Nigerian Journal of Pharmaceutical and Applied Science Research, Vol (No): 86-95; March 2025 ISSN: 2971-737X (Print); ISSN: 2971-7388 (Online).
Available at www.nijophasr.net https://doi.org/10.60787/nijophasr-v14-i1-588

Inhibitory actions of dihydroartemisinin-piperaquine on smooth muscle contractility and other activities in rat models.

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ABSTRACT

Background: Malaria remains a major health challenge in sub-Saharan Africa. While dihydroartemisinin (DHA) and its combinations combat resistant malaria, they can cause side effects like nausea, vomiting, and dizziness. This study investigated the inhibitory effects of DHA and DHA/piperaquine on acetylcholine-induced ileum smooth muscle contraction using in *vitro* and *in vivo* rat models

Methods: I*n-vitro* experiments utilized ileum strips from adult Wistar rats (180–200 g) mounted on organ baths, with responses to acetylcholine, potassium chloride, and barium chloride serving as controls. For in vivo experiments, 60 adult albino mice (20–25 g) were divided into test and control groups (n=5). Test groups were infected with *Plasmodium berghei* and treated with DHA-piperaquine (3.0, 6.0, 9.0 mg/kg) or pure DHA (6.0, 9.0 mg/kg) orally, twice daily for five days. Control groups included non-infected/treated, non-infected/non-treated, and infected/non-treated subgroups, with untreated mice receiving 0.5 mL of distilled water. All animals were fasted for 24 hours before being sacrificed on day seven to harvest ileum tissues for histopathological analysis.

Results: Pure DHA and the DHA/P-Alaxin combination did not exhibit agonist effects on ileum smooth muscles but dose-dependently inhibited contractions induced by acetylcholine, potassium chloride, and barium chloride. DHA showed a significant mean inhibitory response (40.5%) on acetylcholine-induced contractions compared to atropine (77.0%) ($P < 0.05$). Histopathological analysis revealed tissue-friendly effects at moderate therapeutic doses, though mild to moderate cellular changes were observed at 9 mg/kg.

Conclusion: Dihydroartemisinin significantly and dose-dependently inhibited acetylcholine-induced contractions. It demonstrated a safe cellular profile on ileum smooth muscle at therapeutic doses.

Keywords: Contractility, Ileum smooth muscle, Dihydroartemisinic (DHA), Piperaquine, Rat.

1.0 INTRODUCTION

Malaria is a tropical disease, which is transmitted by the female anopheles mosquitoes. Despite efforts of the medical and Pharmacological researches, malaria still remain an enigma; hence, malaria poses an undiminished threat to people living in or traveling to endemic areas such as Africa [1]. In Africa alone between 1 and 2 million children die from the disease each year. Indeed, it has been estimated that malaria may occur in about 500 million cases and at least 2.3 million of these will be fatal; these figures are almost certainly and under estimates because of the nature of the target population and problems with under reporting in Africa sub region [2].and it is a major public health problem in endemic regions [3]. World malaria report indicate that there

