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# Teratogenic Effect of Artesunate on the Morphometric Parameters of the Body and Developing Olfactory Bulb of Wistar Rats Following Maternal Oral Administration

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## ABSTRACT

Artesunate an antimalarial drug has been reported to cause irreversible brain damage in experimental rats, and to cause other toxic effects like haemolysis and neutropenia. This study was conducted to investigate the morphometric teratogenic effect of the antimalarial agent artesunate (ARTS) on the developing olfactory bulb of Wistar rats following maternal oral administration. A total of 16 virgin female and 8 male Wistar rats weighing 150 g were used for this study. The oestrous cycle of the female rats were determined and at the proestrous phase they were allowed to mate with the male overnight. Pregnant rats were administered ARTS daily from gestational day 8-12 via oral gavages, at test doses of 0, 2, 4, or 8 mg/kg (4 females per group). The results showed significant reduction in the crown-rump length, hind-limb length, fore-limb length and organ-body weight ratio in the pups. There was significant decrease in the length, width, thickness, and weight of the olfactory bulb at <0.001, which was dose dependent, with more effect on the 8 mg/kg group than in the 2 mg/kg group. The effect of artesunate was dose dependent, and has been shown to significantly decrease the crown-rump length, fore-limb length, hind-limb length, and organ body weight ratio of the pups and it also significantly decreased the weight, length, thickness and width of the olfactory bulb with no observable adverse effect level to the 2 mg/kg/day group, thus ARTS might affect embryo and foetal development.

**Keywords:** *Artesunate, Teratogen, morphometry, Wistar rat pup, olfactory bulb*

## INTRODUCTION

Artesunate is part of the artemisinins group of drugs that treats chloroquine resistant malaria. It has been recommended as the first line treatment for severe malaria (WHO 2015). Artesunate is a potent and rapidly acting blood schisontocide concentrated in parasitized erythrocytes but has no hypozoiticidal activity. It exerts its anti-malarial activity by generating reactive oxygen species by iron-catalyzed cleavage of the endoperoxide bridge through endoperoxide bond (Nontprasert et al. 1998; Nontprasert et al. 2002). Neurotoxicity is the greatest concern regarding artemisinins, since the administration of high doses in laboratory animals has led to severe and irreversible changes in the brain (Howard and Kuile 1995). Toxic effects including neutropenia, anaemia, haemolysis and elevated levels of liver enzymes, have been noted (Rolling et al. 2013; Bogoniya et al. 2015).

The olfactory bulb is the most rostral part of the brain in most vertebrates, but in humans however, the

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olfactory bulb is on the inferior side of the brain. The major function of the olfactory bulb is to process odour, discriminate between smells, and filter out background smells and deliver the information to various parts of the brain (Mori et al. 2006; Slotnick et al. 2010). This study aimed at investigating the teratogenic effect of different doses of antimalarial agent, artesunate on the morphometric parameters of the body and developing olfactory bulb of Wistar rats following maternal oral administration.

## MATERIALS AND METHODS

### Animals

A total of sixteen (16) virgin female Wistar rats (female Wistar rat in which their virginal orifice were not yet opened) and eight male rats were used for the investigation. The rats were obtained from the Animal house of Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University (A.B.U.), Zaria, Nigeria. The animals were kept in standard animal plastic cages of four animals per cage in the animal house of the Department of Human Anatomy, Faculty of Medicine, A.B.U. Zaria for two weeks for the purpose of acclimatization and adaptation. They were divided into four groups designated as A, B, C and D. Group A serves as the control group, while B, C and D serve as the test groups. The rats were fed with broiler's feed (Grand Cereals Limited, Jos, Nigeria) and given water *ad libitum*. Artesunate tablets (BLISS GVS PHARMA LTD, India. Batch No. ALE-45 and NAFDAC Reg. No. 04-9927) used for this study was purchased from a reputable Pharmaceutical store in Zaria, Nigeria.

