*International Journal of Anatomy and Research,*
*Int J Anat Res 2024, Vol 12(2):8778-92. ISSN 2321-4287*
*DOI: https://dx.doi.org/10.16965/ijar.2023.276*

**Original Research Article**

# Comparative Brain Microanatomical and Neurochemical Alterations Following the Administration of Seven Oral Artemisinin-based Combination Therapies in Swiss Mice

**Innocent A. Edagha [1,2], Promise E. Douglas [1], Aniekan I. Peter [1,3], Aquaisua N. Aquaisua [1], Blessing C. Akpan [4], Edelungudi I. Edagha [5], Moses A. Ataben [1].**

[*1] Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Nigeria.

[2] Department of Human Anatomy, School of Medicine, Moi University, Eldoret, Kenya.

[3] Department of Anatomy, College of Health Sciences, Bowen University, Nigeria.

[4] Department of Chemical Pathology, Federal Neuropsychiatric Hospital, Calabar, Nigeria.

[5] Department of Family Medicine, Faculty of Clinical Science, University of Uyo Teaching Hospital, Nigeria.

## ABSTRACT

Over a decade after artemisinin-based combination therapy (ACT) approval as the most preferred anti-malarial drug. Seven ACTs can be sourced as over-the-counter medications in most African pharmacies without prescription. A comparative neurotoxicity of these ACTs was investigated in an *in vivo* experimental model. Swiss mice numbering 40, weighing 18 - 26 g were allotted to eight groups (n = 5). Group 1 (normal control [NC]), received distilled water 10 mL, while groups 2 to 8 were administered (5.71 mg artesunate+amodiaquine [AA]); (19.29 mg artesunate+mefloquine [AM]); (10.36 mg artesunate+sulfadoxine+pyrimethamine [ASP]); (19.29 mg artesunate+pyronaridine [APy]); (12.5 mg artemisinin+piperaquine [AP]); (15.42 mg dihydroartemisinin+piperaquine [DP]); and (8 mg artemether+lumefantrine [AL]) per kg body weights, respectively orally for 3 days, but for 2 days in group 6. Animals were sacrificed 24 hrs after last administration under ketamine anaesthesia (100 mg/kg, i.p), and excised brains were evaluated for neurochemical and neurohistological alterations. Oxidative stress markers: malondialdehyde and reduced glutathione significantly ($p < 0.05$) increased as well as antioxidant enzymes glutathione peroxidase, catalase, superoxide dismutase in ACT-administered groups compared to NC. Neurohistology of hippocampal cornu ammonis 1 (CA1) and cerebellum demonstrated vacuolations, neuronal hypertrophy and atrophy pyramidal, and Purkinje neurons. Immunohistochemistry with glial fibrillary acidic protein antibody demonstrated mild to mostly severe astrogliosis in the ACT-administered groups. In conclusion, oxidative stress markers and antioxidants were elevated in the order DP>APy>AP>ASP>AA>AL>AM. Together with the neurohistology, neurotoxicity were in the order DP>ASP>APy>AP>AL>AA>AM particularly in the hippocampus compared to the cerebellum.

**KEYWORDS:** Artemisinin-based combination therapies, Cerebellum, CA1 region of the hippocampus, Neurodegeneration, Oxidative stress.

**Corresponding Author:** Innocent A. Edagha, PhD, Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Nigeria.
**E-Mail:** innocentedagha@uniuyo.edu.ng; innocentedagha@mu.ke.ac

| Access this Article online | Journal Information |
|---|---|
| Quick Response code | **International Journal of Anatomy and Research**<br>ISSN (E) 2321-4287 \| ISSN (P) 2321-8967<br>**https://www.ijmhr.org/ijar.htm**<br>**DOI-Prefix:** https://dx.doi.org/10.16965/ijar |
| **DOI:** 10.16965/ijar.2023.276 | **Article Information** |
| | Received: 25 Dec 2023 — Accepted: 10 Feb 2024<br>Peer Review: 29 Dec 2023 — Published (O): 05 Jun 2024<br>Revised: 13 Jan 2024 — Published (P): 05 Jun 2024 |



## INTRODUCTION

Nearly half of the global population is susceptible to malaria infections, and after HIV/AIDS it is the leading cause of death from infectious diseases on African continent[1] with the most preferred drug for malarial treatment

being artemisinin-based combination therapies (ACTs) [2], for over a decade now. WHO recommends oral administration of ACTs for the treatment of uncomplicated malaria due to *P. falciparum* and *P. vivax* parasites. ACTs act to arrest parasites multiplication via the combined action of two active ingredients which are both fast and slow acting on their biochemical targets [3,4].

The current anti-malarial medications are artesunate and amodiaquine, artesunate and mefloquine, artesunate and pyronaridine,

artesunate with sulfadoxine and pyrimethamine, artemisinin and piperaquine, dihydroartemisinin and piperaquine, and artemether and lumefantrine, respectively), although some have been associated with undesirable outcomes. Since malaria is endemic in Nigeria, the frequent exposure to anti-malarial is common and it has been associated with low to moderate neurotoxicity in different literatures [5,6] as most are administered orally.

Artemisinins are hydrolysed to the active metabolite dihydroartemisinin and its by-products artemether, artemotil, and artesunate respectively which are partially soluble in water (artesunate) or oil (artemether and artemotil) [7,8]. Artemisinins are proposed to act through a mechanism that involve the cleavage of their endoperoxide bridge by free heme and C-centred radicals which then interacts with several cellular targets [9], and through the inhibition of parasite mitochondria transport chain (complex-IV) through reactive oxygen species (ROS) synthesis [9,10]. Artemisinin is rapidly absorbed via the gastrointestinal tract and dispensed in the liver, bile, and kidney with about 80% of the medication eliminated within 24 hours of administration through urine and faeces [4,11]. Generally, artemisinin and its derivatives possess a short elimination half-life of roughly 2 - 5 hours and their partner drug has long elimination half-life with mefloquine (17 - 25 days), amodiaquine (16 - 18 days), lumefantrine (4 - 6 days), piperaquine (14 - 21 days), pyronaridine (7 - 9 days) and sulfadoxine – pyrimethamine (3 - 9 days) to prevent recrudescence of malaria parasites [12,13].

Excessive production of free radicals resulting from insufficient level of antioxidant defense system results in oxidative stress, and the brain is most prone to this challenge due to its high oxygen uptake and lipid content. Excess free radical result in the production of ROS-related compounds like superoxide anions (O2•–), hydrogen peroxide ($H_2O_2$) which can interconnect with cellular biomolecules and subsequently cause damages to them [14]. Lipid peroxidation is one of the major mechanisms involved in oxidative stress, which involves disarrangements of intramolecular structures that result in conjugated diene production following polyunsaturated fatty acids attack by ROS [14,15]. Antioxidants are present in low concentrations in the body and can help delay or prevent substrate oxidation and are responsible for preventing and blocking ROS, oxidative stress, and other parameters of cell death [16]. Some vital first line of defense for body include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and peroxiredoxins which constitute the enzymatic antibodies [17]. ACTs has been reported to alter the enzymatic and non-enzymatic antioxidants concentrations [18-20] and exposing the cells to oxidative stress [20].

Artemisinin and its derivatives in animal's studies reported to induce neuronal damage and fatal outcomes in adults or embryos irrespective of the specie used like mouse, rat, dog, and non-primates [21].

These neurological effects associated with artemisinin exposure for 24 hrs are reported to appear after 7 days and worsen after 14 days [21,22] Their seriousness and time to onset are dose-dependent [23] and intravenous administration seems more neurotoxic than the orals[5,24]. Several studies have shown that African children are long term survivors of cerebral malaria and elicit various neurological deficits including attention, memory, and visual skills [25,26].

Mefloquine has been reported to induce ototoxicity in healthy subjects and different dose dependent neuropsychiatric adverse reactions [26,27]. It has also been reported that co-administration of artemeter-lumefantrine

maybe neurotoxic to neurons in successive dosing by its artemisinins derivatives [20,26,28,29].

The brain is one of the most vital and sensitive body organs that are highly susceptible to ACTs toxicities [30,31]. The cerebellum is the part of the brain that controls coordination of muscles as well as the maintenance of body equilibrium [32,33]. Whereas the hippocampal cornu ammonis 1 (CA1) is the brain region involved in the formation and consolidation of memory [32,33] and its neurons very sensitive to damage. Thus, toxicities directed toward these areas of the brain may hinder and/or distort their normal physiology with attendant effects that may even impact on the quality of life [6,32].

It became pertinent to investigate the comparative neurochemical of the whole brain, and microstructural alterations of the hippocampal CA1 region and cerebellum following oral therapeutic doses of the seven ACTs commonly available in Nigeria to determine the least and most neurotoxic.

## MATERIALS AND METHODS

### Drug acquisition and preparations:

The seven ACTs were: Camosunate®, (artesunate+amodiaquine [Geneith Pharmaceutical Limited, Oshodi, Lagos] Lot No.: 440720); Artequin® (artesunate+mefloquine [Ateco, Switzerland] Lot No.: 20055175); Pyramax® (artesunate+pyronaridine [Shin Poong Pharmaceutical Co. Ltd, Korea] No.: 01750087300132), Co-Arinate® (artesunate +sulfadoxine+pyrimethamine [*Famar Italia* Spa, *Italy*] Lot No.: 220021); Artequick® (artemisinin+piperaquine [Artepharm Co. Ltd, China] Lot No.: 20191103), P-Alaxin® (dihydroartemisinin+piperaquine [Bliss Pharm Ltd, India] Lot No.: J1A1W021), and Coartem® (artemether+lumefantrine [Novartis Pharmaceutical Limited, Switzerland] Lot No.: KR471), respectively. The drugs (normally in solid forms) were pulverized in a mortar and pestle. The powdered drugs were separately dissolved in 400 mL of distilled water to constitute the stock solution.

