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Effect of artemether on the mechanical contractility of the isolated uterine smooth muscle in pregnant mice models

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ABSTRACT

Background: Artemether is widely used in combination therapy as anti-malarial agent. The effect of artemether/ lumefantrine on smooth muscle contractility to drug in pregnant female mice was investigated using an isolated uterine smooth muscle strips mounted on organ bath. The study further investigated the effect of artemether on histopathology of the uterus using standard in vivo procedures/ protocols in mice infected with *Plasmodium berghei berghei* .

Method: Twenty-five (25) matured pregnant female albino mice (25 -30g) were utilized for both in vitro and vivo studies. The mice in the test group for in vivo study were treated with 1.5mg/kg, 3.0mg/kg and 6.0 mg/kg of artemether/lumefantrine respectively for five days. Data obtained from the study were represented as Mean ± SEM in table and graphs.

Results: The results of this study revealed that artemether/ lumefantrine ($4x10^{-4}$ to $4x10^{-1}$ mg/ml) alone did not produce contractile responses, but caused significant inhibition on contractile responses induced by oxytocin ($4x10^{-7}$- $4x10^{-4}$ I.U/ ml.) and acetylcholine ($1x10^{-4}$ to $1x10^{-1}$ mg/ml) on uterine smooth muscle tissue in mice in a dose dependent manner ($p<0.05$); also, the Emax of Oxytocin ($4x10^{-4}$ I.U/ ml) is 22.5 ±. 2.0 mm Artemether significantly reduced the Emax of oxytocin ($4x10^{-4}$ I.U/ ml) and acetylcholine ($1x10^{-1}$ mg/ml) respectively on uterine smooth muscle strips to 11.0 ±. 1.0 mm and 2.9 ± 1.0 mm respectively, ($p<0.05$- 0.01); histopathological examination revealed normal uterine smooth muscle profile.

Conclusion: The results from this study revealed significant inhibitory contractile responses of artemether on oxytocin and acetylcholine induced contraction in isolated uterine smooth muscle tissue in mice in a dose dependent manner and artemether has safe profile.

Keywords: Artemether, contractility, *Plasmodium berghei berghei*, and Uterine smooth muscles.

1. INTRODUCTION

Malaria is a tropical disease; and it is a major public health problem in endemic regions [1].World malaria report indicate that there were 219 million cases of malaria globally in 2019 and 438,000 malaria deaths [2]. Plasmodium falciparum, is the most clinically significant causative organism and has been reported to demonstrate an unusual propensity to acquire resistance to antimalarial therapy [3, 4] Currently artemisinin and its derivatives (artemether, arteether, artesunate and dihydroartemisinin) present a new series of antimalarial drugs with a high level of activity against chloroquine-resistance strains of malaria parasite [5]. The World Health Organization currently recommends artemisinin-based combination therapy (ACT) as first line treatment for uncomplicated malaria, which has also resulted to increase in the use of artemisinins [6]. Artemisinins generally have good permeability; artemether is lipid

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–soluble and poorly water-soluble [7]. Furthermore, treatment of malaria with artemisinins has been implicated in various unwanted effect or adverse effects, including that of muscle weakness, malaise, vomiting, diarhoea etc most of these effects are on smooth muscles activities. Research work on the mechanical activity of muscles on ancient anti-malarials such as chloroquine, mefloquine, quinine in animal model have been carried out extensively [8, 9, 10; 11, 12]. Studied the effects of chloroquine on the rat urinary bladder strip. On the contrary, few studies so far have been carried out on the effect of Artemisinin derivatives on muscle contractility; Ejiofor studied the effects of artemether on uterine muscle[13].The effect of artesunate on guinea pig ileum was investigated [14].This present work was designed to investigate the effect of artemether on isolated smooth muscle contractility of uterine strips with a view of elucidating the probable mechanism of the drug action and to ascertain the safety profile of artemether.

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 artemether (Novartis Pharm., New York) -purchased from the University of Uyo Health Centre, Akwa Ibom State, Nigeria. Atropine, Calcium channel blockers e.g. Verapamil, α-adrenoceptor blocker such as Phentolamine etc. Acetylcholine Chloride were obtained from Sigma Chemical Co.(USA), Calcium chloride (CoPharm): Magnesium chloride (Hopkin Williams, U.K) and Potassium chloride (Sigma USA). The Physiological solutions used in this study were Dejallon'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 drug -Arthemeter was administered to all the animals in the test groups, with the aid of 23G stainless steel oropharyngeal cannula (via oral route).