### Determination of Oestrous, Mating and Pregnancy

After two weeks of acclimatizaton, oestrous phase of the female Wistar rats were determined, and at the proestrous phase the animals were allowed to mate overnight following the method of Adebisi (2008). Pregnancy was determined following the method of Adebisi (2008). Pregnancy in rats was confirmed by examining the vaginal cytology from each rat after two days and a continuous diestrous phase of oestrous cycle implied that the rat's regular 4-days reproductive cycle has been altered by pregnancy. And also the weight of the rats was taken daily and a progressive increase in body weight was also attributed to pregnancy according to Mesembe et al. (2004).

### Administration of Artesunate

At embryonic day eight (E8), which is the beginning of the second trimester in Wistar rats, the rats in group A were given 0.2 ml distilled water, the test groups B, C and D were orally administered 2 mg/kg, 4 mg/kg and 8 mg/kg body weight of artesunate respectively (Table 1), from gestational day 8 to gestational day 12 (five days). Adminsitration was from the second week of pregnancy in order to target organogenesis, which begins from this period of pregnancy in Wistar rat. All the experimental procedures and protocols used in the study were in accordance with the Institutional Animal Ethical Committee who had given approval for the research.

**Table 1: Grouping of Experimental Animal**

| Groups | Description of dosage |
|---|---|
| Group A (Control) | Negative control group (0.2 ml) of distilled water |
| Group B | Low dose (2 mg/kg) of artesunate |
| Group C | Medium dose (4 mg/kg) of artesunate |
| Group D | High dose (8 mg/kg) of artesunate |

### Morphometric Studies

Pregnant Wistar rats were allowed to litter normally. The pups from the different groups were weighed using an electronic sensitive weighing scale (Acculab Sartorious group. ME-36S RE-1ug, Scientech Balance, Massachusetts, U.S.A.). The crown-rump length, tail length, fore-limb length and hind-limb length of the pups were taken using digital Vernier caliper (6''/150MM Elite digital Vernier caliper, Shenzen Wiysond Technology Co. Ltd, Guangdong, China). The pups were then humanely decapitated under aneathesia, and the brains were gently removed from the cranium using dissecting kits. The olfactory bulb was carefully removed from the frontal lobe of the cerebrum and weighed using Acculab Sartorius group digital scale, ECS-500 (Scientech). The anterio-posterior length, breadth, width of the olfactory bulb of the litters were measured using Elite digital Vernier caliper.

### Data Analysis

Data obtained were analysed using statistical package, Statistical Package for Social Science (SPSS) version 22 (IBM, Cooperation, NY) for windows. The data were expressed as mean ± SEM. Differences among the mean were determined by one way analysis of variance, and values were considered significant if p-value is less than 0.05 ($p < 0.05$). Tukey's Post Hoc test was used to determine where the significant levels.

**Table 2: Morphometry of Pups Following Maternal Oral Administration of Different Doses of Artesunate**

| | Artesunate (mg/kg) | | | | |
|---|---|---|---|---|---|
| Pup's variables | Control 0.2ml water Mean ± SE | Low dose 2mg/kg Mean ± SE | Medium dose 4mg/kg Mean ± SE | High dose 8mg/kg Mean ± SE | *p*-value |
| Body weight (g) | 6.51± 0.18 | 4.99 ± 0.11 | 5.47 ± 0.19 | 3.82 ± 0.19 | <0.001 |
| Crown rump length(cm) | 4.23± 0.16 | 3.59 ± 0.02 | 3.51 ± 0.00 | 3.56± 0.10 | <0.001 |
| Fore-limb length (cm) | 1.01± 0.01 | 0.93 ± 0.21 | 0.82 ± 0.04 | 0.73 ± 0.04 | <0.001 |
| Hind-limb length (cm) | 1.09± 0.03 | 0.99 ± 0.02 | 0.88 ± 0.03 | 0.89± 0.04 | <0.001 |
| Organ-body ratio (%) | 0.88± 0.05 | 1.07 ± 0.07 | 0.72 ± 0.03 | 0.48 ± 0.02 | <0.001 |

## RESULTS

This study showed the mean body weight, crown-rump length, fore-limb length, hind-limb length and organ-body weight ratio of the pups in the control, low dose (2 mg/kg), medium dose (4 mg/kg) and high dose (8 mg/kg) groups respectively (Table 2). There was significant decrease in the morphometry of the artesunate treated group at $p < 0.001$.

