# Evaluation of Liver and Brain Structural Integrity in Plasmodium Berghei infected Rats administered Antimalaria and Vitamins

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**ABSTRACT**

**Introduction:** Inappropriate prescription, dispense and administration of antimalarial lead to treatment failure. Plasmodium affects different organs in the human body the course of its life cycle. In light of these, the present study aimed to evaluate the effects of Plasmodium berghei infestation on structural integrity of the two key organs (liver and brain) in rats administered chloroquine, artesunate or seed extract of *Phyllanthus amarus* combined with vitamins A, C, B or E.

**Materials and Methods:** Malaria parasitized rats and some non-parasitized rats were randomly distributed into experimental groups (n=5) as follows. Parasitized control (untreated), Chloroquine (20mg/kg), *Phyllanthus amarus* seed extract (PSE) (300 mg/kg), Chloroquine/Vit. A (66.67 IU/kg), Chloroquine/Vit. C (0.056mg/kg), Chloroquine/Vit. E (28.57 IU/kg), Chloroquine/Vit. B (10mg/kg), PSE/Vit. A, PSE/Vit. C, PSE/Vit. B, PSE/Vit. E, Artesunate/Vit. A, Artesunate/Vit. C, Artesunate/Vit. B, Artesunate/Vit. E, Artesunate (20mg/kg) and Un-inoculated. All the drugs and extract were administered orally once daily for 5 days. Twenty hours after the last administration, brain and liver were dissected for histomorphological investigation.

**Results:** A common histopathological lesion in parasitized rats administered antimalarial with vitamins is capillary congestion. Other lesions include vascular congestion and perivascular infiltrates (leucocytes) in groups administered chloroquine/Vit C, marked congested vessel in those administered *P. amarus*/Vit C and marked inflammatory cells coupled with marked activation of Kupffer cells in rats administered artesunate/Vit C. Conclusion: It could be concluded that some combination of vitamins with antimalarial may hamper the mechanism of action of the antimalarial and not be beneficial. This may affect vital organ integrity like liver and brain, and ultimately their functions.

**Keywords:** malaria, *Phyllanthus amarus,* liver, brain, histopathology, apoptosis

## INTRODUCTION

Malaria unarguably is a foremost infectious disease plaguing humanity, especially in the tropics. The history of malaria dates back to about 30 million years.[1] The advent of non-indigenes to endemic areas, like the tropics, resulted in high mortality birthing the name, white man's grave.[2] This led to research efforts, till date, aimed at reducing morbidity, mortality and /or total eradication of the disease. The disease is caused by, a vector-borne (mosquito) parasite, Plasmodium. Evidences have shown that the parasite infects vertebrates including man.[3] Human related species include *P. falciparum*, *malariae*, *ovale* and *vivax*. *Plasmodium knowlesi* have been traced to macaques and is adjudged to be the causative agent of zoonotic malaria in human.[4] The outcome of some studies opined *P. knowlesi* malaria fever as a possible reason for the continued increase in morbidity and treatment failure, as a result of misdiagnosis, in the malaria-endemic area especially in the tropics.[5, 6] Rodents related Plasmodium species, namely *yoelii*, *berghei*, *vinckei and chabaudi*, are valuable in replicating the human infection in laboratory animals. This has been valuable in studying the pathogenesis of the infection and drug development aimed at eradicating the disease condition. [4]

In the course of its life cycle in human, the parasite traverses organs/tissues that are directly involved in the cycle (liver, blood) and other remote organs (brain) that may not be directly involved in the cycle.[7] This comes with pathologic consequences not only in these organs but also on other functionally-related organs/ systems (kidney). The traversing of the organs/systems leads to assault on the functional and sometimes structural integrity of the organs/systems with attendant consequences.[8, 9] The plasmodium parasitic burden is directly proportional to the severity of malaria. However, there are exceptional cases of individuals with a high parasitic burden not showing manifestations of severe illness and some with low parasitic burden culminating in ultimate fatal infections.[10]