The method of calculation of volume (ml) of the drug to be given to each animal 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 dosage 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 Body weight [15].

2.2 Methods

2.2.1 Parasite inoculum preparation
The method of Peter [16]. As described by Udobang [17] 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 breed male and female albino mice (weight - matched). They were all healthy, hence diseases-free. Twenty- five (25) mice (20-25 g), were purchased from the animal house of the Department of Pharmacology and Toxicology University of Uyo; they were used for both in vitro organ bath study and the in vivo models; 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- grower's 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 °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. artemether/lumefantrine 1.5, 3.0 and 6.0mg/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 urethane anaesthesia 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 mice 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 follow: clear incision were made into the abdominal cavity of the mice up till the border near the tail of the rats. Fresh ileum, uterus, and portion of large intestine were 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 describe by Yakubu [18]. 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 [19, 20].which were applicable in the use of 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 be 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 Dejallon'salone; It was allowed to stabilized for some about thirty (30) to sixty (60) minutes before investigation commences. Isolated muscle preparations from mice in vivo model were used for this study: Precisely the smooth muscle preparations were obtained from the reproductive organ system e.g. isolated mice uterine muscle in mice models.

2.2.5 Recordings of Contractile Responses against the Concentration of the Acetylcholine

The methods as described by Unekwe [19].were adopted. A vertical strand of isolated ileal 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 Artemether. 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.

2.2.6 Recordings of Contractile Responses against the Concentration of the Oxytocin.

The methods as described by [19, 20], were adopted. Before the concentration response tests to oxytocin, the organ bath was set up and the equilibration time in e case was 30 - 60 minutes during which time the tissue preparation immersed in Dejallon's solution, was wash several times till the normal rhythm city of the pendular movement become steady. Then, the graded dose - response relationships with isolated muscle tissues at specified concentration of 10^{-7} to10^{-3} M, this was prepared from a stock oxytocin in 10 mls of normal saline, by a ten-fold serial dilution of the stock solution.

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 Artemether. The initial observations with agonist alone served as control values, which were used to

compare the effect of artemether 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 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 Artemether alone.
In another sets of experiment; the tissue was pretreated for about 5-15 minutes with artemether($2.1x10^{-6}$ 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 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' [21].

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 shows that artemether 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 artemether on oxytocin induced contraction in pregnant uterine muscle strip in mice models
Artemether alone at a concentration of $4.0x10^{-1}$g/ml produced no contractile responses on the isolated uterine muscle strip within 30 to 45 minutes of drug contact in the organ bath containing Dejalon's solution. Artemether $4.0x10^{-1}$g/ml significantly antagonist the contraction induced by oxytocin at concentration of 4×10^{-7} to 4×10^{-3}I.U (Table 1).

3.2.1 Effect of Artemether on acetylcholine induced contraction on rat ileum
Artemether at a concentration of $4.0x10^{-1}$g/ml significantly antagonised the contraction induced by oxytocin and 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. (Table 1and Table 2)

3.2.2 The Effect of Artemether on Muscle tissues in Mice :
Administration of artemether (6 mg/kg ,3 mg/kg and 1.5 mg/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 uterine smooth muscles irrespective of the dosage regimens administered in this study (Figure 3 and 4); however, the glandular areas of the uterus was moderately affected.

Table 1: Effect of Artemether on Oxytocin-induced contractions in isolated uterine strip in mice model

				Responses of artemether on oxytocin induced contractions.		
FBC Oxytocin (I.U./ml) Control	-log	* Maximum height (mm)	% of max	FBC of artemether (mg/ml) on oxytocin induced contractions	-Log (M)	* Maximum height (mm)
$4.0x10^{-7}$	6.4	4.6 ±3.0	20 ± 0.4	$4.0x10^{-4}$	3.4	1.0 ± 0.0
$4.0x10^{-6}$	5.4	8.0 ± 2.0	35 ±0.6	$4.0x10^{-3}$	2.4	5.2 ±0.7
$4.0 x10^{-5}$	4.4	13.5 ±0.1	60 ±0.1	$4.0x10^{-2}$	1.4	9.0±0.3*
$4.0 x10^{-4}$	3.4	22.5 ± 2.0	100 ±0.0	$4.0x10^{-1}$	0.4	11.0±1.0*

Control response: graded concentration of Oxytocin (I.U/ ml.), Test response: responses concentration of artemether in g/ml; $\overline{X}$ ± SEM of 4 values, *p ≤0.05; maximum height .22.5 ±2.0 mm for Oxytocin.