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were 219 million cases of malaria globally in 2019 and 438,000 malaria deaths [4]. Malaria causes about 250 million cases of fever and approximately one million deaths annually [5].The disease kills between 660,000 – 1.2 million people every year, many of whom are children in Africa under 15 years old[6]. There are no accurate data available in many rural areas and many cases are not documented, hence the actual number of deaths is not known with certainty. Malaria is commonly associated with poverty and may also be a major hindrance to economic development; however it is a preventable and curable disease [7]. In Africa, malaria is present in both rural and urban areas, though the risk is lower in the larger Cities [8].. About 80% of all malaria cases occur in just 17 countries. In the year 2010, countries with the highest death rate per 100,000 population were Ivory Coast (86.15%), Angola (56. 93%) and Burkina Faso (50.66%), all in Africa [9]. Malaria mortality rates have fallen by over 25% since year 2000 and in the WHO Africa region, rates have dropped by 33%. The malaria burden is being reduced in many parts of the world mainly due to increased malaria prevention and control measures. Travelers from malaria –free areas that enter endemic areas are especially vulnerable to severe symptoms when they become infected [7]. The level of endemicity of malaria in Africa varies from country to country and sometimes from one region to another within the same country. A number of factors have been implicated to be responsible for the variation in endemicity from one place to the other, namely; rainfall in drier areas, and outbreaks of malaria have been predicted with reasonable accuracy by mapping rainfall [10]. Other factors are Stagnant water, High humidity, Constant high humidity; Poor hygienic conditions, and Human activities. All these factors enable mosquito larvae to readily mature/multiply, as they provide the environment necessary for their continuous breeding.[11].The causative parasite of malaria is *Plasmodium*, which is further subdivided into four species of *plasmodia parasites*, namely: *plasmodium vivax* which is extensively distributed, *plasmodium Falciparum* is also widespread; *plasmodium ovale* is mainly confined to Africa and is less prevalent, and lastly, *plasmodium malariae*, which causes the least severe but most persistent infections, also occurs widely [7]. *Plasmodium falciparum*, is the most clinically significant causative organism and has been reported to demonstrate an unusual propensity to acquire resistance to antimalarial therapy [12]. *Plasmodium species* also infect birds, reptiles, monkeys, chimpanzees and rodents. Rodents' malaria parasites are principally parasites of thicket rats. It was first isolated from the blood of thicket rats in Katanga, Africa by Venice in 1948. Models of malaria using non-human malaria parasites were developed to serve as convenient laboratory reference for the provision of biological insight into the human forms of the disease that would either be practically or ethically, difficult to obtain.(Peter,[13].Successful and widely used models include a wide range of species that infect laboratory rodents include among others P*lasmodium berghei-berghei, Plasmodium chibaudi, Plasmodium yoelii and Plasmodium vinckei.* P*lasmodium berghei-berghei* is said to be the best model for research on the developmental biology of malaria parasites [14]. The unprecedented spread of chloroquine-resistant strains of *Plasmodium falciparum* has severely weakened the range of drugs available to treat the disease and has increased interest in newer agents such as the Artemisinin Derivatives. Drugs so far used for the treatment of malaria range from the ancient chloroquine, mefloquine, primaquine, sulfadoxine, pyrimethamine, proguanil, amodiaquine, quinine and agents like Doxycycline, among others. Currently, there seems to be increasing interest in the use of Artemisinin derivatives (Artemether, Arteether, Artesunate and Dihydroarthemisinin) which are a new series of antimalarial drugs with a high level of activity against chloroquine resistant strains of malaria parasite [2,15]. World Health Organization (WHO) recommended treatment schedule containing an Artemisinin-combination therapy (ACTs) is now the recently used standard treatment in the world for *plasmodium falciparum* malaria. As a response to increasing levels of resistance to antimalarial medicines, WHO recommends that all countries experiencing resistance to conventional monotherapies, such as chloroquine, amodiaquine or sulfadoxine and pyrimethamine, should use combination therapies, preferably those containing artemisinin derivatives (ACTs – artemisinin-based combination therapies) for *falciparum malaria*. Thus, WHO currently recommends the following combination therapies as listed below:

- Artemether/lumefantrine,
- Artesunate plus amodiaquine in areas where the cure rate of amodiaquine monotherapy is greater than 80%.
- Dihydroartemisinin/piperaquine;
- Artesunate plus sulfadoxine and pyrimethamine especially in areas where the cure rate of sulfadoxine/pyrimethamine is greater than 80%. All these are available in tablet formulations for oral use especially for treatments of uncomplicated malaria due to plasmodium falciparum [7].

On the other hand, treatment of severe falciparum malaria requires parenteral artemether or artesunate, or parenteral quinine. The intravenous artesunate is the drug of choice in low to moderate transmission areas or outside malaria endemic areas. Parenteral antimalarials are also used to initiate treatments in patients unable to take oral treatment [15]. Despite efforts of medical and pharmacological researchers, malaria still remains as a major health challenge; hence, malaria poses an undiminished threat to people living in or traveling to endemic areas such as Africa. The unprecedented spread of chloroquine-resistant strains of *Plasmodium falciparum* has severally weakened the range of drugs available to treat the disease and has increased interest in newer agents such as the Artemisinin Derivatives [2]. Extensive work on the effects of chloroquine on the rat urinary bladder

strip; the effects of mefloquine on the mechanical activity of the mouse isolated rectal smooth muscles and other monotherapy has been documented: the results showed that chloroquine and mefloquine possessed anticholinergic and appreciable calcium channel blocking drugs. Research on Quinine, Chloroquine, and Mefloquine mainly used as antimalarial agents shows they exert several effects on muscle mechanics.[16]. On the other hand, the effect of Artemisinin derivatives on muscle tissue has not been fully investigated; particularly dihydroartemisinin either used as a combination (dihydroartemisinin and piperaquine) or as a drug in itself (dihydroartemisinin) had been reported to have clinical adverse effect which include: nausea, headache, abdominal pain, vomiting diarrhea, decrease of reticulocyte count, and dizziness etc. All these pose a great research problem that needs to be fully investigated/validated. The present study was designed to investigate the actions of dihydroartemisinin, a major Artemisinin Derivatives on smooth muscle activity (contractility) in isolated smooth muscle preparations in experimental animal models, with a view of elucidating the mechanisms of actions of Dihydroartemisinin on the smooth muscle tissues in different animal models and to ascertain the histopathologic toxicity effect. This study extensively investigated the effect of pure dihydroartemisinin and DHA/piperaquine which is used in various ACTs combination on contractility to drugs in different isolated smooth muscle preparations in experimental animal models.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Equipment

Organ bath apparatus Orchid Scientifics, Aerator (Type r. 301. USA), Slow-moving kymograph (C.F Palmer LTD England), Mettler balance P165 (Gallenkamp, Germany, UK).