Early initiation of effective therapy has proved effective in the prevention of progression to cerebral malaria, a result of the assault on structural integrity on the brain.[11] Also, such therapy has been shown to avert structural/ functional damage to hepatic tissue or resolve hepatic injury and revert the defects when initiated shortly after the onset of parasitaemia. The protective and /or repair of effects on organs/systems correlates with reduced/clearance of parasitaemia leading to less assault on the organs/systems. Aggressive antimalarial drug development exercises have led to the discovery of drugs-natural, synthetic and semi-synthetic. A number of these drugs target the infective phase, erythrocytic phase, that is responsible for the symptomatic illness. Drugs targeting this stage are germane in the therapy of malaria caused by all species of Plasmodium. Prophylactic drugs target the hepatic stage (hypnozoites) of the parasite which can remain dormant in hepatic tissues for a prolonged period. Inappropriate prescription, dispense and administration, however, of the drugs have culminated in parasitic resistance to erstwhile effective antimalarials. Furthermore, some palliative co-administered drugs with antimalarial have been observed to render them less effective.[12] This necessitated the World Health Organisation's recommendation of Artemisinin-based Combination Therapy (ACT) as the gold standard for the therapy of malaria. In light of these, the present study aimed to evaluate the effects of Plasmodium *berghei* infestation on the structural integrity of the two key organs (liver and brain) in rats administered chloroquine, artesunate or seed extract of *Phyllanthus amarus* combined with vitamins A, C, B or E.

## MATERIALS AND METHODS

*Phyllanthus amarus* was sourced from Abraka, Delta State, Nigeria and authenticated at Botany Department, Delta State University, Nigeria (1013568-herbarium number). Fresh seeds of the plant were sorted, air-dried and pulverized. The pulverized seed was Soxhlet extracted with ethanol, filtered with Whatmans filter paper (Cat No: 1011125) and concentrated with Rotary evaporator to obtain the seed extract which was refrigerated until ready for use.

### Animal Handling and Parasite Inoculation

Ethical approval for this study was in line with the recommendation of the Institution Review Board, Faculty of Basic Medical Sciences, Delta State University (PHA/FBMS2017/09/234). Wistar rats (200-250g) were procured from the Animal House, Faculty of Basic Medical Sciences, Delta State University, Nigeria and allowed to acclimate for two (2) weeks prior to commencement of the experiment.[12]

Some of the laboratory rats were inoculated by a passage with *P. berghei* (NK65 strain) obtained from Nigeria Institute of Medical Research (NIMR), Nigeria. The inoculation was as described by Basir [13] with slight modifications. In brief, 1 mL of blood collected from the preservation animal was normal-saline dilute (3mL) at ratio 1:3. A volume of 0.1mLwas inoculated intra-peritoneally into each of the rats. Three days after inoculation the test animals were assessed and confirmed

for malaria parasitaemia according to CDC microscopic diagnosis.[14]

Malaria parasitized rats and some non-parasitized rats were randomly distributed into experimental groups (n=5) as follows. **Group 1**: Parasitized control (untreated), **Group 2**: Parasitized treated with Chloroquine (20mg/kg), **Group 3**: Parasitized treated with *Phyllanthus amarus* seed extract (300 mg/kg), **Group 4**: Parasitized treated with Chloroquine + Vit. A (66.67 IU/kg), **Group 5**: Parasitized treated with Chloroquine + Vit. C (0.056mg/kg), **Group 6**: Parasitized treated with Chloroquine + Vit. E (28.57 IU/kg), **Group 7**: Parasitized treated with Chloroquine + Vit. B (10mg/kg), **Group 8**: Parasitized treated with *Phyllantus amarus* seed extract + Vit. A, **Group 9**: Parasitized treated with *Phyllanthus amarus* seed extract + Vit. C, **Group 10**: Parasitized treated with *Phyllanthus amarus* seed extract + Vit. B, **Group 11**: Parasitized treated with *Phyllanthus amarus* seed extract + Vit. E, **Group 12**: Parasitized treated with Artesunate + Vit. A, **Group 13**: Parasitized treated with Artesunate + Vit. C, **Group 14**: Parasitized treated with Artesunate + Vit. B, **Group 15**: Parasitized treated with Artesunate + Vit. E, **Group 16**: Parasitized treated with Artesunate (20mg/kg) and **Group 17**: Normal control (un-inoculated).