Table 2: Effect of Artemether on Acetylcholine induced contractions in isolated uterine strip in mice model

				Responses of artemether alone			
FBC Acetylcholine (mg/ml) Control	-log	* Maximum height (mm)	% of max	FBC ofartemether0.4 (mg/ml) on Ach. induced contractions	-Log (M)	* Maximum height (mm)	**% of max**
$4.0x10^{-4}$	3.4	1.2 ± 0.1	27	$4.0x10^{-4}$	3.4	0.8 ±0.0	18
$4.0 x10^{-3}$	2.4	3.1 ±0.2	69	$4.0x10^{-3}$	2.4	2.0 ±0.2	44
$4.0 x10^{-2}$	1.4	3.7 ± 0.4	83	$4.0x10^{-2}$	1.4	2.4 ±0.18*	53
$4.0 x10^{-1}$	0.4	4.5 ± 0.1	100	$4.0x10^{-1}$	0.4	2.9 ±0.2*	64

Control response: graded concentration of Acetylcholine (mg/ml), Test response: responses concentration of artemether in mg/ml; $\overline{X}$ ± SEM of 4 values, *p ≤0.05: maximum height 4.5 ± 0.1 mm(Acetylcholine).

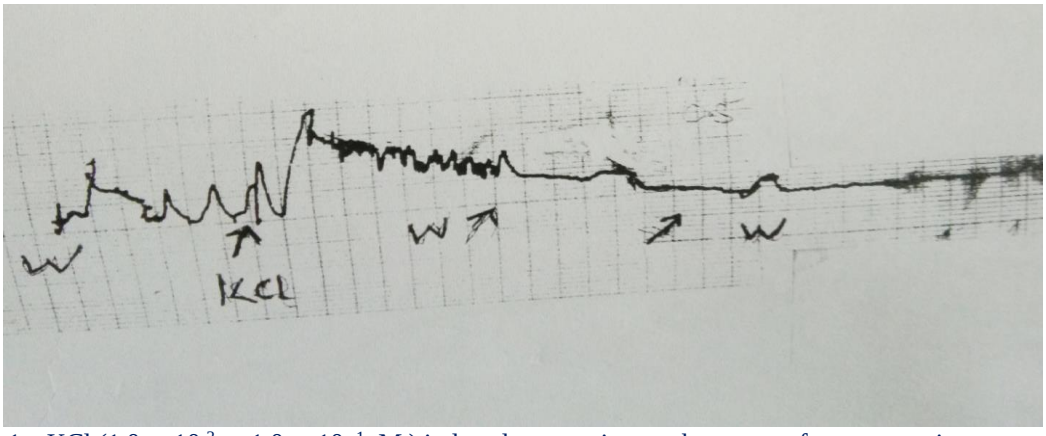

Figure 1a: KCl (1.0×10^{-3} - 1.0×10^{-1} M) induced contraction on the uterus of pregnant mice.

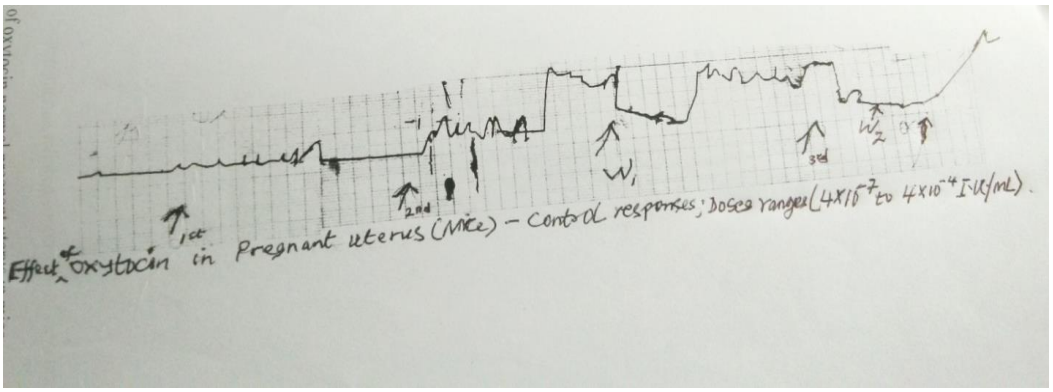

Figure 1b: The effect of artemether on oxytocin ($0\ 4 \times 10^{-7}$ -4.0×10^{-4}) induced contraction on the uterus of pregnant mice.