**Experimental Animals:** The forty (40) male Swiss mice (18 – 26 g) were sourced from the Animal House, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria and randomized into eight groups of five mouse each. All animals were weighed, marked for identification, and placed in a standard plastic cage for acclimatization under optimum pathogen-free environment and maintained in a 12 h light/dark cycles of 25 – 27 °C at relative humidity of 40 – 60 % measured using CEM hydrometer (DT 615, Shenzhen China), one week before the start of the experiment allowing access to diet and water. The animal cages were maintained adequately by changing sawdust, leftover feed and drinking water, daily. All animals were fed with pelletized grower mash (Grand Cereal Vital® Feed Ltd. Jos) and provided drinking water *ad libitum*. The study design and protocols were approved by the Department of Human Anatomy, University of Uyo, Nigeria, in tandem with the globally accepted guideline [34]. The experiment conformed with the Basic and Clinical Pharmacology and Toxicology policy for experimental studies [35].

**Experimental Design:** The animals were allotted into eight groups for placebo and drug administrations, as follows:

| Group | Treatment | Dose (mg/kg/day) | Duration (days) |
|---|---|---|---|
| 1 | DW (NC) | 10 mL | 3 |
| 2 | AA | 5.71 | 3 |
| 3 | AM | 19.29 | 3 |
| 4 | ASP | 10.35 | 3 |
| 5 | Apy | 10.29 | 3 |
| 6 | AP | 12.5 | 2 |
| 7 | DP | 15.42 | 3 |
| 8 | AL | 8 | 3 |

Legend: NC = Normal control; DW = distilled water;
AA = artesunate+amodiaquine;
AM = artesunate+mefloquine;
ASP = artesunate+sulphadoxine+pyrimethamine;
APy = artesunate+pyronaridine;
AP = artemisinin+piperaquine;
DP = dihydroartemisinine+piperaquine;
AL = artemether+lumefantrine.

**Administration of experimental drugs:** Drug administrations were performed through oral gavage for three days based on each mouse's body weight using digital compact scale (Zeiss, Germany) and determined by the dose calculation: desired over have formula method

described by Toney-Butler et al. [36].

All drugs were administered using at required therapeutic doses for a period of three days; however, the Group 6 (AP) was administered for two days as per instruction on drug leaflet for each drug.

**Necropsy of experimental animals:** The animals were euthanized with ketamine (100 mg/kg i.p.) 24 hrs after the last oral therapeutic administration of ACTs. Intracardiac perfusion was performed with phosphate buffered saline (PBS) for 2 minutes. A 4% paraformaldehyde was then utilized via a second drip line as fixative until tail stiffness was achieved. This allowed for whole brain fixation which were excised and transferred to specimen bottle of same fixative for 72 h. Paraffin embedded brain tissue were sectioned at 5 µm using the rotary microtome (Microtome Thermo Scientific – Microm HM 325**,** England), with every fifth ribbon picked from a warm water bath unto an albumenized slide and thereafter stained with haematoxylin and eosin as described by Everitt and Gros, [37].

Likewise, some whole brains were weighed using an electronic weighing balance (Scout Tm Pro; Microstep (pty) Ltd; Circifense, Switzerland) and the volumes were determined with a measuring cylinder and the brain average and volumes were recorded. The bi-parietal and fronto-occipital indices were obtained with an electronic vernier caliper.

**Determination of morphometric indices:** Organosomatic indices describe proportional changes in organ weights as reflecting the status of organ systems which may increase or decrease more rapidly than an organism's weight. This was determined as described by Ariweriokuma et al. [38] for the brain and body weights ratio of each mouse using the formula: brain weight/ body weight of mouse x (multiplied by) 100

**Antioxidant and oxidative stress markers assessments:** Four brain homogenates obtained from the control group and the groups orally exposed to the seven different ACTs were assessed for endogenous concentrations of oxidative stress markers), reduced glutathione (GSH) and malondialdehyde (MDA), and antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). This was to determine the degree of exposure-associated neurochemical alterations. The eviscerated frozen fresh brain tissues were homogenized using a ceramic mortar and pestle with a 5 mL phosphate buffer was added into the ceramic mortar using a syringe. The resulting homogenate poured into a plain tube and centrifuged at 3000 rpm for 10 minutes to produce supernatants which were decanted into another plain tube using a syringe, and then used for enzymatic studies.

The GSH activity was determined as described by Sedlak and Lindsay [39].

To the homogenate 10% TCA was added, centrifuged. Thereafter, 1.0 mL of brain supernatant was treated with 0.5 mL of Ellmans reagent (19.8 mg of 5,5-dithiobisnitro benzoic acid (DTNB) in 100 mL of 0.1% sodium nitrate) and 3.0 mL of phosphate buffer (0.2M, pH 8.0) and an absorbance read at 412 nm.

The MDA for lipid peroxidation was determined as described by Buege and Aust [40]. A 1.0 mL of brain supernatant was added to 2 mL of (1:1:1 ratio) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24N HCl and 15% TCA) tricarboxylic acid- thiobarbituric acid- hydrochloric acid reagent boiled at 100$^{o}$C for 15 min and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 minutes. Resulting supernatant was removed, and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA- complex of $1.56 \times 10^{5}\ M^{-1}CM^{-1}$.

The SOD was determined as described by Sun and Zigma [41] via its ability to inhibit the auto-oxidation of epinephrine and absorbance read at 480 nm. The reaction mixture contained 2.95 mL 0.05 M sodium carbonate buffer pH 10.2, 0.02 mL of brain homogenate and 0.03 mL of epinephrine in 0.005 N HCL to initiate the reaction. The reference cuvette contained 2.95 mL buffer, and 0.02 mL of water. The SOD activity was calculated by measuring the change in absorbance at 480 nm for 5 minutes.

The CAT concentration was colorimetrically determined as described by Sinha [42] at 620 nm and expressed as µmoles of $H_2O_2$ consumed/min/mg protein at $25^0$C. The reaction mixture contained 1.0 mL of 0.01M phosphate buffer (pH 7.0), 0.1 mL of tissue homogenate and 0.4 mL of 2M $H_2O_2$. The reaction was stopped by the addition of 2.0 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). The GPx determination protocol is as described by Rotruck et al [43].

**Histopathological assessments**

**Haematoxylin and eosin (H&E) stain:**

Fixed brain tissues were dehydrated in ascending grades of ethanol 50%, 70%, 95% and 100% two changes for 5 minutes each. Thereafter tissues were cleared in two changes of xylene for 2 minutes each and infiltrated with paraffin-wax and embedded overnight. Blocked tissues were sectioned tissues at 5 µm unto a warm water bath and every fifth picked ribbons were carefully picked unto albuminized slides and rehydrated in descending grades of alcohol 100%, 95%, 70%, and 50% for five minutes each. Thereafter tissues were transferred to running tap water for 15 minutes to remove any infiltrating agent left in the tissue before they were stained with hematoxylin for 5 minutes following which tissues were rinsed in running tap water (bluing). Tissues were then rinsed with 1% acid alcohol for two seconds and rinsed in the running tap water for 2 minutes. Tissues were stained using eosin for 10 minutes before being transferred to increasing alcohol 50%, 70%, 95% alcohol for 1 minute and cleared in xylene for 1 minute. Tissues were allowed to dry and mounted using DPX (dibutylphthalate polystyrene xylene) mountant and cover slipped [44].

**Immunohistochemical assessment:**

The expression of glial fibrillary acid protein (GFAP) antibody was demonstrated as described by Edagha et al [45] for positive astrocytic reactions hippocampus CA 1 region and cerebellar cortex. To inactivate the endogenous peroxidase, the brain tissue sections were treated in 0.1% $H_2O_2$ (Deventer, The Netherland) for 15 minutes and thereafter allowed to set in 5% normal goat serum in PBS for 1 h. The sections were then incubated overnight at 4 °C in PBS containing 1% normal goat serum and rabbit anti-glial fibrillary acidic protein (GFAP) (1: 500; Dako, Lot 00083681), used to observe the reactive astrocytes. The brain sections were incubated for 2 h at room temperature using a biotinylated secondary antibody (diluted 1:200) after washing. Thereafter, the sections were then reacted in an avidin biotin solution (ABC kit, Vectastain) and finally using 3, 3'-diaminobenzidine as chromogen and peroxide. Tissues on charged slides were then dehydrated in ascending grades of alcohol 50%, 70%, 95%, and 100%, respectively, and then cleared in xylene, mounted on gelatinized slides, and coverslipped using DPX. The photomicrographs of regions of the brain (CA1 of the hippocampus and cerebellum) were captured with Leica DM750 microscope attached with an ICC50W digital camera (Wetzlar, Germany).

**GFAP immunostaining scores:** The semi-quantitative scoring method based on the intensity of immune expression of the anti-GFAP antibody as described by Klein et al [46], and Rizzardi et al [47]. The photomicrographs were scored based on the following criteria: (a) percentage of astrocytic reactions labelled (0 = lack of labelling of astrocytes; 1 = less than 30 % of astrocytes reactive; 2 = 30 to 60 %; and 3 = more than 60 %); and (b) intensity of the immunostaining (0 = no staining; 1 = weak; 2 = mild; and 3 = strong staining). The sum of the two values (a + b) totalled the eventual score for each photomicrograph, ranging from 0 to 6. To forestall any bias, at least two independent histopathologists undertook the analyses of the brain images.