**Drugs Administration and Sample Collection**

All the drugs used in this study were procured from local retailers. All the drugs and extract were administered orally once daily for 5 days. Twenty hours after the last dose administration of the drugs/extract, the rats were euthanized by cervical dislocation. The brain and liver dissected and fixed in 10% formal-saline for histomorphological investigation. [7, 13]

## RESULTS

Hepatic photomicrograph of untreated parasitized rats showed inflammatory cells infiltrates and a marked activation of Kupffer cells (Fig. 1). The hepatic histo-architecture of rats administered chloroquine (Fig. 2), *P. amarus* (Fig. 3) and artesunate (Fig. 16) appeared normal. It was observed that histo-morphology of rats in other groups showed lesions ranging from vascular congestion and perivascular infiltrates (leucocytes/leukocytosis) in groups administered chloroquine/Vit C (Fig. 5), marked congested vessel in those administered *P. amarus*/Vit C (Fig. 9) and marked inflammatory cells coupled with marked activation of Kupffer cells in rats administered artesunate/Vit C (Fig. 13). Also observed in animals administered antimalarial/Vit E are mild inflammatory cell infiltrates, mild Kupffer cells activation in chloroquine/Vit E (Fig. 6), congested portal vessel, peri-portal inflammatory cells infiltrate, activation of Kupffer cells in *P.amarus*/Vit E (Fig. 11) and mild congestion in artesunate/Vit E (Fig. 15). The hepatic lesions in Vit A co-administration include moderate Kupffer cells activation, mild inflammatory cell infiltration in chloroquine/Vit A (Fig. 4), periportal inflammatory cells infiltration in *P. amarus*/Vit A (Fig. 8) and congested vessel, bi-nucleated hepatocyte, activation of Kupffer cells in artesunate/Vit A (Fig. 12). Photomicrograph of rats in co-administration with Vit B showed moderate activation of Kupffer cells in chloroquine/Vit B (Fig. 7), congestion of central vein, mild activation of Kupffer cells, mild perivascular infiltration in *P. amarus*/Vit B (Fig. 10) and perivascular inflammatory cells infiltration, congested central vein in artesunate/Vit B (Fig. 14).

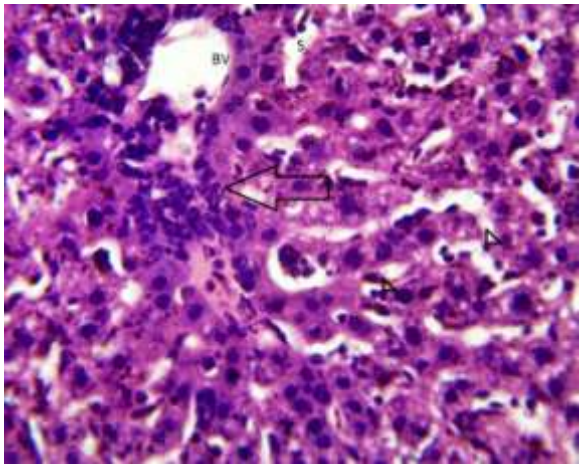


**Figure 1: X400 H & E Photomicrograph of liver of malaria parasitized and untreated rat: marked inflammatory cells infiltrates (arrow) and marked activation of kupffer cells (arrow head).**

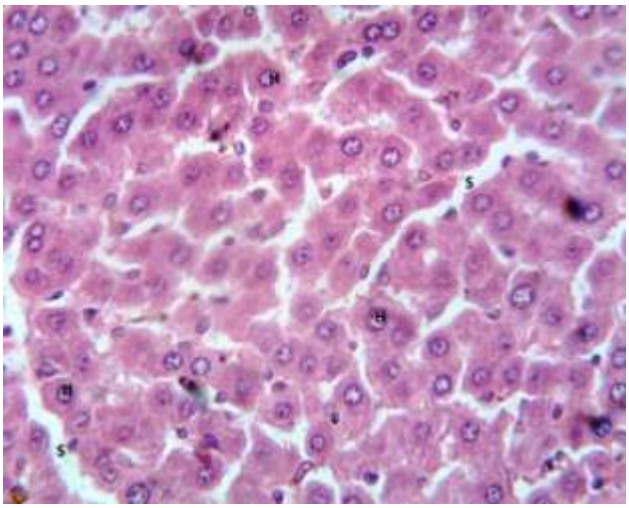

**Figure 2: X400 H & E Photomicrograph of liver of malaria parasitized rat administered chloroquine: hepatocytes (H), sinusoids (S) that are normal.**