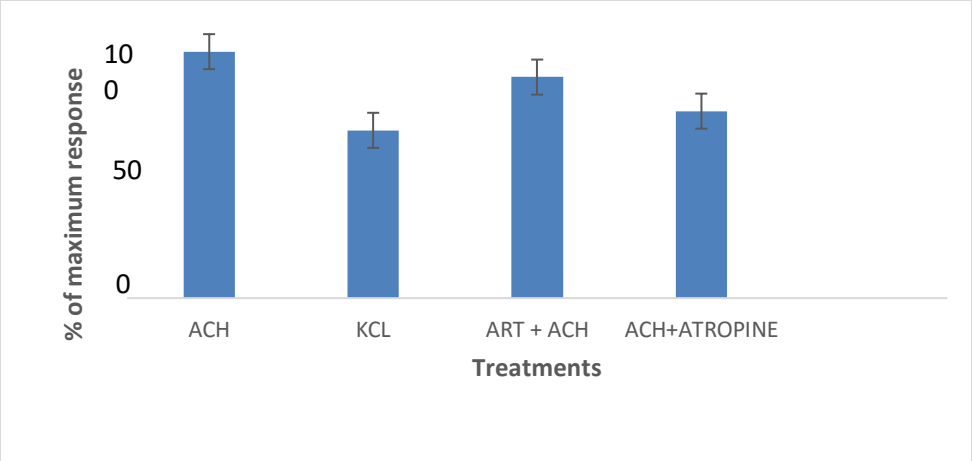

Figure 2: Bar chart showing selected maximum responses ofsome agonists / antagonist and artemether(ART.).

3.2.3 The histopathological examination of the effect of arthemeter on Uterus

The photomicrographs of the administration of arthemeter (6 mg/kg,3 mg/kg and 1.5 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 (Figure 3-4)

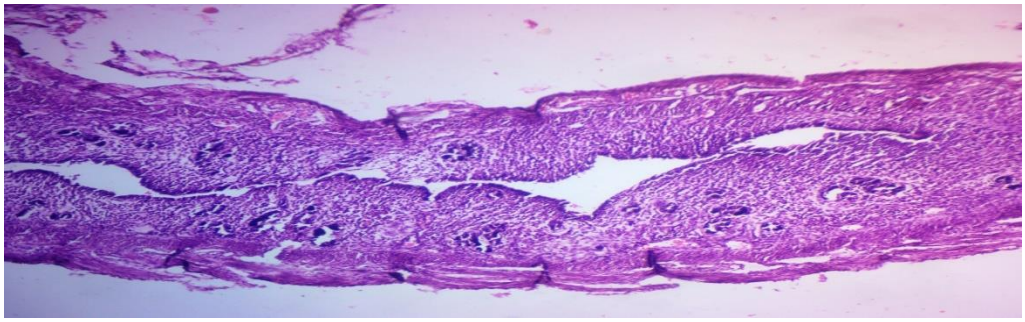

Figure 3. Photomicrographs of smooth muscle of the Uterus non-Infected and 6 mg/kg of artemether at mag. A(x100) & B(x400) stained with H& E method. Revealed normal cellular profile with no abnormality seen.

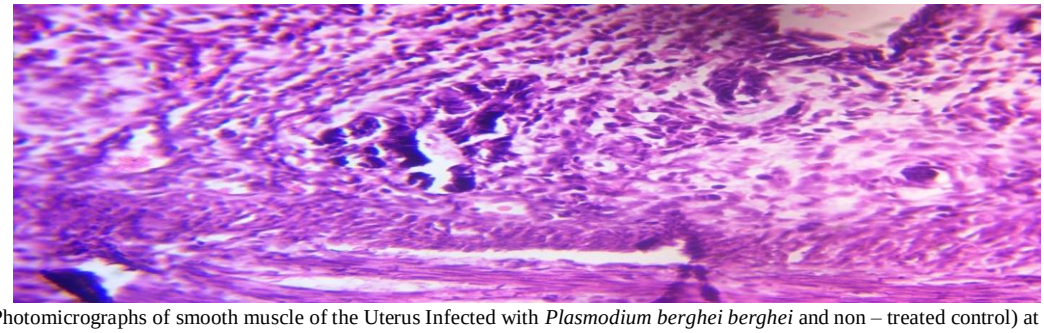

Figure 4. Photomicrographs of smooth muscle of the Uterus Infected with *Plasmodium berghei berghei* and non – treated control) at Mag. A (x100) & B (x400) stained with H & E method. (Negative