**Statistical analysis:** Data was analysed with the GraphPad Prism version 10.2.0 software. The one-way analysis of variance (ANOVA) was used to determine the difference in the means across the groups. Tukey post-hoc test was performed for between groups comparison and expressed as mean ± standard error of mean (SEM) with significance set at $p < 0.05$.

## RESULTS

**Comparative effect of oral administration of some ACTs on body weights:** The body weight was not significantly changed in the AL, AM, and APy-administered groups except in the ASP-group, which was significantly increased when compared with NC, while a decreasing trend (not significant) in AA, AP, and DP-administered groups, respectively (Fig. 1).

**Comparative effect of oral administration of some ACTs on brain morphometry:** There was a no significant change in the brain morphometry analysed for ACT-administered groups compared to NC (Fig. 2).

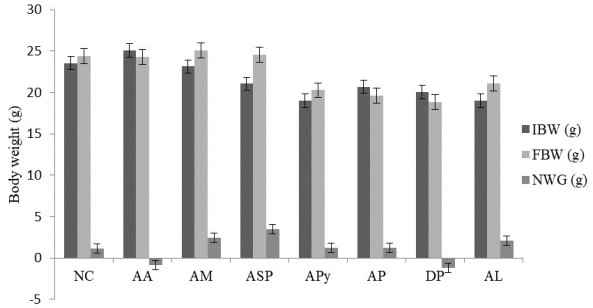


**Fig. 1:** Comparative effect of oral administration of some ACTs on body weights (g) Values are expressed in Mean ± SEM; α = ASP (+ 3.52 g) significantly ($p < 0.05$) increased compared to DP (- 1.18 g) and AA (- 0.82 g).

**Legend:** IBW = initial bodyweight;
FBW = final body weight; NWG = net weight gain.
NC = Normal control; AA = Artesunate amodiaquine;
ASP = Artesunate sulfadoxine pyrimethamine;
APy = Artesunate pyronaridine;
AP = Artemisinin piperaquine;
DP = Dihydroartemisinin piperaquine;
AL = Artemether lumefantrine.

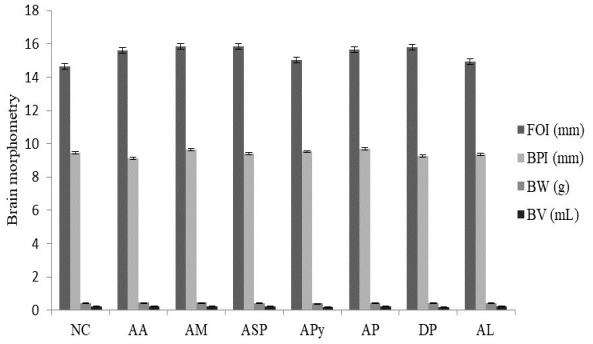


**Fig. 2:** Comparative effect of oral administration of some ACTs on brain morphometry Values are expressed in Mean ± SEM. Legend:
FOI = fronto-occipital index;
BPI = biparietal index; BW = brain weight;
BV = brain volume. Brain morphometrics were not significantly different.

**Comparative effect of oral administration of some ACTs on oxidative stress markers:** There were significantly increased oxidative markers; malondialdehyde (MDA) maker for expression of lipid peroxidation activity and reduced glutathione (GSH) concentrations in the ACT-administered groups compared to the normal control (NC) as shown in (Figs. 3 and 4).

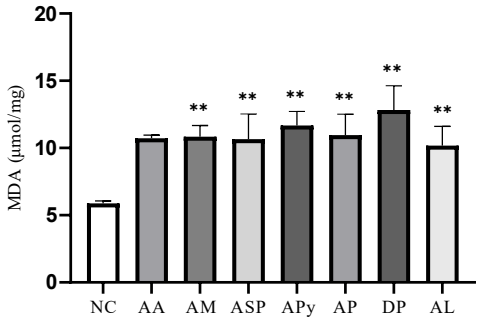


**Fig. 3:** Comparative effect of oral therapeutic administration of some ACTs on malondialdehyde (MDA) concentration in Wistar rats. Values are expressed in Mean ± SEM, with significance at $p < 0.05$; ** = significant from NC.

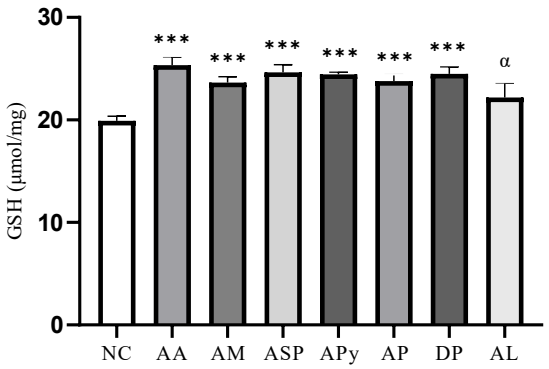


**Fig. 4:** Comparative effect of oral therapeutic administration of some ACTs on reduced glutathione (GSH) concentration in Wistar rats. Values are expressed in Mean ± SEM, with significance at $p < 0.05$; *** = significant from NC. α = significant from AA.

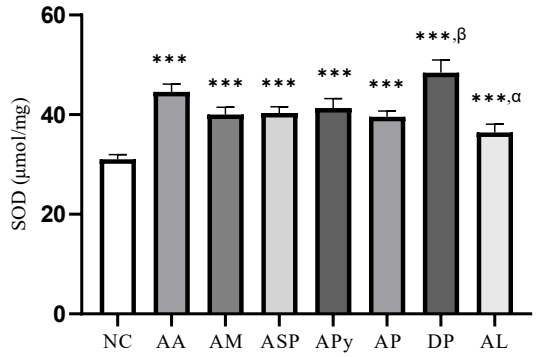


**Fig. 5:** Comparative effect of oral therapeutic administration of some ACTs on superoxide dismutase (SOD) concentration in Wistar rats. Values are expressed in Mean ± SEM, with significance at $p < 0.05$; *** = significant from NC. α = significant from AA.

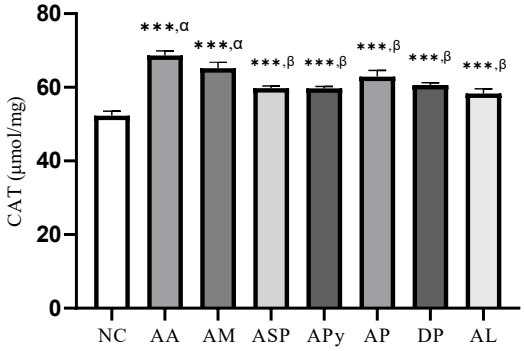


**Fig. 6:** Comparative effect of oral therapeutic administration of some ACTs on catalase (CAT) concentration in Wistar rats. Values are expressed in Mean ± SEM, with significance at $p < 0.05$; *** = significant from NC. β = significance from AA; α = significance from NC; ASP and AL.

**Comparative effect of oral administration of some ACTs on antioxidants concentrations:** Likewise, there were significant increase in antioxidant concentration of SOD, CAT, and GPx respectively in all the ACT-administered groups when compared with NC as shown in Figs. 5 – 7.

**Comparative effect of oral administration of some ACTs on hippocampal CA1 and cerebellar GFAP antibody expressions:** The demonstration of immunohistochemical scores for GFAP antibody expression in the CA1 region of the hippocampus and cerebellar neuropils (Tables 1 and 2), indicated that AA, ASP, APy, AP, DP and AL-administered groups had high expressions compared to the NC and AM-administered groups.

**Table 1:** Comparative effect of oral administration of some ACTs on hippocampal CA1 GFAP antibody expression.

| Group | % of IHC (A) | Intensity of IHC (B) | Final Score (A+B) | GFAP Antibody Expression |
|---|---|---|---|---|
| NC | < 30 % (1) | Weak (1) | 2 | Low |
| AA | > 60 % (3) | Mild (2) | 5 | High |
| AM | < 30 % (1) | Mild (2) | 3 | Low |
| ASP | > 60 % (3) | Strong (3) | 6 | High |
| APy | > 60 % (3) | Strong (3) | 6 | High |
| AP | > 60 % (3) | Strong (3) | 6 | High |
| DP | > 60 % (3) | Strong (3) | 6 | High |
| AL | > 60 % (3) | Strong (3) | 6 | High |

NC = normal control; AA = artesunate + amodiaquine; AM = artesunate + mefloquine; ASP = artesunate + sulphadoxine + pyrimethamine; APy = artesunate + pyronaridine; AP = artemisinin + piperaquine; DP = dihydroartemisinin + piperaquine; AL = artemether + lumefantrine. Key: % IHC: 0 = 0%; 1 = < 30%; 3 = > 60% Intensity of IHC: 0 = No reaction; 1 = Weak; 2 = Mild; 3 = Strong. Final Score: Range = 0 - 6; 0/6 = Negative Reaction 1/6, to 3/6 = Low expression 4/6, to 6/6 = High expression.