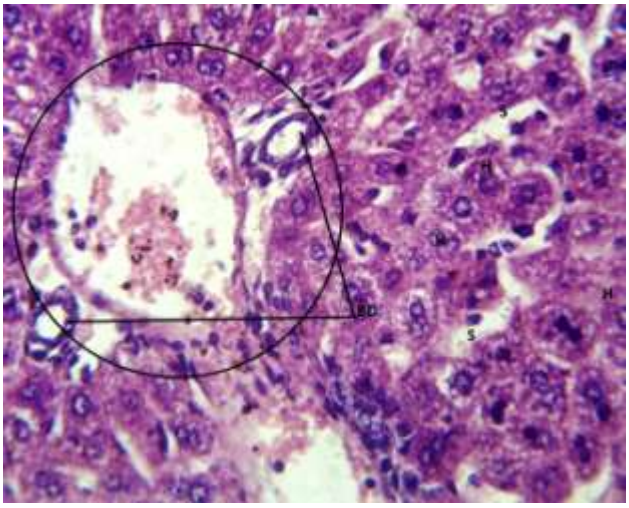

**Figure 3 : X400 H & E Parasitized rat administered *P. amarus*: Section appear normal. Portal triad (Circle), bile duct (BD), sinusoid (S) and Hepatocytes (H).**

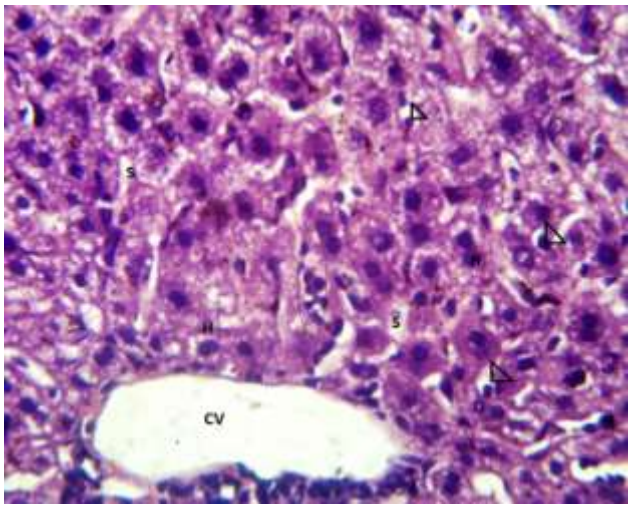


**Figure 4: X400 H & E Photomicrograph of plasmodium parasitized rats treated with chloroquine/Vit A: sections of the hepatic tissue composed of a defined central vein (CV), hepatocytes (H) disposed in sheet and are separated by the sinusoids (S). There is moderate activation of the kupffer cells (arrow head) and mild infiltration of inflammatory cell (circle).**

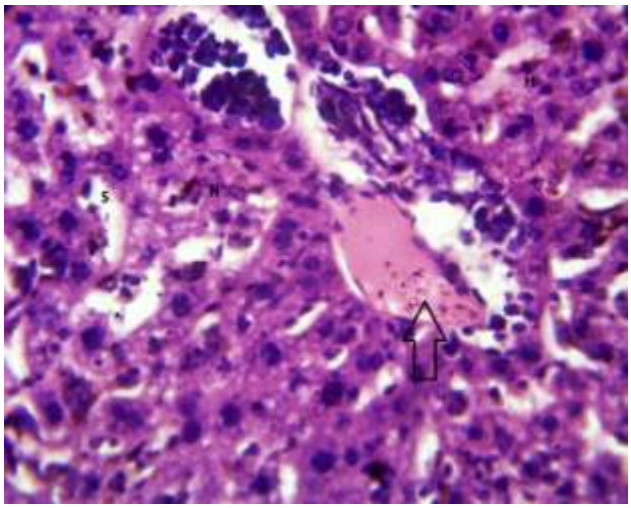

**Figure 5: X400 H & E Photomicrograph parasitized rat administered chloroquine/Vit C: vascular congestion and perivascular infiltrates. The hepatocytes (H) and sinusoids appear normal.**