2.1.2 Drugs

The drugs purchased include dihydroartemisinin/piperaquine (Novartis Pharm., New York) -purchased from the University of Uyo Health Centre, Akwa Ibom State and pure dihydroartemisin powder (Afrab Chem. Ltd., Lagos), Nigeria. Atropine, Calcium channel blockers e.g. Verapamil, α -adrenoceptor blocker such as Phentolamine etc. Acetylcholine Chloride was obtained from Sigma Chemical Co. (USA), Calcium chloride (Co.Pharm): Magnesium chloride (Hopkin Williams, U.K) and Potassium chloride (Sigma USA)-Some chemicals were obtained from the Pharmacology and Toxicology Laboratory, Universityof Uyo, Uyo, Nigeria. The Physiological solutions used in this study were Tyrode's solution. All chemicals were of high analytical grade and were dissolved in either deionized distilled water or normal saline at the required concentrations.

2.1.3 Preparation of Drugs

The completely homogenous test drugs –Dihydroartemisin and DHA/Piperaquine were administered to all the animals in the test groups, with the aid of a rubber oropharyngeal cannula attached to a calibrated syringe (via oral route).

The volume (mL) of the drug to be given to each animal was calculated as follows:

$$\text{Volume administered (ml)} = \frac{\text{Weight of rat in kg x Required dose in mg per kg}}{\text{Concentration of the drug stock}}$$

The doses used for the *in vivo* study were determined from the data obtained based on standard doses used for animal models relating to body weight in previously established dosages in similar studies on the effect of Artemisinin-Based Combination therapy in Wistar albino rats which suggested treatment dose of between 3 mg /kg -6 mg /kg of Bodyweight [17]; Also the doses for the *in vitro* study were obtained based on previous work carried out by Tologbonse *et al*.[18].

2.2 Methods

2.2.1 Parasite inoculum preparation

The method of Peter [13] as described by Udobang [19] was adopted; each mouse that was used in the *in vivo* experiment (test group) was inoculated intraperitoneally with 0.2 ml of infected blood containing about $1x10^7$*Plasmodium berghei berghei* parasitized erythrocytes. The percentage parasitaemia was determined by counting the number of parasitized red blood cells against the total number of red blood cells. The research was carried out using matured experimental animals of both sexes namely: home bred male and female albino mice (weight - matched). They were all healthy, hence disease-free. Thirty- (30) mice (20-25 g), were purchased from the animal house of the Department of Pharmacology and Toxicology University of Uyo ; they were used for the *in vivo* studies, while rats were utilized for the *in-vitro* organ bath study; the mice were divided randomly into groups according to their body weights in a proper range. The animals were fed with a standard pelleted feed- growers mash from Agro Feeds Limited, Lagos and provided distilled water for drinking *ad libitum*. All

animals were well acclimatized having been kept in clean metabolic polypropylene cages with laboratory-grade pine shavings as beddings, contained in well-ventilated house and maintained under standard conditions (temperature:25 ±3 ^{o}C; photoperiod: 12-h natural light and 12-h dark cycle; humidity: 35-60 %) for two weeks prior to drug treatments.

2.2.2 Grouping of Animals for in vivo Study

The mice were divided into two groups for the in-vivo experiment. The first set served as the Control group - the mice received only distilled water (no drug treatment), and the second group comprises of the test group animals. The test group animals were divided into three (3) sub-groups containing four (4) mice per group infected with *Plasmodium berghei bergiei*; this order was followed for the three set of drug treatments- i.e.Dihydroartemisinin3.0 ,6.0 and 9.0mg/kg per body weight for low, moderate and high doses regimen respectively. The animals in the control groups were also divided into 3 subgroups; -

i. Non-infected/ Non-treated mice,
ii. Non-infected/Treated mice.
iii. Infected mice /Non-treated according to the dosage regimens respectively.

At the end of all treatment exposures, animals were sacrificed under Chloroform and by cervical dislocation. All drugs were given twice daily via oral route for five (5) consecutive days adding up to 120 hours. The animals of all the groups were observed for 2 days post-administration. The animals were fasted overnight and sacrificed on the 8th day. The rats were also assessed for the integrity of muscles using standard histological tissues staining procedures.

2.2.3 Histopathological Studies and Collection of samples

Samples were collected in the in vivo experiment after treatment as follows: clear incisions were made into the abdominal cavity of the mice up till the border near the tail of the rats. Fresh ileum was removed from the rats and immediately fixed in 10 % formalin in specimen containers for 3 days (72 hours). These organs were cut laterally and longitudinally to examine the internal structure as described by Yakubu [20]. They were processed for histological evaluation by pathologist in the University of Uyo Teaching Hospital, Uyo, Nigeria.

2.2.4 Experimental procedures in vitro animal models

This study was carried out using standard experimental procedures as described by Unekwe and Nwafor [21, 22] which were applicable in the use of an Organ bath with a slow-moving kymograph, a basic instrument for measuring muscle tension. The organ bath was properly washed using distilled water and filled with appropriate physiological solutions. A vertical strand of isolated muscle tissue of 2 cm was picked gently using forceps, needle and white thread was passed through the tissue and tied through the arm of the frontal lever to the tissue holder. The tissue holder was then placed in the tissue organ bath. The tissue was observed closely for the contractile response in the Tyrode's solution alone; It was allowed to stabilized for some about thirty (30) to sixty (60) minutes before investigation commences. Isolated muscle preparations from rat in vitro model were used for this study: Precisely the ileum smooth muscle preparations were obtained from the rat models used.