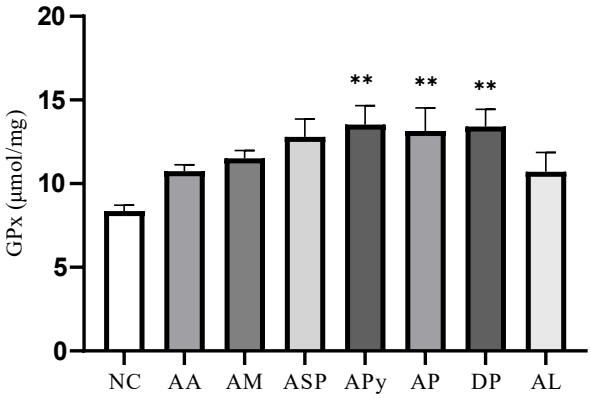


**Fig. 7:** Comparative effect of oral therapeutic administration of some ACTs on glutathione peroxidase (GPx) concentration in Wistar rats. Values are expressed in Mean ± SEM, with significance at $p < 0.05$; ** = significant from NC.

**Table 2:** Comparative effect of oral administration of some ACTs on cerebellar GFAP antibody expression.

| Group | % of IHC (A) | Intensity of IHC (B) | Final Score (A+B) | GFAP Antibody Expression |
|---|---|---|---|---|
| NC | < 30 % (1) | Weak (1) | 2 | Low |
| AA | > 60 % (3) | Strong (3) | 6 | High |
| AM | < 30 % (1) | Mild (2) | 3 | Low |
| ASP | > 60 % (3) | Strong (3) | 6 | High |
| APy | > 60 % (3) | Strong (3) | 6 | High |
| AP | > 60 % (3) | Mild (2) | 5 | High |
| DP | > 60 % (3) | Strong (3) | 6 | High |
| AL | > 60 % (3) | Strong (3) | 6 | High |

NC = normal control; AA = artesunate + amodiaquine; AM = artesunate + mefloquine; ASP = artesunate + sulphadoxine + pyrimethamine; APy = artesunate + pyronaridine; AP = artemisinin + piperaquine; DP = dihydroartemisinin + piperaquine; AL = artemether + lumefantrine. Key: % IHC: 0 = 0%; 1 = < 30%; 3 = > 60% Intensity of IHC: 0 = No reaction; 1 = Weak; 2 = Mild; 3 = Strong. Final Score: Range = 0 - 6; 0/6 = Negative Reaction 1/6, to 3/6 = Low expression 4/6, to 6/6 = High expression.

**Haematoxylin and Eosin:** The brain tissue section of the CA1 portion of the hippocampus in the control group showed normal molecular, pyramidal, and polymorphic layers, and the pyramidal cells (Fig. 8). ASP 10.36 mg administered group showed atrophied pyramidal cells and vacuolation at pyramidal layer, and APy 19.29 mg administered group showed hypertrophied pyramidal cells. ASP 10.36 mg and AL 8.00 mg administered groups showed vacuolations at the molecular layer. DP 15.42 mg administered group showed normal histoarchitecture when compared with NC (Fig. 8).

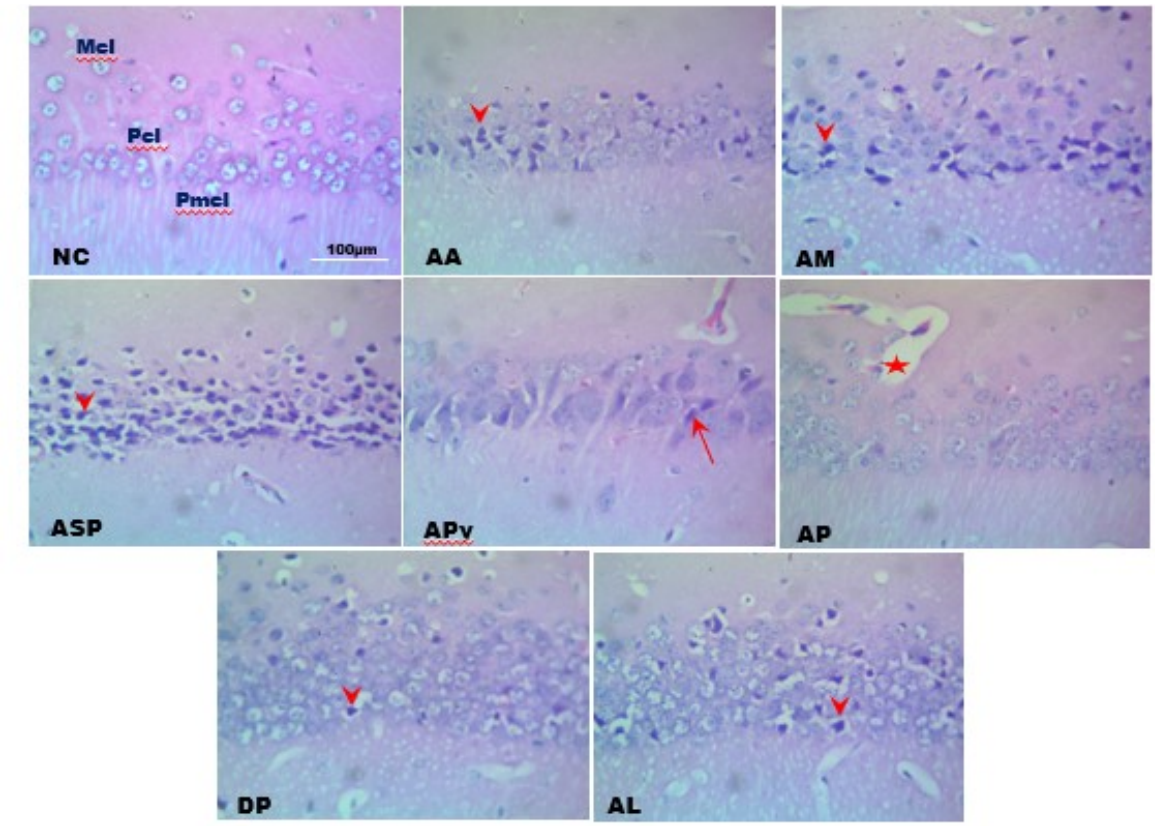


**Fig. 8:** Photomicrographs of the transverse section of the hippocampal CA 1 following oral administration of some ACTs in Swiss mice, H&E x400. Red arrow = hypertrophy; Red arrowhead = atrophy and neuronal shrinkage. Mcl = molecular cell layer; Pcl = pyramidal cell layer; Pmcl = polymorphic cell layer and Bv = blood vessel.

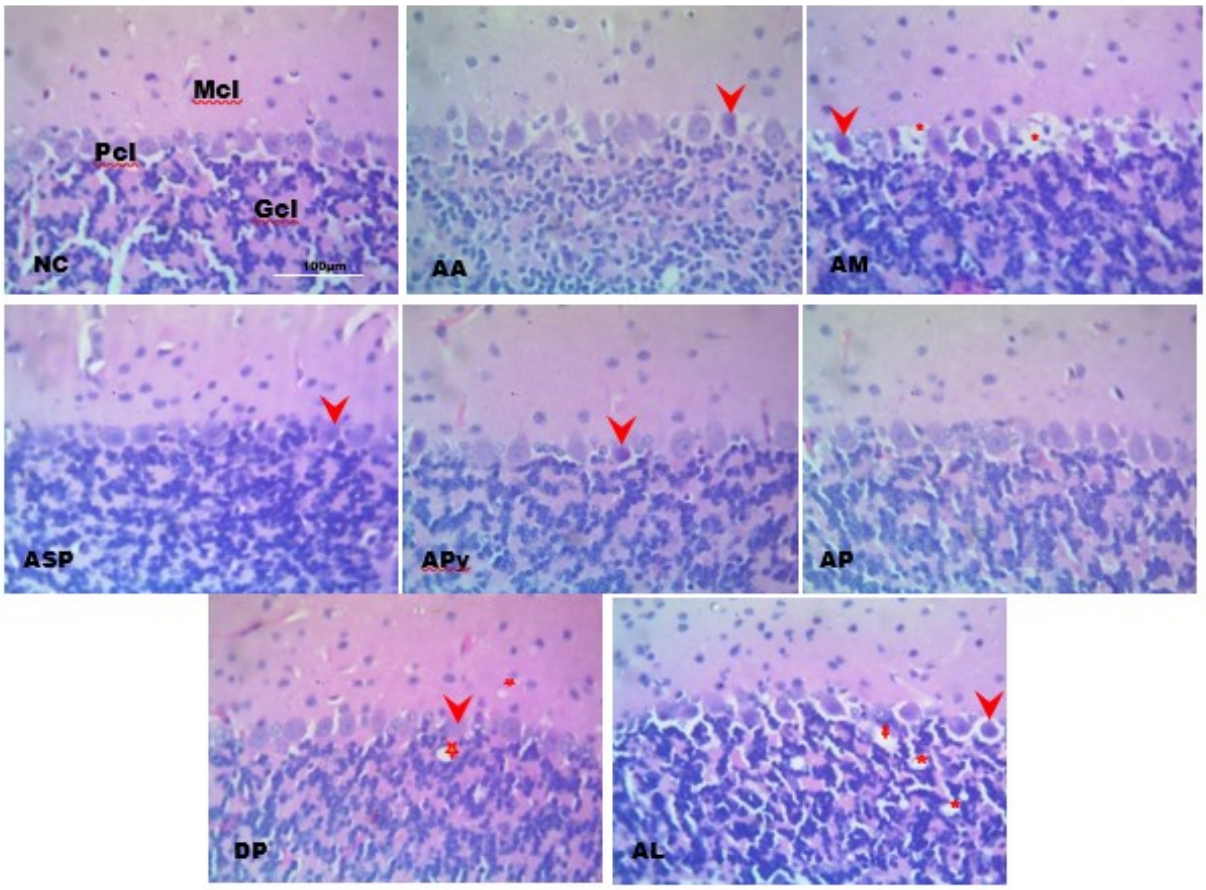


**Fig. 9:** Photomicrographs of the transverse section of the cerebellum following oral administration of some ACTs in Swiss mice, H&E x400. Red arrowhead = atrophy and neuronal shrinkage; Mcl = molecular cell layer; Pcl = Purkinje cell layer and Gcl = granule cell layer.

