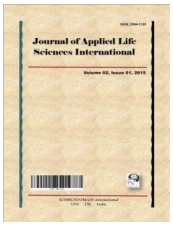

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Histomorphological Effects of Artesunate on the Developing Hypothalamus of Wistar Rat Foetuses

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Authors' contributions

This research work was carried out in collaboration between all the authors. Author AOI designed the study and supervised all aspects of the study, author AEI wrote the protocol and the first draft of the manuscript. Author EMS performed all the Laboratory studies, author SPA managed the analyses of the study and author UKE managed the literatures. All the authors read and approved the final manuscript.

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ABSTRACT

Artesunate is one of the first choice treatments among pregnant women with malaria in Nigeria as they are more susceptible and likely to develop anaemia and other complications. The present study was aimed to investigate the morphological and histological changes due to artesunate on the developing hypothalamus of Wistar rat fetuses. Twenty healthy female Albino Wistar rats of average weight of 165 g were grouped into 4 groups with 5 rats in each Group. The rats were fed daily and water was provided *ad libitum*. The animals were cycled and mated overnight with sexually matured males. The rats were separated into different cages after confirmation of pregnancy. Oral doses of 0.2 mg/kg, 0.4 mg/kg and 0.8 mg/kg body weight of artesunate were administered to the pregnant rats in Groups 2, 3 and 4 respectively from the 8th day to the 12th day of pregnancy. Rats in Group 1 were used as the Control, and received distilled water on the same days as the experimental

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Groups. The result showed that litter sizes were reduced and the number of malformed litters of Dams in the treatment Groups were increased when compared to the Control Group while the litters in the treatment Group 4 showed some malformations and resorption of the litters. The results of histological examinations revealed distortion and fusion of the hypothalamic sulcus, narrowing of the hypothalamic region, distortion of the hypothalamic nuclei when compared to the Control Group. The distortion of the hypothalamic nuclei and the reduction of the hypothalamic area could be due to the reduction in sizes of the hypothalamic nuclei thereby leading to compromised functions of the affected nuclei. The results showed that artesunate when administered at high dosage could be dangerous to the ontogenetic development of the hypothalamus in the fetuses.

Keywords: Artesunate; Wistar rats fetuses; hypothalamus; Morphology; Histology.

1. INTRODUCTION

Artesunate and other artemisinin derivatives are potent antimalarial drugs, but concern has been raised as to their neurotoxic potential [1,2]. Several studies have shown that some doses of artemisinin derivates can produce neurologic defects such as gaint disturbances, damage to brain stem centers in mice and in rats [3,4], loss of spinal cord and pain-response mechanism [2]. Eweka and Adjene [5] had shown the histological effects of oral administration of artesunate on the visual relay centers of adult Wistar rats and reported cell clustering, cellular hypertrophy, and intercellular vacuolation appearing in the stroma of superior colliculi of rats. Thus they concluded that varying doses and long administration of artesunate may have some deleterious effects on the neurons of the intracranial visual relay center and that may have some effects on the visual sensibility by its deleterious effects on the cells of the superior colliculi of adult Wistar Rats [5, 6].

Ekong, et al. [7] had reported that amodiaquine plus artesunate caused destruction of purkinje cortical layers, alteration in the cerebellum which may result in cerebellar dysfunction manifesting, as some motor problems like dizziness, gaint disturbances and convulsion. Ekanem, et al. [8], had reported that artequine a combination therapy comprising of artesunate and mefloquine, induced a dose dependent reactive astrocytes formation in the hippocampus which may impair uptake of neurotransmitters and altered neuronal environment thus altering hippocampal functions such as learning and memory [9].

Adebisi [10] had evaluated the toxicity of artesunate on bone development and reported impairment of the bone development and retarded calcification. Few cases of missing ribs, vertebrae, phalanges, non-ossified tarsals and metacarpals were also reported. A morphometric study of the teratogenic effects of artesunate on the Central Nervous System of the Wistar rats' fetuses by Mesembe, et al. [11] had shown that high doses of artesunate may cause severe intrauterine growth retardation and may be neurotoxic to the developing nervous system of Wistar rats. Work by Nosten and White [12] in Rodents and Rabbits had revealed that artemisinin given in critical period in early stage of gestation may also cause limb deformation. Also in primates, doses of 12-13 mg/kg body weight daily given continuously between 20-50 post coitum which is equivalent to 20-56 days in human pregnancy cause fetal resorption and mild long bone shortening. Clark [13] also reported that rats and rabbits have consistently shown severe embryo-fetal toxicities leading to a high resorption rate and low incidence of cardiovascular malformation and skeletal abnormalities as also observed by Okafor [9].

