LE. Acute uncomplicated Cystitis in an era of increasing antibiotic resistance. A proposed approach to emperical therapy. Clin Infet Dis. 2004, 39(1): 75-80.
20. Georgewill UO, Ebong NO,Adikwu E. Antiplasmodial activity of desloratidine-dihydroartemisinin-piperaquine on plasmodium *berghei* infected mice. JABB 2021;9(2):169-173.
21. Ryley, J.F., Peters, W. The antimalarial activity of some quinolone esters. *Ann Trop Med and Parasit.* 1970; 84: 209-222.
22. Knight, D.J., Peters, W. The antimalarial action of N-Benzyl oxydihydrotriazines and the studies on its mode of action. Ann of Tropical *Med and Parasit. 1980;* 74: 393-404.
23. Peters, W. Rational methods in the search for antimalarial drugs. *Trans of Royal. Soc Trop Med and Hyg*. 1967; 61: 400-410.
24. Word Health Organization (WHO), 2001 Drug resistance in malaria
25. Nzila,A., Ma, Z.,& Chibale, K. Drug repositioning in the treatment of malaria and TB. Future Medicinal Chemistry, 2011, 3(11), 1413-1426.
26. Udeme U. Georgewill, Elias Adikwu. Potential antimalarial activity of artemether-lumefantrine-doxycycline on mice infected with *Plasmodium berghei*. Advance pharmaceutical journal 2021b; 6(1):22-28

27. Craig AG, Grau GE, Janse C, Kazura JW, Milner D, Barnwell JW, Gareth T, Jeem L .(2012). The role of animal models for research on severe malaria. PLos Pathog 8 (2): e1002401.doi:10.1371/Journal.ppat.1002401.

28. Waako P, Gumede B, Smith P, Folb P. The in vitro and in vivo antimalarial activity of Cardiospermum halicacabum L. and Momordica foetida Schumch. Et Thonn. *J* Ethnopharmacol. 2005;99(1):137–143.
29. Lamb, T.J., Langhorne, J. The severity of malarial anaemia in *Plasmodium chabaudi* infections of BALB/c mice is determined independently of the number of circulating parasites. Malar J. **7,** 68 (2008), 1-9
30. Scaccabarozzi D , Deroost K, Corbett Y, Lays N , Corsetto P , Salè FO, Van den Steen PE and Taramelli D. Diferential induction of malaria liver pathology in mice infected with Plasmodium chabaudi AS or Plasmodium berghei NK65 l. Malar J. 2018, 17:18, 1-9
31. Meshnick, S.R. The mode of action of antimalarial endoperoxides. Transactions of Royal Society of Tropical Medicine and Hygiene, 1994; 8(1) 31-32.
32. Tarning J. (2007). Piperaquine-bioanalysis, drug metabolism and pharmacokinetics. Institute of neuroscience and Physiology, Department of Pharmacology, the Sahlgrenska Academy at Goteborg, Sweden.
33. Gardner, M.J., Hall, N.,Fung,E,.White, O., Berriman, M., Hyman, R.W., Carlton, J.M., pain A., Nelson, K.E., Bowman, S., Paulsen, I.T.., James, K.,Eisien, J.A.,

Rutherford, B. Genome sequence of the human malaria parasite plasmodium falciparum. Nature, 2002, 419(6906), 498-511.
34. Vennerstrom, L.J.; Eaton, J.W. Oxidants, oxidant drugs, and malaria. J. Med. Chem. 1988, 31, 1269–1277.
35. Sarma, G.N.; Savvides, S.N.; Becker, K.; Schirmer, M.; Schirmer, R.H.; Karplus, P.A.(2003). Glutathione reductase ofthe malarial parasite Plasmodium falciparum: Crystal structure and inhibitor development. J. Mol. Biol.328, 893–907.