There was statistical significant decrease in the weight (g) of the Pups in the artesunate treatment groups 2 mg/kg, 4 mg/kg, 8 mg/kg, was significantly higher compared to control group (distilled water) (Table 2). There was significant difference in the morphometry (length, width, thickness and weight) of the olfactory bulb of pups in the artesunate treated groups at $p < 0.001$ (Table 3).

## DISCUSSION

This study showed that prenatal consumption of artesunate significantly reduce the weight, crown-rump length, hind-limb length, fore-limb length and organ-body weight ratio of pups, except for the tail length, with increase in concentration of artesunate administered at p-value less than 0.05 ($p < 0.05$). Bigoniya et al. (2015) reported that sub-chronic oral artesunate administration of 2, 4 and 8 mg/kg/day for 42 days treatment caused decrease in body weight. An increase in the incidence of pup visceral and skeletal variations observed in the 4 mg/kg and 8 mg/kg groups occurred in a dose-dependent manner, which suggests that foetal development was adversely affected by artesunate and it is in support with the findings of Adebisi (2008), who reported that animal experiments (Wistar rat model) show considerable toxicity upon application of artesunate. Chung et al. (2013) also reported that artesunate has adverse effect on the overall visceral and skeletal development in albino rats (Sprague-Dawley rats). Other reports show that artesunate caused embryo lethality and malformations when administered orally to rats during organogenesis (White et al. 2006; Li et al. 2008).

The present study shows that prenatal consumption of artesunate significantly ($p < 0.001$) reduce the weight, length, breadth and thickness of the olfactory bulb of the pups, with increase in concentration of artesunate administered. This could lead to overall decrease in the normal proportion of the olfactory bulb content. In a previous study, oral administration of different doses of artesunate in pregnant Wistar rats showed histological changes in the olfactory bulb of litters of pregnant Wistar rat exposed to high dose of the drug (Musa et al. 2015). Variations observed in the 4 mg/kg and 8 mg/kg groups occurred in a dose-dependent manner. This support the findings of Mesembe et al. (2004), that reported artesunate to cause significant decrease in the length, diameter, breadth, width and weight of the central nervous system of Wistar rat pups.

In conclusion, exposure of pregnant Wistar rats to 2 mg/kg, 4 mg/kg and 8 mg/kg of artesunate has prenatal effects on developing olfactory bulb of Wistar rat dams. Statistical analysis for the pups, and

**Table 3: Morphometry of the Olfactory Bulb of Wistar Rat Pups Following Maternal Oral Administration of Different Doses of Artesunate**

| | Artesunate (mg/kg) | | | | |
|---|---|---|---|---|---|
| | Control 0.2ml water | Low dose 2mg/kg | Medium dose 4mg/kg | High dose 8mg/kg | |
| Olfactory bulb variables | Mean ± SE | Mean ± SE | Mean ± SE | Mean ± SE | *p*-value |
| Length (mm) | 4.76 ± 0.09 | 4.51 ± 0.08 | 3.79 ± 0.04 | 3.04 ± 0.02 | <0.001 |
| Width (mm) | 1.85 ± 0.08 | 1.67 ± 0.01 | 1.52 ± 0.01 | 1.40 ± 0.01 | <0.001 |
| Thickness (mm) | 1.30 ± 0.05 | 1.30 ± 0.01 | 1.27 ± 0.01 | 1.17 ± 0.01 | <0.007 |
| Weight (g) | 0.06 ± 0.00 | 0.05 ± 0.00 | 0.04 ± 0.00 | 0.02 ± 0.00 | <0.001 |

olfactory bulb morphometries indicated that artesunate had significantly impact on the weight, crown-rump length, fore-limb length, hind-limb length and organ-body weight ratio of the pups and olfactory bulb's length, thickness, width and weight. These findings on developing olfactory bulb teratogenic morphometric abnormalities signify safety concern. It is therefore recommended that further studies are required to be done to assess the safety in vulnerable populations.

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## Conflict of Interest

None declared.

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