Previous studies have shown that artemisinin and its derivatives including artesunate are generally safe [14,15], there are yet many research works that have proved that the drugs, especially artesunate have some effects on some parts of the body systems [16,17,18]. The aim of the present study was to investigate the effect of artesunate on the developing hypothalamus of Albino Wistar rat fetuses. Though there have been some research works on the effects of artesunate on the different parts of the body, there is little or no study specifically directed to investigate the effects on the developing hypothalamus, the part of the brain that controls very vital body parts and vital daily life activities since it plays a big role in the body by influencing both the endocrine and nervous systems. Also, the WHO [19], has recommended that artemisinin group of drugs and their derivatives be subjected to regular and widespread study so as to assess their potential toxicity and therapeutic effectiveness from time to time.

2. MATERIALS AND METHODS

2.1 Experimental Design

Twenty (20) female Wistar rats of average weight of 160 g were bought from the Animal House of the Department of Human Anatomy, Ahmadu Bello University Zaria. The animals were kept in smaller animal holdings of the Department and were divided into 4 groups, namely Groups 1, 2, 3 and 4, each Group containing 5 animals. The animals were fed with commercial rat feeds and clean tap water was provided *ad bilitum*, and weight assessment was done throughout the experiment period using EC-500 digital weighing scale (Scientech Balance, Massachusetts, U.S.A). The experimental procedure was approved by the ethics Committee of the Faculty of Veterinary Medicine, Ahmadu Bello University Zaria.

The animals were cycled and during the pro-estrous phase of the estrous cycle, they were caged overnight in groups of three per one sexually matured adult male rat in each Group. Confirmation of pregnancy was done according to the method of Clark, et al. [20] in which the presence of sperm plug in the vaginal smears obtained the following morning confirmed coitus and the sperm positive day was designated as day zero of pregnancy. Following the confirmation of pregnancy, the animals were separated into different cages and fed individually, so as to create a disturbance and stress-free environment.

2.2 Drug Preparation and Administration

Artesunate was purchased from F. Pharmacy, Samaru Zaria, was manufactured by B. G. PHARMA LTD; Batch No: 45 and NAFDAC Reg.No-04-9927. Each tablet weighed 50 mg and was dissolved in 20 ml of distilled water. Low, Medium and High Dose Groups, received the Oral artesunate dosages of 0.2 mg/kg, 0.4 mg/kg and 0.8 mg/kg body weights respectively. The administration was for 5 days, from the 8^{th} to the 12^{th} day of gestation and was by oral gavage using the orogastric tube. Group 1 was used as the Control and received 2.0 ml/kg body weight of distilled water on the same days of administration.

On the 20^{th} day of gestation, the pregnant rats were humanely sacrificed using ketamine anesthesia. The fetuses were harvested by maternal abdominal incision and the average litter numbers, number of resorbed, dead, and malformed fetuses were recorded. Physical observations of the fetuses and the measurement of some anatomical parameters were taken.

The retrieved fetuses were weighed and fixed in 10% formol saline. The heads were opened through a mid-sagittal incision for easy removal of the hypothalamus and then fixed. The tissues were then processed for histological examination and were stained with hematoxylin and eosin (H & E) routine stain.

2.3 Statistical Analysis

Data obtained from morphological parameters were presented as mean ± standard error of mean (SEM). Data were analyzed using Statistical Package for Social Sciences (SPSS) IBM version 20. One-way Analysis of Variance (ANOVA) was employed, followed by Tukey post hoc test, and values were considered significant at $p \leq 0.05$.

3. RESULTS

3.1 Morphological Observations

The results of the morphological effects of artesunate treatment on the Wistar rat fetuses in the Control (Group 1), Low dose (Group 2), Medium dose (Group 3) and high dose (Group 4) are shown in Fig. 1. The result showed that the sizes of the fetuses were reduced and the number of malformed litters of Dams in the treatment Groups were increased when compared to the Control Group. The treatment Groups 2, 3 and 4 were smaller in sizes when compared to those in the Control Group 1 as shown in Fig. 2A, B and C while the high-dosed group (Group 4) showed 6 malformations and 1 resorption as shown in Fig. 1 and Fig. 2D respectively.