2.2.5 Recordings of Contractile Responses against the Concentration of the Acetylcholine

The methods as described by Unekwe [21] were adopted. A vertical strand of isolated ileum smooth muscle tissue of about 2 cm was gently picked using forceps, needle and white thread was passed through the tissue and tied through the arm of the frontal lever to the tissue holder. After equilibrium, the concentration-response test to acetylcholine alone at a concentration of $4x10^{-6}$ to10^{-3}M was conducted separately before the addition of DHA. The least dose (10^{-6} M) was added first to the fluid bathing the tissues and the effect was observed for 0.5-2 minutes and this was followed by 2-3 washings, after which the next higher dose of the agonist was added and the procedure was repeated for about 5 doses of the agonist.

2.2.7 Contractile Responses against the Concentration of the other Agonists

Concentration-response tests to potassium chloride and barium chloride were also conducted separately before the addition of Dihydroartemisinin. The initial observations with agonist alone served as control values, which were used to compare the effect of Dihydroartemisinin on agonist - induced contractions. For graded dose-response relationships, a specified dose of potassium chloride (KCI) or barium chloride ($BaCI_2$) were added to the fluid bathing the tissues and the effect was observed for 0.5-2.0 minutes and this was followed by 3-5 washings, after which the next higher dose of the agonist was added and the procedure was repeated for about 5 doses of the agonist. The interval between successive doses was 5-10 minutes.

2.2.8 Recordings of Contractile Responses against the Concentration of Dihydroartemisinin/Piperaquine *alone.*

In other sets of experiments; the tissue was pretreated for about 5-15 minutes with Dihydroartemisinin ($1.6x10^{-6}$ g/mL) and the whole procedure was repeated as described for acetylcholine. In each case threads was attached to the bottom of each piece of muscles; one thread was tied to the aerator hook and the other to a

transducer/frontal writing lever. The lever was balance by appropriate load (e.g., plasticine) of about 0.5 -1.0g which was applied to the lever to maintain vertical tension as described by [19, 20].

2.2.9 Ethical Consideration

All the animals received humane care and the study protocols were designed to comply with the institution's guidelines for the use of laboratory animals (Faculty of Pharmacy, University of Uyo, ethical committee's clearance was obtained), in line with the 'Principle of Laboratory animal care [23].

2.3 Statistical Analysis

The results of this study were expressed as Mean ±SEM and presented based on statistical computation using one way analysis of variance test (ANOVA) followed by Turkey-Kramer multiple comparison test using Graph Pad software. A probability level of less than 5% were considered significant.

3. RESULTS

The result of this study showed that dihydroartemisinin at a concentration of $4.0x10^{-4}$to $4.0x10^{-1}$g/mL produced no significant contractile responses on the isolated uterine smooth muscles within 30- 45 minutes of drug contact in the organ bath containing the appropriate physiological solution.

3.1 Effect of Dihydroartemisinin on acetylcholine induced contraction on rat ileum

Dihydroartemisinin at a concentration of $4.0x10^{-1}$g/ml significantly antagonised the contraction induced by acetylcholine at concentration range of 4×10^{-7} to 4×10^{-4} U.I and 4×10^{-4} to 4×10^{-1} mg/ml respectively. *P ≤ 0.05, when compared to control. (Tables 1 and2)

3.2 The Effect of Dihydroartemisin on Muscle tissues in Mice

Administration of Dihydroartemisinin (9 mg/kg ,6 mg/kg and 3.0mg/kg of body weight) to mice for 5 days in non-infected/ non-treated mice, infected/ treated mice and in infected/non- treated mice did not produced any significant effect on the integrity of the ileum smooth muscles in the range of 3.0 mg/kg and 6.0mg/kg doses administered in this study (Figure 4 to 6); however, the cellular profile of the ileum was moderately affected with the 9 mg/kg dose administered.

Table 1: Effect of Dihydroartemisininon acetylcholine-induced contractions in isolated ileum strip in rat model

					Responses of DHAon Ach induced contractions.		
FBC (mg/ml) Control	Acetylcholine	-log	*Maximum height (mm)	% of max	FBC of DHA (1.6 $x10^{-4}$mg/ml) on acetylcholine induced contractions	-Log (M)	*Maximum height (mm)
$4.0x10^{-10}$		9.4	1.5 ±0.5	50 ± 0.4	$4.0x10^{-10}$	9.4	0.7± 0.2
$4.0x10^{-9}$		8.4	2.0 ± 0.1	66 ±0.7	$4.0x10^{-9}$	8.4	1.0 ±0.1
$4.0 x10^{-8}$		7.4	2.5 ±0.0	83 ±0.3	$4.0x10^{-8}$	7.4	1.1±0.1*
$4.0 x10^{-7}$		6.4	3.0 ± 0.1	100 ±0.0	$4.0x10^{-7}$	6.4	1.4±0.5*