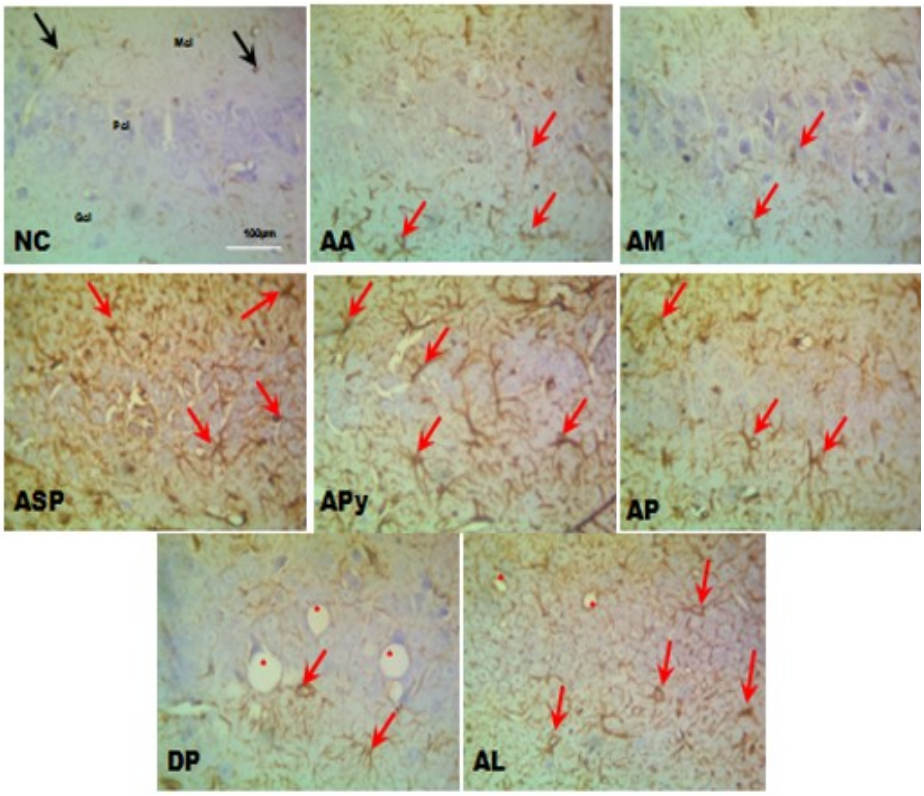


**Fig. 10:** Photomicrographs of the transverse sections of hippocampal the CA1 portion following oral administration of some ACTs in Swiss mice, GFAP x400. Black arrows = low/weak GFAP antibody expression/intensity. Red arrows = strong intensities and reactive astrogliosis. Mcl = molecular cell layer; Pcl = pyramidal cell layer; and Pmcl = polymorphic cell layer.

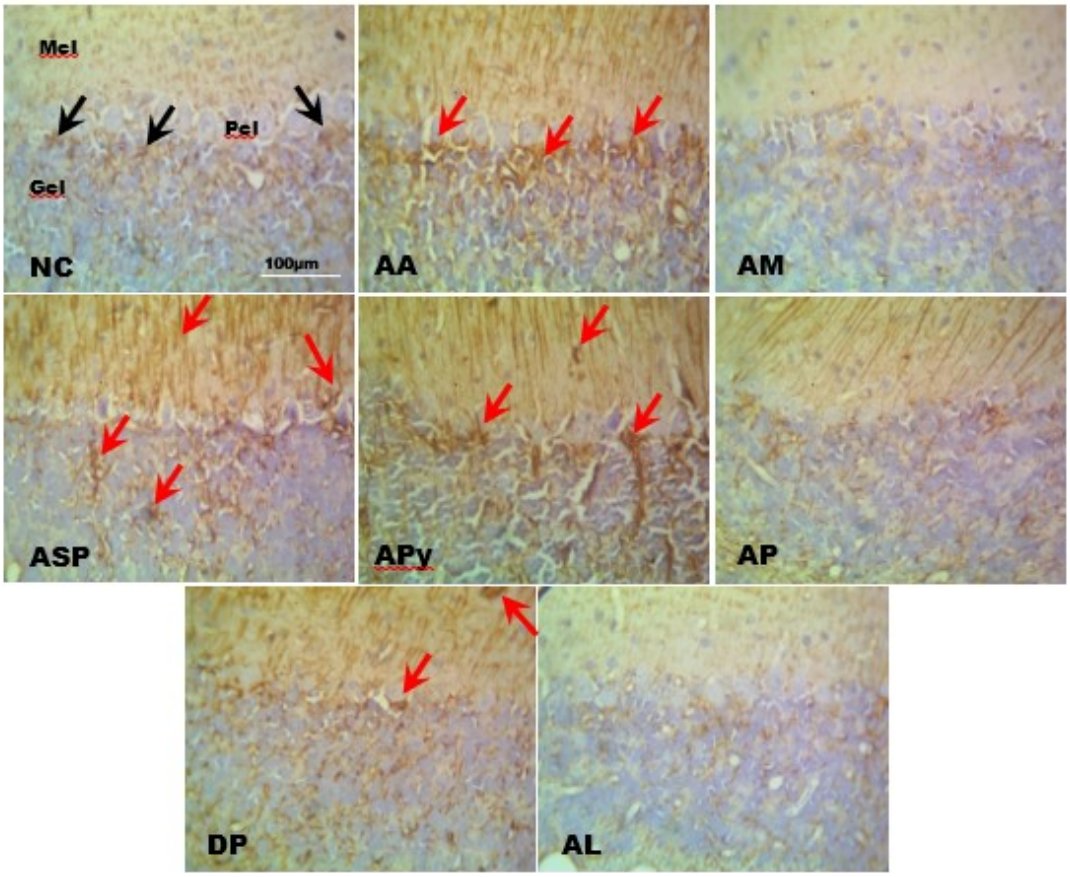


**Fig. 11:** Photomicrographs of the transverse section of the cerebellum following oral administration of some ACTs in Swiss mice, GFAP x400. Black arrows = low/weak GFAP antibody expression/intensity. Red arrows = strong intensities and reactive astrogliosis. Mcl = molecular cell layer; Pcl = Purkinje cell layer and Gcl = granule cell layer.

The cerebellar cortex of the NC showed normal histological features. The molecular, Purkinje, and granular cell layers, Purkinje cells, stellate cells, granular cells, and white matter were normal (Fig. 9). The groups administered with AA 5.71 mg and AM 19.29 mg showed small vacuolations and mild distortions in the granular and Purkinje cell layers, respectively when compared to NC (Fig. 9). The group administered with ASP 10.36 mg showed atrophying Purkinje cells, AP 12.5 mg administered group showed hypertrophying Purkinje cells, DP 15.42 mg and AL 8.00 mg administered groups showed atrophy of the Purkinje cells, and vacuolations in the granular layer compared to NC (Fig. 9).

**Immunohistochemical Labelling of Glial Fibrillary Acidic Protein:** The CA1 portion of the hippocampus of the control group demonstrated normal expression of astrocytes, with no reactive astrogliosis (Fig. 10). All test groups demonstrated high GFAP intensity for reactive astrogliosis except in AM-administered group and NC, and this was similar in the CA1 region of the hippocampus (Fig. 10), as well as in the cerebellar neuropil (Fig 11).

## DISCUSSION

Nearly half of the global population are susceptible to malaria infections, and because of sustained resistance to the initial monotherapies, and the toxicological profiles of earlier anti-malaria drugs, almost all malaria-endemic countries now employ the WHO-approved ACTs as one of their anti-malaria management strategies as reported by WHO [2]. ACTs are officially the most preferred drug for malaria treatment but not without side effects [48-49] including neurotoxicity [26,50] and advocated during the recent COVID-19 pandemic as reported by Ong et al., [51] with attendant neurologic complications. There is even recently the consideration for the deployment of triple ACT in Southeast Asia and Africa due to the potential spread of antimalaria drug resistance as reported by Tindana et al., [52]. The neurotoxicological profile of seven over-the-counter ACTs available in pharmacies without prescription were evaluated in this study; artesunate +amodiaquine (AA), artesunate +mefloquine (AM), artesunate+ sulphadoxine+ pyrimethamine (ASP), artesunate+ pyronaridine (AP), artemisinin+piperaquine (AP), dihydroartemisinin+piperaquine (DP) and artemether+lumefantrine (AL) on oxidative stress levels, brain antioxidants concentrations and the histomorphology of the hippocampal CA1 region and cerebellum of an experimental animal model.