3.2 Histological Studies

The histological examination of the sections of the developing hypothalamus showed altered tissue sections in the artesunate-treated groups when compared to the Control, while those of the higher treatment groups demonstrated greater extent of alteration when compared to those

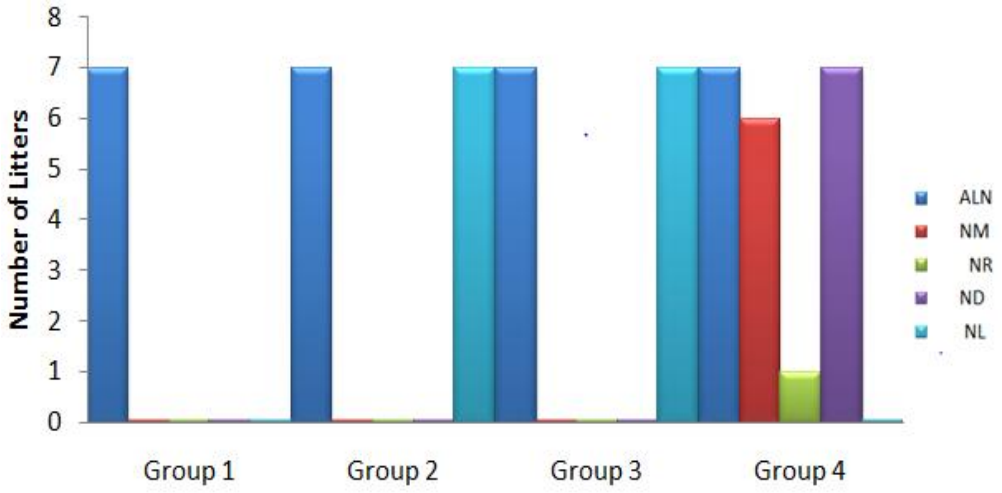

Figure 1: The morphological Observations of the Wistar Rat foetuses

KEY: ALN = Average litter number; NR = Number resorbed; NM = Number malformed; ND = Number dead; NL = Number living.