$\overline{X}$ ± SEM of 5 values, *p ≤0.05; maximum height3.0 ±0.1 mm for Acetylcholine

Table 2: Effect of Dihydroartemisinin/Piperaquine compared to Acetylcholine response contractions in isolated ileum strip in rat model

				Responses of DHA/piperaquine alone .			
FBC Acetylcholine (mg/ml) Control	-log	*Maximum height (mm) of contractions	% of max	FBC of DHA/Piperaquine (mg/ml) on the ileum baseline contractions	-Log (M)	*Maximum inhibitory height (mm) of relaxation	% of max
$4.0x10^{-10}$	9.4	1.5 ± 0.5	50 ± 0.4	$1.6x10^{-6}$	5.8	0.00 ±0.0	0.0
$4.0 x10^{-9}$	8.4	2.0 ±0.1	66± 0.7	$1.6x10^{-5}$	4.8	0.03 ±0.1	5.0
$4.0 x10^{-8}$	7.4	2.5 ± 0.0	83± 0.3	$1.6x10^{-4}$	3.8	0.04 ±0.1*	72.3
$4.0 x10^{-7}$	6.4	3.0 ± 0.1	100 ± 0.0	$1.6x10^{-3}$	2.8	0.17 ±0.1*	100

Mean ± SEM, of n=5, *p ≤0.05: maximum height 0.17 ± 0.1 mm(Acetylcholine)

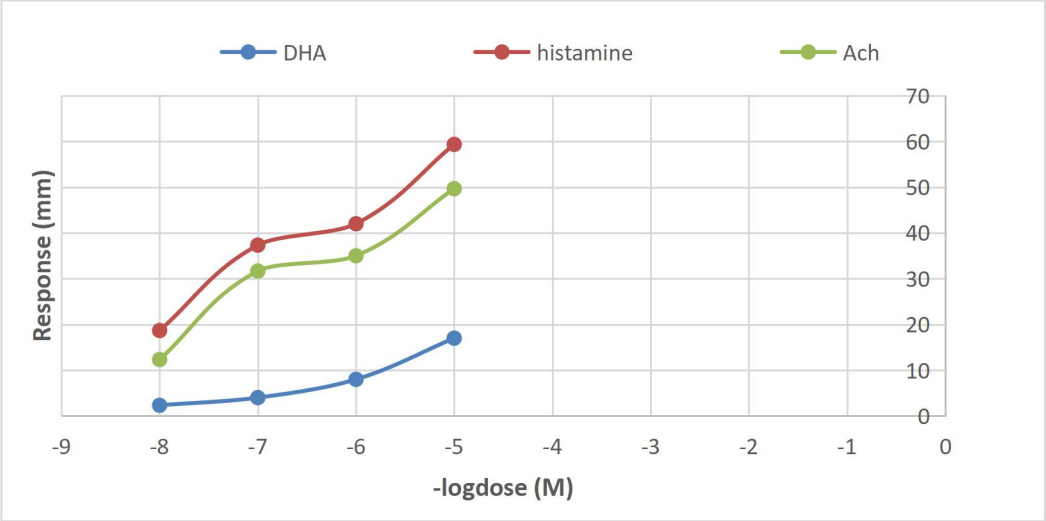

Figure 1: Comparative effect of DHA on Acetylcholine induced contraction on rat ileum, .*p ≤ 0.05 significant relative to the control.

3.2.3The histopathological examination of the effect of arthemeter on Uterus

The photomicrographs of the administration of dihydrortemisinin (3.0 mg/kg,6.0 mg/kg and 9.0 mg/kg of body weight) in mice for 5 days in non- infected/ non-treated mice ,infected/ treated mice and in infected/non-treated mice did not produced any significant effect on the integrity of the smooth muscles(p >0.05) irrespective of the dosage regimens administered in this study (Figure2-6)

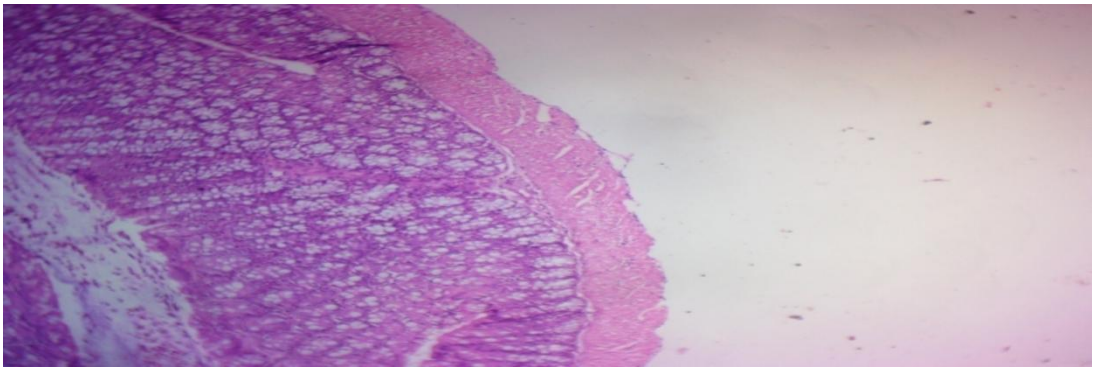

Figure 2. Photomicrographs of smooth muscle of the Ileum of non-Infected and 6 mg/kg of dihydroartemisinin at mag. A(x100) & B(x400) stained with H& E method. Revealed normal cellular profile with no abnormality seen.