The ACT mechanism of action is facilitated by the endoperoxide moiety in artemisinins which are highly reactive, also artemisinins act by generating free radicals and carbon-centred radicals. Other mechanism involved the disruption of mitochondrial membrane potential and inhibition of calcuim-dependent SERCA-like ATPase PfATP6 with the activation of Iron (II) from haemoglobin [53-54]. The increase in oxidative stress marker across administered groups when compared to NC group suggest that ACTs penetrate the blood-brain barrier describe by Jiang et al., [55] from an earlier report due their nonselective inhibitors of phospholipase $A_2$ and lipophilic nature.

Antioxidants act to mitigate oxidative cellular damage associated with metabolism reported by Mamta et al., [56]. Ayala et al., [57] reported that malondialdehyde (MDA) helps to measure the extent of lipid peroxidation and eventual tissue damage. The significant increase in lipid peroxidation evident by increased MDA concentrations in all ACT-administered groups especially in AP, APy and DP groups when compared to normal control (NC), may suggest that these drugs separately or in combination can trigger the MDA generation, a lipid peroxidation by-product. Increase in MDA concentration will suggest the generation of free radical leading to oxidative damage. Generally, lipid facilitate in replacing worn-out membranes and serve as electrical insulator which help to allow depolarization of action potentials along myelinated nerves [58]. Furthermore, disruption in this function can elicit neurodegeneration and eventually leading to deficits in brain capacity demonstrated by severity of structural changes in histopathological assessment in the APy and DP-administered groups. Popoola and

Imosemi [6] AA at therapeutic dose reportedly caused decreased body weight, induced oxidative stress, elicited karyolysis and reduced Purkinje cells with astrogliosis in cerebellum of Wistar rats. The significant increases in SOD, CAT, GSH and GPx activities in all the ACT-administered groups in the present study suggest that these antioxidants may have been overwhelmed [59].

The oral ACTs effect on the hippocampal neuronal cells demonstrated vacuolation, pyknosis and atrophy in AA, AM, ASP APy and AL-administered groups. According to Kumar et al., [60] neurons react to injury by the displacement of their nuclei to the periphery, and they become swollen and rounded off. This may be due to damage resulting from increase in lipid peroxidation leading to oxidative stress. The hippocampus pyramidal cells in the CA1 region form the cognitive map of contextual, emotional informal and spatial encoding that transmit throughout the brain [60-61]. Degeneration of neurons as shown in AL and ASP-administered groups is associated with neuroinflammatory response, which is a mechanism of protection by host cells to mitigate injury and necrotic cells [60]. Artemisinin has also been reported to trigger apoptosis through p53-dependent and independent pathways [61-62] therefore altering the basic function of the hippocampus CA1 region such as learning and memory [60].

Also, the cerebellum demonstrated in DP, AL, and ASP-administered groups suggest that the drugs cause reduction in size of the Purkinje cells in processes that possibly involving vacuolation and apoptosis with neuro-inflammation. Furthermore, the reduction of size of Purkinje cells may be due to complication resulting from increase in lipid peroxidation and oxidative stress as seen with an increase in MDA concentrations in AA, AM, ASP, and AL-administered groups and more significant increase in DP, APy and AP-administered groups when compared to NC group. Damage to the Purkinje cells as seen in the histopathological assessment of the administered groups especially the DP, APy and AP groups may result from de-myelination and disruption of the cortico-cerebellar circuit due to brain injury and resulting to alteration in functions of the Purkinje cells as reported by Özen et al., [63].

Astrocyte plays an important function in the CNS such as glutamate and potassium utilization, generation of growth factors, some cytokines and extracellular matrix proteins in health and injured states as reported by Ahadiat and Hosseinian [64]. The moderate to severe expression of astrocytes in the sections of the cerebellum in the ASP, APy, AA, DP, AP and AL-administered groups, and sections of the hippocampus CA1 region in the AA, AL, and ASP-administered groups suggest injury to neurons. Hypertrophied cell bodies and thickened dendritic processes as shown in APy, DP and AP-administered groups when compared to NC group are evidence of astrocytic expression and microglia in response to neuronal injury reported by Kumar et al., [65]. This finding supports the reports that brain tissue undergoes reactive astrogliosis as the on-set of neuronal loss [65] as seen in immunohistochemical assessment of AP, APy and DP-administered when compared with NC groups and other administered groups of the hippocampus CA1 region and cerebellum.

Artemether demonstrated increased mortality, renal necrosis, and brain hemorrhage at $\geq$ 30mg/kg/day with no persistent effects in surviving animals described by Beckman et al., [24], which can be as a result of cumulative high levels following repeated daily dosing. Dihydroartemisinin as been reported to exhibit significantly more toxicity than artemether or arteether, with markedly increased in vitro neurotoxicity of artemether and arteether in the presence of liver metabolizing enzymes which in turn results in a reduced exposure to the parent drug and an elevated exposure to active drug metabolite(s) generated by the induced enzyme (or by subsequent metabolizing steps in the same metabolic pathway) reported by Talevi and Bellera, [66]. Arthemether-lumefantrine dispensible tablet (20 mg artemether/ 120 mg lumefantrine) given twice daily for three days demonstrated adverse events of mild to moderate severity in infants and children, although the authors

concluded on the dosage as generally well tolerated compared to artemether and dihydroartemisinin with respect to preclinical safety margins for neurotoxicity reported by Tiono et al., [67] and likewise AL has been reported to elicit ototoxicity while used in the treatment of falciparum malaria in a randomized trial reported by Gurkor et al., [68]. Nonetheless what is in practice and reality is that artemisinins and its derivatives are now used in combination with a potentially neurotoxic partner antimalarials thus making distinct neurotoxicity difficult wherein these medications affect diverse brain parts differentially. The ACTs used in the present study were more neurotoxic to the hippocampal CA 1 compared to the cerebellum, with ASP, DP APy and AL more neurotoxic than AP, AA and AM. Similar findings were reported even in the midst of *Plasmodium berghei*- induced hyperparasitemia in rodentia experimental malaria study on the vital organs of first-pass demonstrating that ACTs markedly increased liver transaminases and altered hepato-renal histoarchitectures with ASP, DP and AL-administered groups with impoverished outcomes when compared to AP, AM and AA antimalarials as reported by Edagha et al., [69].

## CONCLUSION

It is concluded from this study that the standard regimen of the seven ACTs generally upregulated all the oxidative stress parameters and antioxidant concentrations in the order of DP>APy>AP>ASP>AA>AL>AM in Swiss mice. Comparatively the histological evidence from the most to the least neurotoxic ACTs were in the order DP>ASP>APy>AP>AL>AA>AM. Moreover, the ACTs were more neurotoxic to the hippocampal CA region compared to the cerebellum. An extensive regional brain comparison of these ACTs versus in a withdrawal phase warrants a further critical study to ascertain the pharmacokinetics of these drugs with potential demonstrable neuropathologies/ neuromorphological alterations on the differential brain regions viz impact on their functions in the short and long-term besides neurochemically established neurotoxicity.

## Author Contributions

Research design: Innocent A. Edagha, Blessing C. Akpan, Aniekan I. Peter, Aquaisua N. Aquaisua, and Edelungudi I. Edagha. Methodology and Investigation: Promise E. Douglas, Blessing C. Akpan, Moses A. Ataben. Reviewing and Editing: Innocent A. Edagha, Aniekan I. Peter, Aquaisua N. Aquaisua, and Edelungudi I. Edagha. Manuscript was read and approved by all authors.

**Data availability:** Data will be available on request.

## ORCiD

**Innocent A. Edagha:** 0000-0002-5979-7048
**Promise E. Douglas:** 0009-0004-6979-2007
**Aniekan I. Peter:** 0000-0002-1155-6368
**Aquaisua N. Aquaisua:** 0009-0001-9470-2307
**Blessing C. Akpan:** 0009-0003-2868-7019
**Edelungudi I. Edagha:** 0009-0001-4508-3378
**Moses A. Ataben:** 0000-0001-9312-2362

**Funding:** Self-funded research.

## ACKNOWLEDGEMENTS

Authors acknowledge Dr. Jonathan Madukwe of the Department of Histopathology, National Hospital, Abuja and Mr. Collins Igajah, Department of Biochemistry, University of Calabar, Nigeria for their technical assistance.