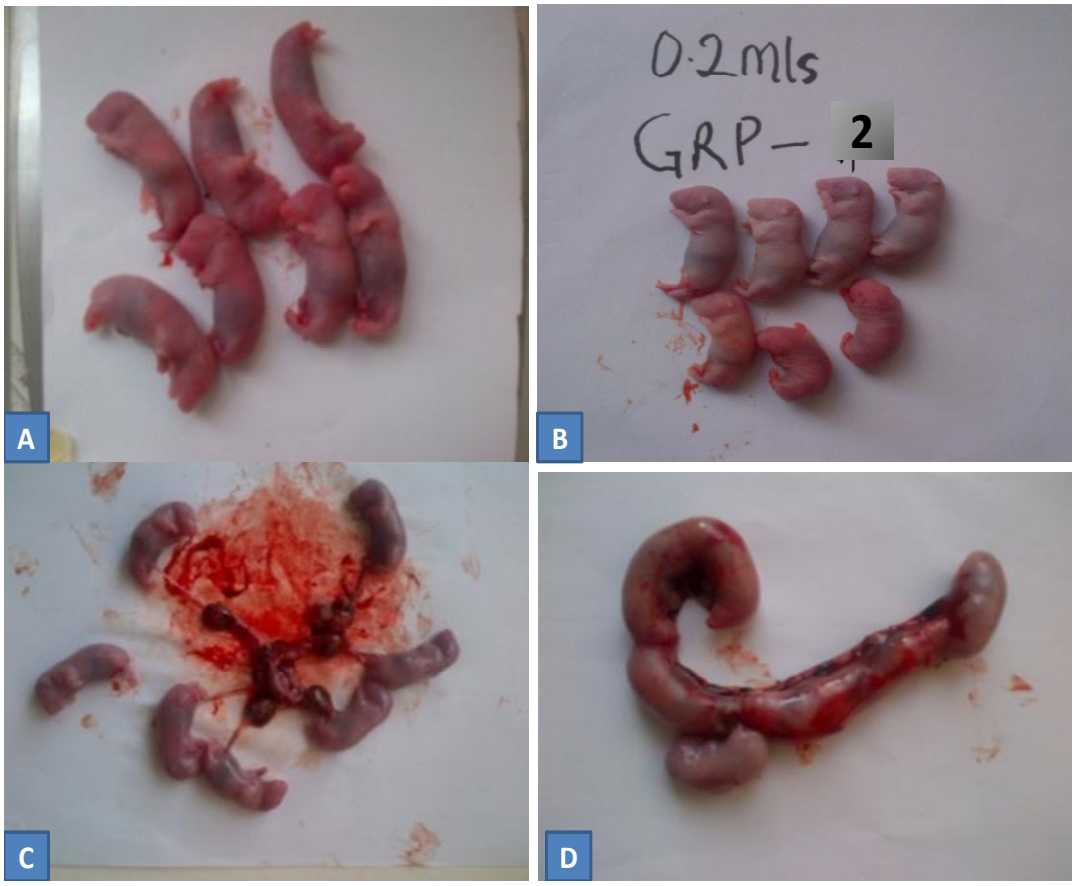

Fig. 2. Showing the control group 1 (A), with larger body sizes and healthier Litters. B: Low dose (Group 2) showing a relatively reduced body sizes. C: Medium dose (Group 3) showing greatly reduced litter sizes. D: High dose (Group 4) showing malformed and resorbed fetuses

of low treatment Group. Fig. 3 show the histological sections of the developing hypothalamus of Wistar rat fetuses in the four different groups following maternal administration of artesunate during the brain-sensitive period of development in pregnancy.

Fig. 3A show the normal structure of the hypothalamic sulcus from the Control (Group 1), which leads from the interventricular sulcus of lateral ventricles down into third ventricle up to the region of the optic chiasma, and is important for the normal flow pathway of the cerebrospinal fluid produced from the choroid plexus into the fourth ventricle and spinal cord. The result from the histological section shows distinct landmarks of the hypothalamic nuclei and the entire hypothalamic region as shown from Fig. 3A.

Fig. 3B, displayed the hypothalamic tissue section from the low dose group (Group 2), showing a mildly distorted structure of the hypothalamic sulcus, as well as reduced clarity of the landmarks of the hypothalamic nuclei. While Fig. 3C show a section of the hypothalamic tissue from the medium dose group (Group 3) revealing a partially fused hypothalamic sulcus, as it appears greatly narrowed with a clearly reduced hypothalamic region when compared to the Control Group Fig. 3A. Fig. 3D showed a section of the hypothalamic tissue from the high dose group (Group 4) showing a completely distorted and fused hypothalamic sulcus, and also a narrowed entire hypothalamic region when compared to the Control Group (Fig. 2A) and Group 2 (Fig. 2B).

4. DISCUSSION

The results from the present study have demonstrated the teratogenic potentials of artesunate by causing significant decrease and reduction in the litter numbers and body sizes with increasing dosage of the treatment. Thus, the present study has revealed some level of intrauterine growth retardation following artesunate administration and this was in agreement with the earlier studies and reports by Mesembe, et al. [11] and Adebisi [10].

The result of the histological observations of the hypothalamus from the present study showed distortional changes of the developing hypothalamus of Wistar rat fetuses following the administration of artesunate to the dams in varying doses of low, medium and high from the 8^{th} to 12^{th} day of gestation. The changes observed include the distortion of the hypothalamic sulcus, narrowing of the hypothalamic region and fading of the landmarks of hypothalamic nuclei with increasing dosage of artesunate, which are clearly and prominently present in the Control tissues. Although studies reporting the effect of artesunate on the developing hypothalamus are scarcely available, there are yet similar works that have revealed the teratogenic effects of the drug, artesunate on the other parts of the brain, thus revealing the vulnerability and susceptibility of the developing hypothalamus to some other agents administered during the critical period of pregnancy and neurulation [21]. These observed effects could imply compromised functions of the hypothalamus, leading to many other complications. For example, the distortion of the hypothalamic sulcus could imply restriction to the normal flow of Cerebrospinal fluid (CSF) from the lateral and 3^{rd} ventricles into the 4^{th} ventricle and spinal cord region, thus resulting in the retention and accumulation of the cerebrospinal fluid within the brain and then leading to hydrocephalus and other related complications [22]. Also, the reduced hypothalamic region could be due to the shrinkage or rather reduction in sizes of the hypothalamic cells and nuclei, leading to a compromised functionality of each of such affected nuclei, like abnormal appetite for food when the lateral and dorsomedial nuclei are affected [23,24]. The hypothalamus is a small part of the brain located below the thalamus on both sides of the third ventricle in the ventral part of diencephalon and is formed by groups of nuclei, scattered in the walls and floor of third ventricle which extends from the optic chiasma to the mamillary body [25,26].