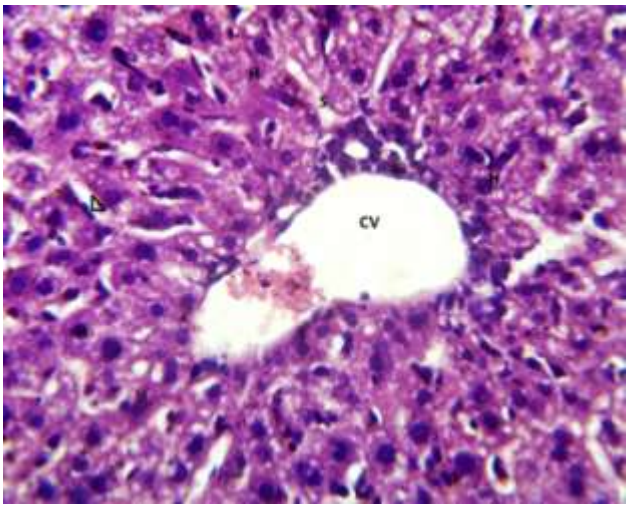


**Figure 6: X400 H & E Photomicrograph of plasmodium parasitized rat administered chloroquine/Vit E: mild activation of kupffer cells within the sinusoids (arrow head) and mild perivascular inflammatory cell infiltrates (leukocytes).**

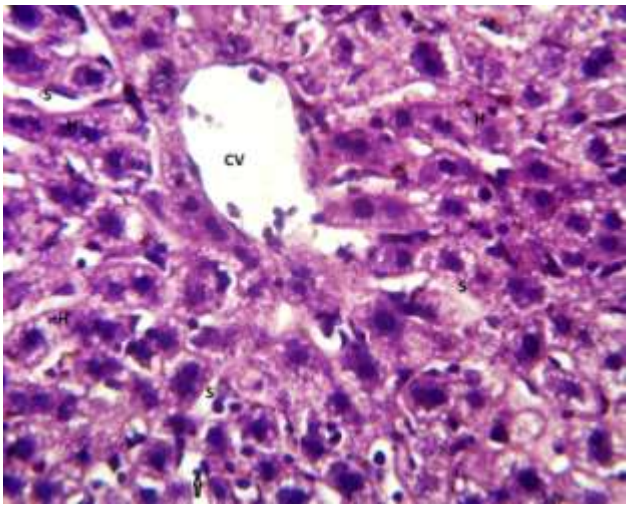


**Figure 7 : X400 H & E Section of rat liver parasitized and treated with chloroquine/Vit B: moderate activation of kupffer cells (arrow). The sinusoids (S), hepatocytes (H) and central vein are essentially normal.**

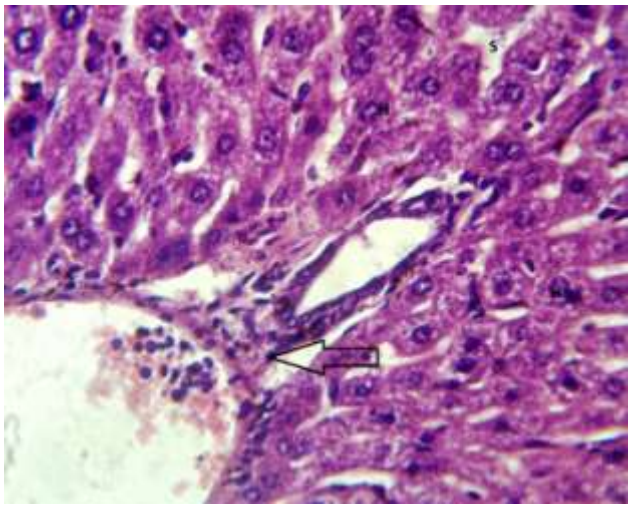

**Figure 8: X400 H & E Photomicrograph of rat parasitized and treated with *P. amarus*/Vit A: peri-portal inflammatory cells infiltrates (leukocytes) (arrow). The hepatocytes and sinusoids are normal.**