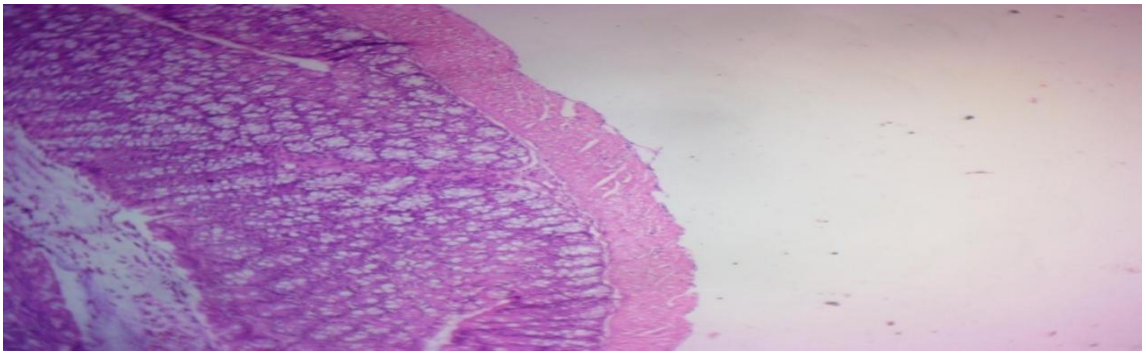

Figure 3: Photomicrographs of smooth muscle of the Ileum **Non –Infected** and treated with **3mg/kg of M.D of DHA/piperaquine** at mag. A(x100) & B(x400) stained with H& E method.**Keys:** Lumen (**L**), Nucleus (**N**), Gastric gland (**Gg**), Gastric pit (**Gp**),Muscularis mucosa **(MM)** .Revealed normal cellular profile, hence no abnormality seen

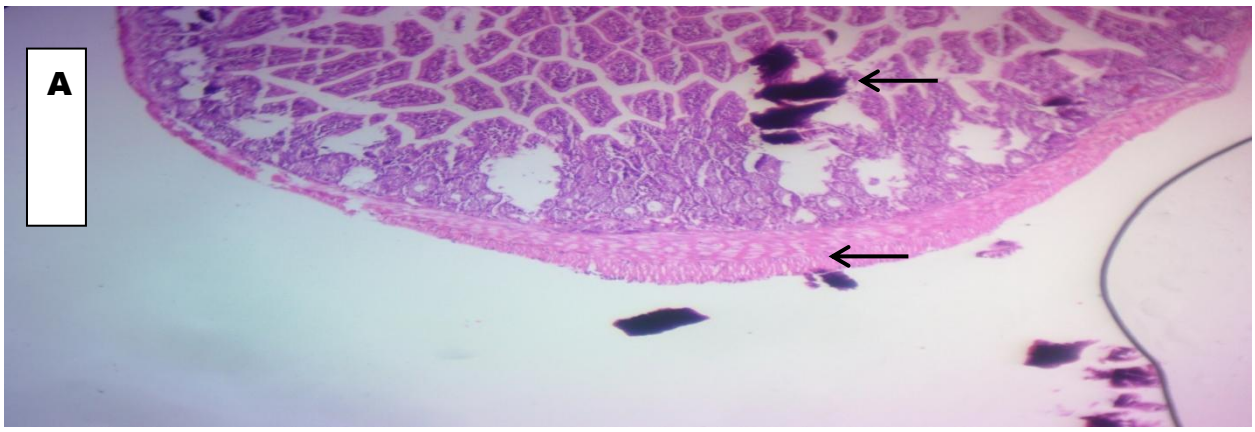

Figure 4. Photomicrographs of smooth muscle of the Ileum of non-Infected and 9 mg/kg of dihydroartemisinin at mag. A(x100) & B(x400) stained with H& E method. Revealed mild to moderate inflammation of cellular profile .