## Conflicts of Interests: None

## REFERENCES

[1]. World Health Organization (WHO). World malaria report 2022. World Health Organization, Geneva, 2022.
[2]. World Health Organization (WHO). Q & A on the malaria vaccine implementation programme (MVIP). Retrieved from https://www.who.int/malaria/media/malaria-vaccine-implementation-qa/en/ (accessed on 30 October 2022), 2022.
[3]. Charle P, Berzosa P, Descalzo M, de Lucio A, Raso J, Obono, J. Efficacy of artesunate + sulphadoxine pyrimethamine (AS + SP) and Amodiaquine + sulphadoxine pyrimethamine (AQ + SP) for Uncomplicated falciparum malaria in Equatorial Guinea (Central Africa). Journal of Tropical Medicine 2009; 781865.
https://doi.org/10.1155/2009/781865
PMid:20339460 PMCid:PMC2836821
[4]. Xia M, Liu D, LiuY, Liu H. The Therapeutic Effect of Artemisinin and Its Derivatives in Kidney Disease. Front Pharmacol 2020; 11:380.
https://doi.org/10.3389/fphar.2020.00380
PMid:32296335 PMCid:PMC7136752
[5]. Classen W, Altmann B, Gretener P, Souppart C, Skelton-Stroud P, Krinke G. Differential effects of orally versus parenterally administered quinghaosu derivative artemether in dogs. Experimental and Toxicology Pathology 1999; 51, 507-516.
https://doi.org/10.1016/S0940-2993(99)80128-6

[6]. Popoola AN, Imosemi, IO. Sub-acute evaluation of artesunate, amodiaquine and their combination on muscular coordination and morphology of the cerebellum of Wistar rats. International Journal of Biological and Chemical Science 2020; 14:1498-1510. https://doi.org/10.4314/ijbcs.v14i5.1

[7]. Zeng Q, Qiu F, Yuan L. Production of artemisinin by genetically-modified microbes. Biotechnol Lett 2008; 30:581-592. https://doi.org/10.1007/s10529-007-9596-y PMid:18008167

[8]. Aronson J. Meyler's Side Effects of Drugs Artemisinin derivatives, Sixth Edition. Elsevier, Philadelphia 2016; pp. 701-710. https://doi.org/10.1016/B978-0-444-53717-1.00328-0

[9]. Kavishe R, Koenderink J, Alifrangis M. Oxidative stress in malaria and artemisinin combination therapy: Pros and Cons. FEBS J 2017; 284: 2579 - 2591. https://doi.org/10.1111/febs.14097 PMid:28467668

[10]. Li W, Mo W, Shen D, Sun L, Wang J, Lu S, Gitschier, J, Zhou B. Yeast model uncovers dual roles of mitochondria in action of artemisinin. PLoS Genet 2005; 1:e36. https://doi.org/10.1371/journal.pgen.0010036 PMid:16170412 PMCid:PMC1201371

[11]. LiY. Qinghaosu (artemisinin): chemistry and pharmacology. Acta Pharmacol. Sin2012; 33:1141-1146. https://doi.org/10.1038/aps.2012.104 PMid:22922345 PMCid:PMC4003104

[12]. Davis T, Karunajeewa H, Ilett K. Artemisinin-based combination therapies for uncomplicated malaria. Med J Aust2005; 182:181-185. https://doi.org/10.5694/j.1326-5377.2005.tb06650.x PMid:15720175

[13]. Sugiarto S, Davis T, Salman S. Pharmacokinetic considerations for use of artemisinin-based combination therapies against falciparum malaria in different ethnic populations. Expert Opin Drug Metab Toxicol 2017; 13:1115 - 1133. https://doi.org/10.1080/17425255.2017.1391212 PMid:29027504

[14]. Sidorova Y, Domanskyi A. Detecting Oxidative Stress Biomarkers in Neurodegenerative Disease Models and Patients. Methods Protoc 2020; 24: 3(4):66. https://doi.org/10.3390/mps3040066 PMid:32987935 PMCid:PMC7712543

[15]. Borza C, Muntean D, Dehelean C, Sãvoiu G, ªerban C, Simu G, Andoni M, Butur M, Drãgan S. Lipid Metabolism, Oxidative stress and lipid peroxidation-A lipid metabolism dysfunction. Rijeka, Croatia 2013; pp. 23-38. https://doi.org/10.5772/51627

[16]. Pieme C, Tatangmo J, Simo G, Nya P, Moor V, Moukette B, Sobngwi E. Relationship between hyperglycemia, antioxidant capacity and some enzymatic and non-enzymatic antioxidants in African patients with type 2 diabetes. BMC Res. Notes 2017; 10:141. https://doi.org/10.1186/s13104-017-2463-6 PMid:28356165 PMCid:PMC5372257

[17]. Ighodaro O, Akinloye O. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alex. J. Med 2018; 54: 287-293. https://doi.org/10.1016/j.ajme.2017.09.001

[18]. Olayemi S, Arikawe A, Akinyede A, Oreagba A, Awodele O. Effect of malarial treatments on biochemical parameters and plasma pH of mice infected with Plasmodium berghei. Int J Pharmacol 2012; 8: 549 - 554. https://doi.org/10.3923/ijp.2012.549.554

[19]. Bukola A. Biochemical Effect of Artemisinin-based Combination Therapy (ACT) Antimalarial Drugs on Enzymatic Antioxidants in Pregnancy. Advances in Clinical Toxicology 2016; 4: 10. https://doi.org/10.23880/act-16000151

[20]. Edagha I, Offong O, Ekanem A, Edagha E, Asuquo I, Peter A, Azu O. Efficacy of six artemisinin-based combination therapies in the attenuation of Plasmodium berghei-induced testicular toxicity in Swiss mice. European Journal of Anatomy 2019; 23: 289 - 300.

[21]. Schmuck G, Roehrdanz E, Haynes K, Kahl R. Neurotoxic mode of action of artemisinin. Antimicrobial Agents Chemotherapy 2002; 46: 821 - 827. https://doi.org/10.1128/AAC.46.3.821-827.2002 PMid:11850267 PMCid:PMC127487

[22]. Petras J, Young G, Bauman R, Kyle D, Gettayacamin M, Webster H. Artemether-induced brain injury in macaca mulatta. I. The precerebellar nuclei: the lateral reticular nuclei, paramedian reticular nuclei, and perihypoglossal nuclei. Anatomy and Embryology 2000; 201: 383 - 397. https://doi.org/10.1007/s004290050326 PMid:10839633

[23]. Genovese R, Newman B, Li Q, Peggins J, Brewer T. Dose-dependent brainstem neuropathology following repeated arthemether administration in rats. Brain Research Bulletin 1998; 45:199 - 202. https://doi.org/10.1016/S0361-9230(97)00339-0 PMid:9443840

[24]. Beckman D, Youreneff M, Butt M. Neurotoxicity assessment of artemether in juvenile rats. Birth Defects Research Part B Developmental and Reproductive Toxicology 2013; 98:183-99. https://doi.org/10.1002/bdrb.21054 PMid:23495177

[25]. Bangirana P, John C, Boivin M. Rehabilitation for cognitive impairments after cerebral malaria in African children: strategies and limitations. Trop Med Int Health 2006; 11(9) :1341 - 1349. https://doi.org/10.1111/j.1365-3156.2006.01685.x PMid:16930255

[26]. Mwai L, Gwer S, Gatakaa H, Kokwaro G, Newton C. Neurotoxicity of antimalarial drugs: a systematic review. JBI Libr Syst Rev. 2008;6(12 Suppl):1-14. https://doi.org/10.11124/jbisrir-2008-824 PMid:27820468

[27]. Fusetti M, Eibenstein A, CorridoreV, Hueck S, Chiti-Batelli S. Mefloquine and ototoxicity: a report of 3 cases. Clin Ter 1999; 150 :379 82.

[28]. Ezzet F, van Vugt M, Nosten F, Looareesuwan S, White N. Pharmacokinetics and pharmacodynamics of lumefantrine (benflumetol) in acute falciparum malaria. Antimicrob Agents Chemother 2000; 44: 697 - 704. https://doi.org/10.1128/AAC.44.3.697-704.2000 PMid:10681341 PMCid:PMC89749

[29]. Toovey S, Jamieson A. Response to artemisinin derivatives: toxic for laboratory animals, safe for humans? Toxicology Letters 2004; 151: 491 - 492.

[30]. Idro R, Marsh K, John C, Newton C. Cerebral malaria: mechanisms of brain injury and strategies for improved neurocognitive outcome. Pediatr Res 2010; 68(4): 267-74. https://doi.org/10.1203/PDR.0b013e3181eee738 PMid:20606600 PMCid:PMC3056312

[31].Rénia L, Howland S, Claser C, Charlotte A, Suwanarusk R, Hui T, Russell B. Cerebral malaria: mysteries at the blood-brain barrier. Virulence 2012; 3(2) :193-201. https://doi.org/10.4161/viru.19013 PMid:22460644 PMCid:PMC3396698

[32]. Solomon N. Hippocampus: Anatomy and functions. https://www.kenhub.com/en/library/anatomy/hippocampus-structure-and-functions (Retrieved on 22nd August, 2022).

[33]. Standring S. Gray's Anatomy: The Anatomical Basis of Clinical Practice. 41st Edition. Elsevier Limited, London2016; pp 239, 309-311, 331-334.

[34]. National Research Council. Guide for the Care and Use of Laboratory Animals. The National Academies Press, Washington DC 2011; 246p.

[35]. Tveden-Nyborg P, Bergmann TK, Jessen N, Simonsen U, Lykkesfeldt J. BCPT 2023 policy for experimental and clinical studies. Basic Clin Pharmacol Toxicol. 2023 Oct;133(4):391-396. PMid:37732406 https://doi.org/10.1111/bcpt.13944

[36]. Toney-Butler TJ, Nicolas S, Wilcox L. Dose Calculation Desired Over Have Formula Method. [Updated 2023 Jun 20]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan. https://www.ncbi.nlm.nih.gov/books/NBK493162/

[37]. Everitt, J. I. and Gross, E. A. Euthanasia and Necropsy. In: The Laboratory Rat (Second Edition). American College of Laboratory Animal Medicine. 2006;665 - 678. https://doi.org/10.1016/B978-012074903-4/50023-6

[38]. Ariweriokuma V, Arinrotimi A, Gabriel U. Effect of cypermethrin on condition factor and organosomatic indices of Clarias gariepinus. Journal of Agricultural and Social Research 2011; 11: 67-72.