The hypothalamus formed the lower portion of the alar plate, differentiates into a number of nuclear areas that regulate the visceral functions, including sleep, digestion, body temperature, blood pressure and emotional behavior. One of these groups, the mamillary body, forms a distinct protuberance on the ventral surface of the hypothalamus on each side of the midline [25,27]. As such the distortion of the hypothalamic sulcus and hypothalamic nuclei of the developing hypothalamus as seen from the present study could impair some functions of the body like secretion and control of the pituitary gland, control of the adrenal gland, regulation of autonomic nervous system, regulations of the

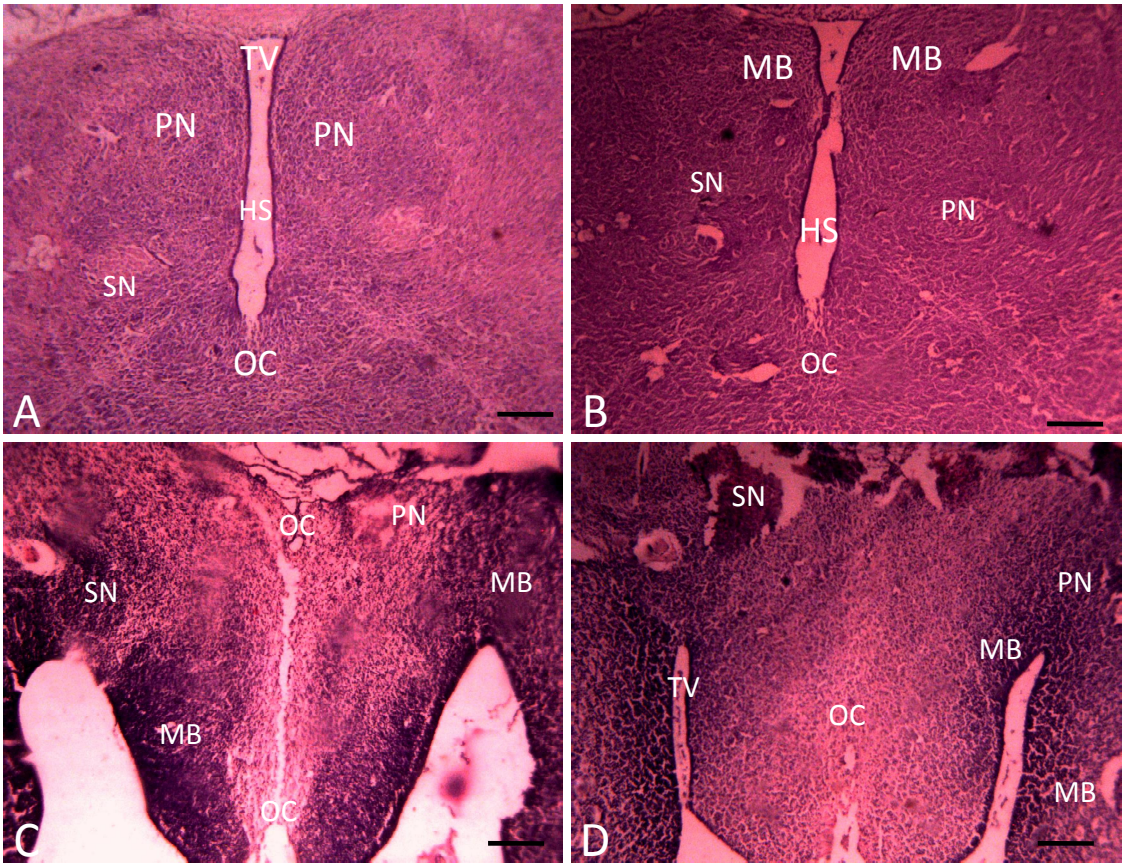

Fig. 3 (A). A section of the developing hypothalamus of Wistar rat fetuses in Group 1 (Control) showing normal orientation of the hypothalamic sulcus (HS) and some visible landmarks of the surrounding hypothalamic nuclei, Paraventricular nuclei (PN), Supraoptic nuclei (SN), Optic Chiasma (OC), Third Ventricle (TV) (H & E x100). Scale Bar: 1 mm=5 µm