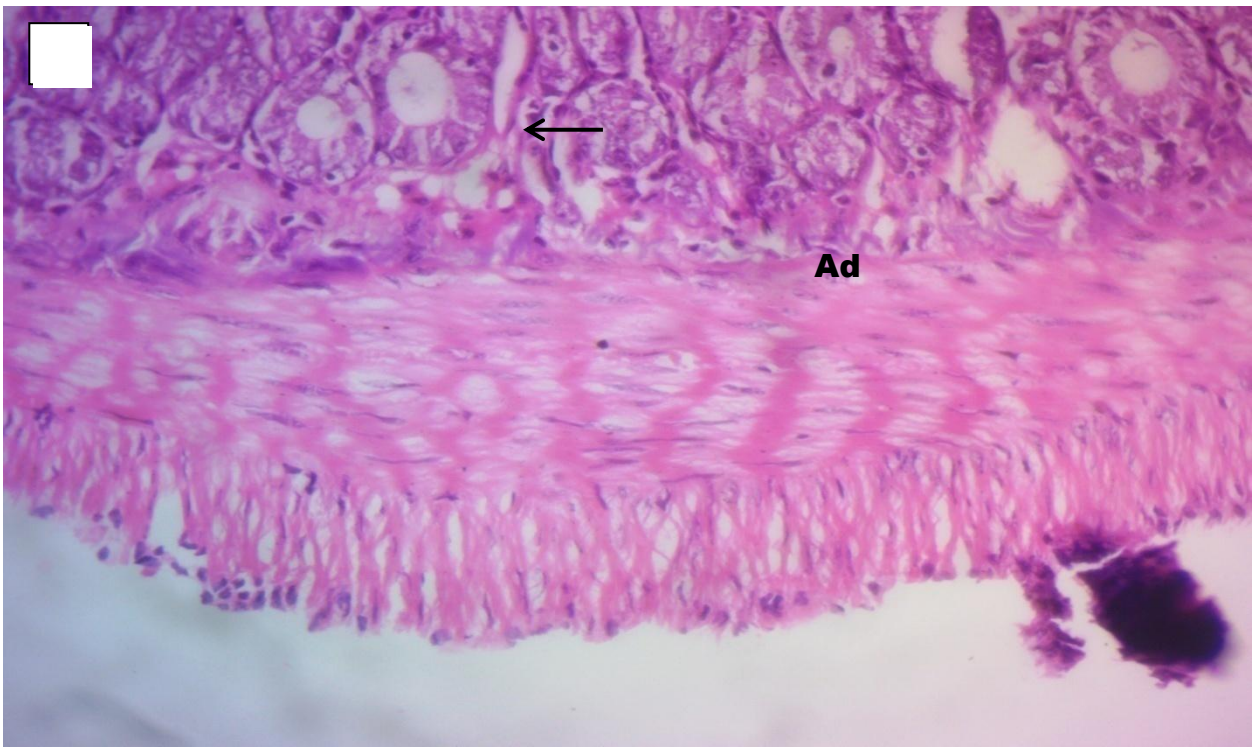

Figure 5. Photomicrographs of smooth muscle of the Ileum of non-Infected and Treated with6mg/kg of dihydroartemisinin at mag. A(x100) & B(x400)stained with H&E method. Revealed mild to moderate inflammation of cellular profile.

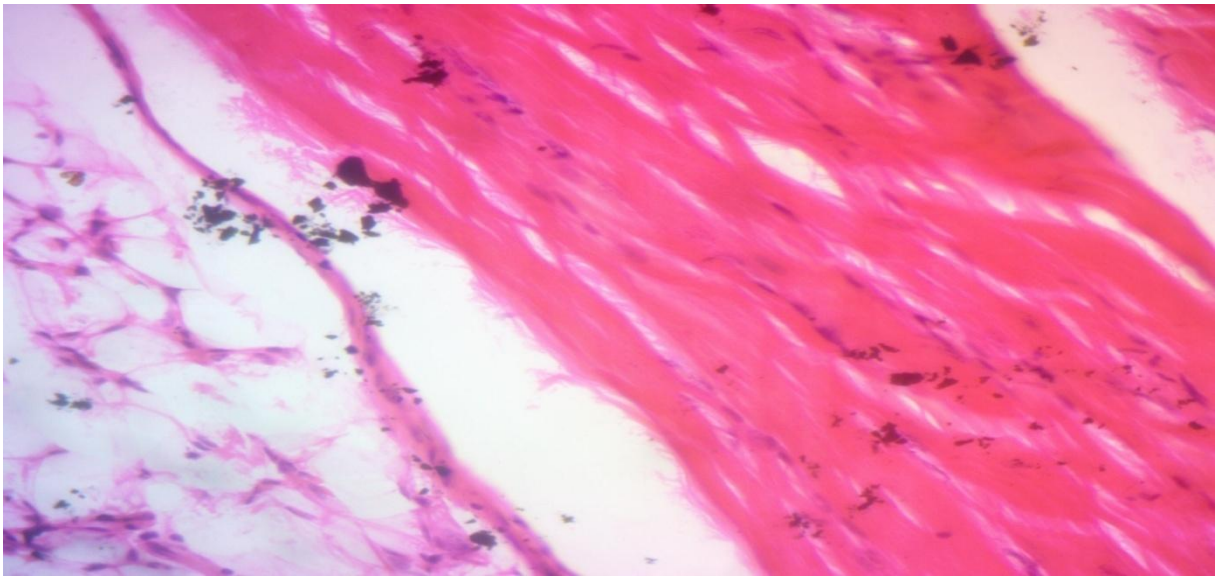

Figure 6. Photomicrographs of smooth muscle of the ileum Infected with *Plasmodium berghei berghei* and non – treated control) at Mag. A (x100) & B (x400) stained with H & E method. (Negative control).Normal cellular profile seen.