[39]. Sedlak, J., Lindsay, R. Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem. 1968;25:192-205. https://doi.org/10.1016/0003-2697(68)90092-4

[40].Buege, J.A., Aust, S.D. Microsomal lipid peroxidation. Methods Enzymol. 1978;52:302-310. https://doi.org/10.1016/S0076-6879(78)52032-6

[41]. Sun, M., Zigma, S., 1978. An improved spectrophotometric assay of superoxide dismutase based on ephinephrine antioxidation. Anal. Biochem. 1978;90:81-89. PMid:727489 https://doi.org/10.1016/0003-2697(78)90010-6

[42]. Sinha, K.A., Calorimetric assay of catalase. Anal. Biochem. 1971;47:389-394 PMid:4556490 https://doi.org/10.1016/0003-2697(72)90132-7

[43]. Rotruck, JT., Pope AL., Ganther, HE., Swanson AB., Hageman DG., and Hoekstra WG. Selenium: Biochemical Role as a Component of Glutathione Peroxidase. Science 1973;179:588-590. https://doi.org/10.1126/science.179.4073.588

[44]. Bancroft D, Layton C, Suvarna S. Bancroft's Theory and Practice of Histological Techniques: Expert Consult: Online and Print, 8e. In Churchill Livingstone 2019; pp. 75 - 129.

[45]. Edagha I, Ekanem A, Usoh I, Umoh V, Ataben M, Akpan A. Brain antioxidants and hippocampal microanatomical alterations following the administration of Efavirenz/Lamivudine/Tenofovir disoproxil fumarate and Lamivudine/Nevirapine/Zidovudine in adult male Wistar rats. IBRO Neurosci Rep 2022; 13 (12): 210-216. https://doi.org/10.1016/j.ibneur.2022.03.004 PMid:35340763 PMCid:PMC8941179

[46]. Klein, M., Picard, E., Vignaud, J.M., Marie, B., Bresler, L., Toussaint, B., Weryha, G., Duprez, A., Lecl'ere, J. Vascular endothelial growth factor gene and protein: strong expression in thyroiditis and thyroid carcinoma. J. Endocrinol. 1999;161:41-49. https://doi.org/10.1677/joe.0.1610041 PMid:10194527

[47]. Rizzardi, A.E., Johnson, A.T., Vogel, R.I. et al. Quantitative comparison of immunohistochemical staining measured by digital image analysis versus pathologist visual scoring. Diagn Pathol 2012;7:42. https://doi.org/10.1186/1746-1596-7-42 PMid:22515559 PMCid:PMC3379953

[48]. Efferth T, Kaina B. Toxicity of the antimalarial artemisinin and its derivatives. Crit Rev Toxicol, 2010; 40:405-21. https://doi.org/10.3109/10408441003610571 PMid:20158370

[49]. German P, Aweeka F. Clinical pharmacology of artemisinin-based combination therapies. Clinical Pharmacokinetics2008; 47: 91-102. https://doi.org/10.2165/00003088-200847020-00002 PMid:18193915

[50]. Grabias B, Kumar S. Adverse neuropsychiatric effects of antimalarial drugs. Expert Opin Drug Saf. 2016 Jul;15(7):903-10. PMid:27077782 https://doi.org/10.1080/14740338.2016.1175428

[51]. Ong W, Go M, Wang Y, Cheah I, Halliwell B. Effects of Antimalarial Drugs on Neuroinflammation-Potential Use for Treatment of COVID-19-Related Neurologic Complications. Mol Neurobiol. 2021;58(1):106-117. https://doi.org/10.1007/s12035-020-02093-z PMid:32897518 PMCid:PMC7477069

[52]. Tindana P, de Haan F, Amaratunga C, Dhorda M, van der Pluijm RW, Dondorp AM, Cheah PY. Deploying triple artemisinin-based combination therapy (TACT) for malaria treatment in Africa: ethical and practical considerations. Malar J. 2021 Feb 27;20(1):119. https://doi.org/10.1186/s12936-021-03649-7 PMid:33639946 PMCid:PMC7910789

[53]. Tilley L, Straimer J, Gnädig NF, Ralph SA, Fidock DA. Artemisinin Action and Resistance in Plasmodium falciparum. Trends Parasitol. 2016 Sep;32(9):682-696. https://doi.org/10.1016/j.pt.2016.05.010 PMid:27289273 PMCid:PMC5007624

[54]. Wang J, Zhang CJ, Chia WN, Loh CC, Li Z, Lee YM, He Y, Yuan LX, Lim TK, Liu M, Liew CX, Lee YQ, Zhang J, Lu N, Lim CT, Hua ZC, Liu B, Shen HM, Tan KS, Lin Q. Haem-activated promiscuous targeting of artemisinin in Plasmodium falciparum. Nat Commun. 2015 Dec 22;6:10111. https://doi.org/10.1038/ncomms10111 PMid:26694030 PMCid:PMC4703832

[55]. Jiang YY, Shui JC, Zhang BX, Chin JW, Yue RS. The Potential Roles of Artemisinin and Its Derivatives in the Treatment of Type 2 Diabetes Mellitus. Front Pharmacol. 2020 Nov 26;11:585487. https://doi.org/10.3389/fphar.2020.585487 PMid:33381036 PMCid:PMC7768903

[56]. Mamta M, Dhillon S, Brar S., Verma M. Antioxidants. Springer Science and Business media, New York, 2014. https://doi.org/10.1007/978-1-4614-8005-1_6

[57]. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014; 2014: 360438. PMid:24999379 PMCid:PMC4066722 https://doi.org/10.1155/2014/360438

[58]. Otuechere CA, Edewor G, Kale OE, Ekor M. Subacute therapeutic dosing of artemether-lumefantrine and artesunate-amodiaquine combination preserves plasma cholesterol, renal antioxidant status, and organ weights in rats. Malar Res Treat;2012:257986. https://doi.org/10.1155/2012/257986 PMid:22848861 PMCid:PMC3400373

[59]. Kavishe RA, Koenderink JB, Alifrangis M. Oxidative stress in malaria and artemisinin combination therapy: Pros and Cons. FEBS J. 2017 Aug;284(16):2579-2591. PMid:28467668 https://doi.org/10.1111/febs.14097

[60]. Kumar K, Abbas K, Aster J. Robbins and Cotran Pathologic Basis of Disease,Seventh edition, Elsevier/ Saunders, Philadelphia 2014; pp 234.

[61]. Mizuseki K, Diba K, Pastalkova E, Buzsáki G. Hippocampal CA1 pyramidal cells form functionally distinct sublayers. Nat Neurosci. 2011 Aug 7;14(9):1174-81. https://doi.org/10.1038/nn.2894

[62].DiSabato DJ, Quan N, Godbout JP. Neuroinflammation: the devil is in the details. J Neurochem. 2016 Oct;139 Suppl 2(Suppl 2):136-153. PMid:26990767 PMCid:PMC5025335 https://doi.org/10.1111/jnc.13607

[63]. Özen I, Mai H, De Maio A. Purkinje cell vulnerability induced by diffuse traumatic brain injury is linked to disruption of long-range neuronal circuits. Acta Neuropathol Commun 2022; 10:129. https://doi.org/10.1186/s40478-022-01435-3 PMid:36064443 PMCid:PMC9446851

[64]. Ahadiat, SA., Hosseinian, Z. Astrocytes' innate role in neurodegenerative disorders. Bull Natl Res Cent. 2023;47:105. https://doi.org/10.1186/s42269-023-01083-0

[65]. Kumar A, Fontana IC, Nordberg A. Reactive astrogliosis: A friend or foe in the pathogenesis of Alzheimer's disease. J Neurochem. 2023 Feb;164(3):309-324. https://doi.org/10.1111/jnc.15565 PMid:34931315

[66]. Talevi, A., Bellera, C.L. Enzyme Induction and Drug Metabolism. In: The ADME Encyclopedia. Springer, Cham. 2012;68-1. https://doi.org/10.1007/978-3-030-51519-5_68-1

[67]. Tiono, A.B., Tinto, H., Alao, M.J. et al. Increased systemic exposures of artemether and dihydroartemisinin in infants under 5 kg with uncomplicated Plasmodium falciparum malaria treated with artemether-lumefantrine (Coartem®). Malar J. 2015;14(157). https://doi.org/10.1186/s12936-015-0682-7 PMid:25886021 PMCid:PMC4407414

[68]. Gürkov, R. Eshetu, T., I. B. Miranda, I. B., Berens-Riha, N. Mamo, Y. Girma, T. Krause, E. Schmidt, M. Hempel, J. and Löscher, T. Ototoxicity of artemether/ lumefantrine in the treatment of falciparum malaria: a randomized trial Malaria Journal 2008;7:179. https://doi.org/10.1186/1475-2875-7-179 PMid:18796142 PMCid:PMC2559845

[69]. Edagha IA, Ekpo AJ, Edagha EI, Bassey JV, Nyong TP, Akpan AS, Obeten RF, Okon AS, Ating BA. Investigating the Comparative Effects of Six Artemisinin-based Combination Therapies on Plasmodium-induced Hepatorenal Toxicity. Niger Med J. 2019 Jul-Aug;60(4):211-218. https://doi.org/10.4103/nmj.NMJ_152_18 PMid:31831942 PMCid:PMC6892336

**How to cite this article**: Innocent A. Edagha, Promise E. Douglas, Aniekan I. Peter, Aquaisua N. Aquaisua, Blessing C. Akpan, Edelungudi I. Edagha, Moses A. Ataben. Comparative Brain Microanatomical and Neurochemical Alterations Following the Administration of Seven Oral Artemisinin-based Combination Therapies in Swiss Mice. Int J Anat Res 2024;12(2):8878-8892. **DOI:** 10.16965/ijar.2023.276