Fig. 3 (B). A section of the developing hypothalamus of Wistar rat fetuses in Group 2 (low-dosed group) showing a mild distortion of the hypothalamic sulcus with relatively indistinct landmarks of the hypothalamic nuclei. hypothalamic sulcus (HS) and some visible landmarks of the surrounding hypothalamic nuclei, Paraventricular nuclei (PN), Supraoptic nuclei (SN), Optic Chiasma (OC), Third Ventricle (TV), Mamillary bodies (MB) (H & E x100). Scale Bar: 1 mm=5 µm

Fig. 3 (C). A section of the developing hypothalamus of Wistar rat fetuses in Group 3 (medium-dosed group) showing a more pronounced distortion of the hypothalamic sulcus, seen as merging and narrowing of the two ends of the sulcus as well as the entire hypothalamic region, with indistinct landmarks of the hypothalamic nuclei. hypothalamic sulcus (HS) and some visible landmarks of the surrounding hypothalamic nuclei, Paraventricular nuclei (PN), Supraoptic nuclei (SN), Optic Chiasma (OC), Third Ventricle (TV), Mamillary bodies (MB) (H & E x100). Scale Bar: 1 mm=5 µm

Fig. 3 (D). A section of the developing hypothalamus of Wistar rat fetuses in Group 4 (high-dosed group) showing a completely merged and obliterated hypothalamic sulcus, as well as the narrowing of the entire hypothalamic region, with indistinct landmarks of the nuclei. hypothalamic sulcus (HS) and some visible landmarks of the surrounding hypothalamic nuclei, Paraventricular nuclei (PN), Supraoptic nuclei (SN), Optic Chiasma (OC), Third Ventricle (TV), Mamillary bodies (MB) (H & E x100). Scale Bar: 1 mm=5 µm

heart rate, blood pressure, body temperature, hunger and food intake, water balance, sleep and wakefulness [27].

The present study agreed with the work of Ajibade, et al. [2] and Samuel et al. [28], who had reported that artesunate when administered to Wistar rats caused neuronal degeneration and reduction of the neuronal population in the cerebral and cerebellar cortices. Ajibade, et al. [29] had reported reduced staining of neuronal cells following artesunate administration. Eweka and Adjene [5] had reported that the treated section of the medial geniculate body showed some decreased cellular population, degenerative changes, cellular hypertrophy, with some vacuolations appearing in the stroma.

Artsunate in earlier studies were reportedly embryolethal and teratogenic in rats especially during the most sensitive days of neurogensis mainly from 10 and 11 days postcoitum [30,31] which correspond to the period of administration in the present work. Artesunate has been reported to cause significant embryo-fetal toxicity [32], causing embryo death and malformations. Adebisi [10] had evaluated the toxicity of artesunate on bone development and reported impairment of the bone and retarded calcification while few cases of missing ribs, vertebrae, phalanges, non-ossified tarsals and metacarpals were also reported. Structural defects and death in the extremely malformed feotuses were observed in the present study. Mesembe *et al*. [11] had shown that high dose of artesunate may cause severe intrauterine growth retardation and may be neurotoxic to the developing nervous system of Wistar rats.

The results from the present study agreed with the work of some Scientists which indicated that early pregnancy exposure could induce fetal resorption which could result in early pregnancy loss which could be due to the specific inhibition of erythropoeisis [20,33]. In primates, doses of 12-13 mg/kg body weight daily given continuously between 20-50 days post coitum which is equivalent to 20-56 days in human pregnancy caused fetal resorption and mild long bone shortening. Clark, et al. [20]; Olumide and Raji [34] had reported that animals treated with artesunate have consistently shown severe embryo-fetal toxicities leading to a high resorption rate with low incidence of cardiovascular malformation and skeletal abnormalities [9,21].

5. CONCLUSION

The results from the present study have shown that artesunate has silent toxic and teratogenic effects including resorption of fetuses, growth retardation as shown from the morphological studies and the distortion of the hypothalamic area, complete fusion of the hypothalamic sulcus, narrowing of the entire hypothalamic region with distortion of the hypothalamic nuclei as shown from the histological studies in developing Wistar rat fetuses, of which these alterations were more in the high dosage Group of the experimental drug showing a dosage dependent effects. These effects could possibly be evident in later developmental stage of the fetuses and in later life. Hence, care must be taken when administering artesunate to pregnant women, paying attention to the dosage and the time of administration in particular during the brain-sensitive period of development from the 3rd to 8th weeks of gestation or the first trimester period as such could possibly lead to similar effects in the developing human embryos.

COMPETING INTERESTS

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.

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