4. DISCUSSION

The results of this study revealed that, the control drug, acetylcholine in the dose range (4×10^{-4} – 4.0×10^{-1} mg/ml), Oxytocin ($0\,4 \times 10^{-7}$–4.0×10^{-4}), Potassium chloride (1.0×10^{-3} - 1.0×10^{-1} M), each caused a marked concentration

– dependent contractions of the isolated uterine muscle strips . In another study, artemether alone at a concentration of ($4x10^{-4}$ –$4x10^{-1}$g/ml) produced no contractile responses on the isolated mice uterine muscle strips within 30 – 60 minutes of drug-tissue contact in the organ bath containing Dejalon'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. This observed results of no contractile responses when artemether 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 by artemether in each instance, for example (Ach. > Oxytocin>KCl) 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 (Table 2 ;Figure 2). It had been an established principle that, contractile responses induced by acetylcholine and carbachol were influenced mainly by the stimulation of muscarinic receptors Unekwe et al., 2007) On the other hand, the observed 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); [22, 23, 24; 25]. also, artemether ($4 \times 10^{-4} – 4 \times 10^{-1}$mg/ml) when applied alone and separately excited marked variable effects on rat uterine muscles strips: the effect of artemether (4×10^{-1}mg/ml) in non-pregnant mice uterus on oxytocin ($4.0 \times 10^{-7} – 4.0 \times 10^{-4}$ I.U.) induced contractions was markedly inhibitory ;these inhibition was significant ($P< 0.01 - 0.05$), (Figure 1b; Table 1) .This findings corroborated 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 the drug (24 - 240 ug/ml) reduced oxytocin-induced contraction of uterine tissues concentration-dependently, particularly in pregnant uteri [13]. 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 , [23, 22, 25,]. The availability of Ca^{2+} is a basic determinant for smooth muscle contraction [26]. The observed result of inhibition by artemether on agonists induced contractile responses were not inhibited by phentolamine ,and atropine in the different set of study- which might likely suggest non-specific antagonism. The histopathological examination revealed no abnormality seen in the uterine smooth muscle profiles.

5. CONCLUSION

The results from this study revealed significant inhibitory contractile responses of artemether on oxytocin and acetylcholine induced contraction in isolated uterine smooth muscle tissue in mice in a dose dependent manner. Artemether and lumefatrine seems to be acting via a non-specific receptor mechanism, interference with extracellular Ca^{2+} influx and possibly with mild interference with transmembrane ion fluxes by a non-specific processes; artemether seems to possess great and safe pharmacological properties that justified their current usage in the treatment of malaria as recommended by the World Health Organization.

DECLARATIONS

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Conflict of Interest

Declare the conflict of interest of the Authors here

Contribution of the Authors

State how each Author contributed to the research work here

REFERENCES

[1] Breman, JG; Alilio, MS, and Mills A. Conquering the intolerable burden of malaria: what's new, what's needed. (2004).

[2] WHO / World Malaria Report World Health Organization. world malaria report 2014/en/Accessed 2019,March 3·Availableathttp://www.who.int/malaria/publications/

[3] Bruce-Chwatt, LJ. Recent Trends of Chemotherapy and Vaccination against Malaria . New lamps for old. British Medical Jounal1985, 291: 1072-1076 .

[4] WHO Severe and Complicated Malaria Transmission. Royal Society of Tropical Medicine. And Gyg. 1986. 80 (suppl). 1-50.

[5] Gary, HP; Michael HP; Northrop, Jeffrey S; Ploypradith, Orally active, hydrolytically stable, semi-synthetic, antimalarial trioxanes in the Artemisinin family. Journal of Medical Chemistry. 1998, 42(2): 300-304.