4. DISCUSSION

The results of this study revealed that, the control drug, acetylcholine in the dose range (4 x 10^{-10} –4.0 x 10^{-6} mg/mL)and Potassium chloride (1.0 x 10^{-3} - 1.0 x 10^{-1} M), both drugs caused a marked concentration – dependent contractions of the isolated ileum muscle strips. In another study, dihydroartemisinin (DHA) alone and DHA/piperaquine at a concentration of (1.6x10^{-6} –1.6x10^{-3}g/mL) produced no contractile responses on the isolated rat ileum muscle strips within 30 – 60 minutes of drug-tissue contact in the organ bath containing Tyrode's solution. In some preparations it showed no response, while in others it produced slight phasic contraction when external calcium (Ca^{2+}) ion was introduced. The slight phasic contractile activity was abolished where verapamil (5x10^{-3}mg/mL) was added to the organ bath fluid. These observed results of no contractile responses when dihydroartemisin was applied alone, was similar with report on chloroquine which produced no contractile responses when applied on the rat urinary bladder strip under baseline conditions [11]. The antagonism bydihydroartemisinin in each instance, for example (Ach. >Histamine) was non-competitive, this is basically proven by the agonist –concentration response curves which were clearly displaced to the right in asymmetric non – parallel fashion,with depressed maxima (Table2; Figure 1). It had been an established principle that, contractile responses induced by acetylcholine and carbachol were influenced mainly by the stimulation of \muscarinic receptors [16]; On the other hand, the observed slight contractile responses, were reversibly abolished due to the introduction of zero Ca^{2+} in physiological solution. KCl-induced contractions are largely reported to be due to a depolarizing action on the plasma membrane of the rat urinary bladder, as a result of which extracellular Ca^{2+} influx occurs via voltage – dependent Ca^{2++} channels (VOCs); [24, 25, 26; 27]. Also, dihydroartemisinin (1.6 x 10^{-6} – 1.6 x 10^{-3}mg/mL) when applied alone and separately had little or no marked variablecontractile effects on rat ileum smooth muscles strips: the effect of dihydroartemisinin/piperaquine (1.6 x 10^{-4}mg/mL) on acetylcholine (4.0 x 10^{-10} – 4.0 x 10^{-6} mg/mL) induced contractions was markedly inhibitory ;these inhibition was significant (P< 0.01 - 0.05, Table 1).This findings is similar with report that artemether (48 -480 ug/ ml) had no agonist effects on the isolated uterine smooth muscles of both non-pregnant and pregnant rats, however artemether (24 - 240 ug/mL) reduced oxytocin-induced contraction of uterine tissues concentration-dependently, particularly in pregnant uteri [13];similarly artesunate (4.0 x 10^{-6} – 4.0 x 10^{-5} mg/mL) had been reported to caused marked significant dose-dependent inhibitory contraction of acetylcholine, potassium chloride and histamine in depolarizing Tyrode's solution [18] .This observed results can further be justified based on earlier reports that, KCl- induced contractions were due to a depolarizing action on the plasma membrane of the guinea pigs and rats isolated ileum, as a result of which extracellular Ca^{2+} influx occurs via voltage – dependent Ca^{2+}- Channels , [24, 25, 27,]. The availability of Ca^{2+} is a basic determinant for smooth muscle contraction [28]. The observed result of inhibition by dihydroaremisinin on agonists induced contractile responses were not inhibited by phentolamineand atropine. in the different set of study- which might likely suggest non-specific antagonism. The histopathological examination revealed no abnormality seen in the ileum smooth muscle profiles at the dose of 3mg/Kg and at 6mg/Kg of DHA/piperaquine administered.

5. CONCLUSION

The results from this study revealed significant inhibitory contractile responses of dihydroartemisinin and DHA/piperaquine combination on acetylcholine induced contraction in isolated ileum smooth muscle tissue in rat. These inhibitory responses were in a dose dependent manner. Dihydroartemisinin seems to be acting by interference with extracellular Ca^{2+}influx and possibly with mild interference with transmembrane ion fluxes by a non-specific process; histopathological studies also revealed that DHA may possess safe pharmacological properties that justified their current usage in the treatment of malaria as recommended by the World Health Organization.

DECLARATIONS

Acknowledgments

Authors are immensely grateful to the TETFUND for approval and sponsorship of this institution-based research work in 2021-2023; we are also grateful to Professor Prince Unekwe and Professor Paul A. Nwafor for providing useful information on the basic protocols for organ bath studies and details of guidelines for ethical approval respectively. Also, we acknowledged Professor H.O.C. Mbagwu for strong suggestion and encouragement to carry out these studies with our under graduate students in the University of Uyo, Uyo Akwa Ibom State.

Conflict of Interests

There is no conflict of interest existing among the Authors in this research work.

Contribution of the Authors

Author 1 is the chief investigator, Author 2 , 3and 4 are the co-investigators; while Authors 5 is our undergraduate student on research work ; author 6 and 7 assisted in the data analysis while Author 8 is the main supervisor.

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