[6] Chen, C. Development of antimalarial drugs and their application in China; a historical review.inf Dis Pov. 2014: 3(9); 3-9.

[7] Rosenthal, PG. Antiprotozoal drugs in; Katzung BG (ed.) Basic and clinical Pharmacolgy. The McGraw-Hill Companies Inc., Singapore. 2004. p. 864-85.

[8] Isaacson, A; Sandow, A. Quinine and Caffein effects on 45 Ca movements in frog Sartorious muscles, sartorious muscles J , Gen. Physiol.1967:50,2109-2128

[9] Huddart, H. The effect of quinine on tension development, membrane potentials and excitation-contraction coupling of crab skeletal muscle fibers. Journal of Physiology (London) 1971: 216:214-257.

[10] Ebeigbe, A; BM; Aloamaka CP; Effects of Chloroquine on mechanical activity the rat portal vein microcirculation 1982: 2, 151-159.

[11] Unekwe, PC; Nwajie EE; Edafiogho, IO; Effect of Chloroquine on rat Urinary bladder strip. Nig. Journal of physiological Sciences, 1990: 6(2) 109-113.

[12] Unekwe, PC; Ogama JO; Chilaka KC; Okonkwo JC. Effect of Mefloquine on the mechanical activity of the Mouse isolated rectal smooth muscle. Nigeria. Journal of Physiol. Sciences. 2007:22(1-2):43-47.

[13] Ejiofor, JI; kwanashie HO; Anuka JA. Effects of Artemether on pregnancy related parameters in albino wistar rats, Journal of Pharmaceutical Sciences, 2006; 17: 97—11 1

[14] Tologbonse, AA; Unekwe PC; Mbagwu, HO C; Udo MO; Essien, GE; Bello, TE. The Effects of Artesunate on Isolated Ileum Smooth Muscle in Guinea pig Model…Absract in International Conference Proceedings of West African Society of Toxicology(WASOT),Uniabuja, 2018. p.65.

[15] Ugian, B; Artemisinin Based Combination Therapy Combinations for Treatment of Malaria: Metaanalysis. 2013; 363(9402):9-17.

[16] Peters, W; Chemotherapy and Drug Resistance in Malaria. London and New York Academic Press, 1970 345-391.

[17] Udobang, JA; Nwafor, PA; Okokon, JE. Analgesic and Antimalarial Activities of Crude Leaf Extract and Fractions of AcalyphaWilkensiana. Journal of Ethnopharmacology, 2010; 127, 373 – 378.

[18] Yakubu, MT; Olajide AT; Akanji MA. Mode of cellular toxicity of aqueous extract of Fadogia agrestis (schwein f.Ex Hiern) stem in male rat liver and kidne. Human and experimental Toxicology, 2009; 28(8), 469-478.

[19] Unekwe, PC; Ogama, JO; Chilaka KC; Okonkwo JC. Effect of Mefloquine on the mechanical activity of the Mouse isolated rectal smooth muscle. Nigeria Journal of Physiological Sciences. 2007; 22 (1-2):43-47.

[20] Nwafor, PA; Okwuasaba, FK. Anti-conceptive and Anti-inflammatory Effects of Methanolic Extract of Asparagus pubescens Root in Rodents. Journal of Ethnopharmacology. 2003; 84, 125 – 129.

[21] NIH Principle of Laboratory animal care (of National Institute of Health-NIH Publication No. 1996; 85-23,) guidelines.

[22] Bolton, TB. Mechanism of Action of Transmitters and other Substances On Smooth Muscles. Physiology Review 1979; 59,:606-718.

[23] Van Breemen, C; Aronson, P, Lutzenbiser, R. Sodium-calcium interactions in mammalian smooth muscles. Pharmacology. Rev. 1977; 30: 167-208

[24] Brading, AI; Srieldon, P. Evidence for Multiple Sources of Calcium for Activation of the Contractile Mechanism for Guinea pig Taenia coli on Stimulation with Carbachol. British Journal Pharmacology 1980; 70:229-240.

[25] Cauvin, C; Lukeman S; Cameton J; Hwang O; Masheri K; Yamamoi H; Van Breeman, C. Theoretical bases for Vascular Selectivity of Ca^{2+}antagonists, Journal of Cardiovascular Pharmacology, 1984; 6:5630 -5638.

[26] Marshall Ionic basis for smooth muscle contraction. Pharmacol. Text, 1980.