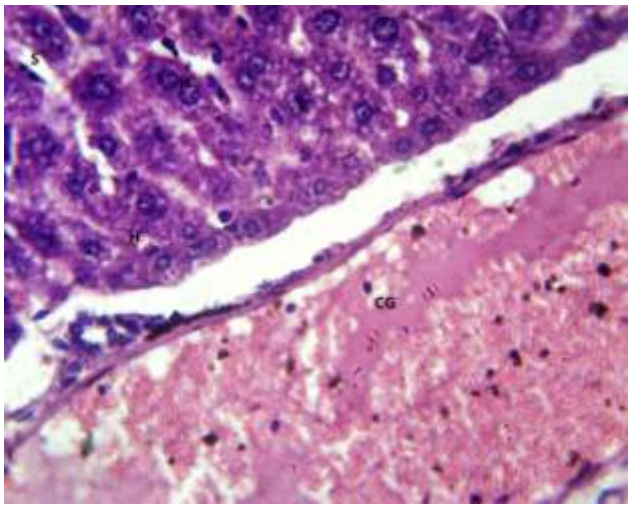


**Figure 9: X400 H & E Photomicrograph of rat parasitized and administered *P. amarus*/Vit C: marked congested vessel (CG). Hepatocytes (H) and Sinusoids (S) appear normal.**

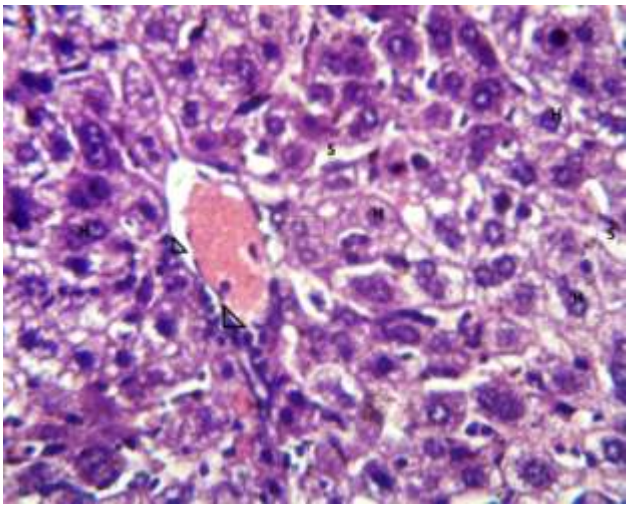

**Figure 10: X400 H & E Photomicrograph of parasitized rat administered *P. amarus*/Vit B: congestion of the central vein (arrow) mild activation of kupffer cells and mild perivascular infiltration (arrow head). The sinusoids (S) are free from collection and hepatocytes are normal.**

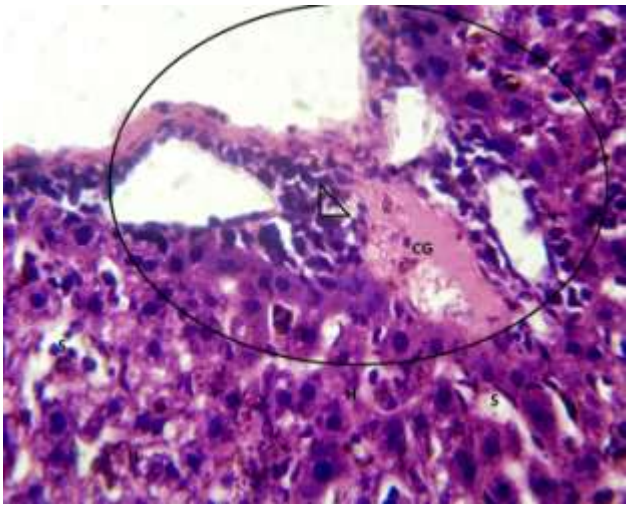


**Figure 11: X400 H & E Photomicrograph of parasitized rat administered *P. amarus*/Vit E: composed of the portal triad (Circle); the portal vessel is congested (CG), peri-portal inflammatory cells infiltrate (leukocytes) (arrow head) and activation of kupffer cells. The sinusoids (S) and Hepatocytes (H) are normal.**

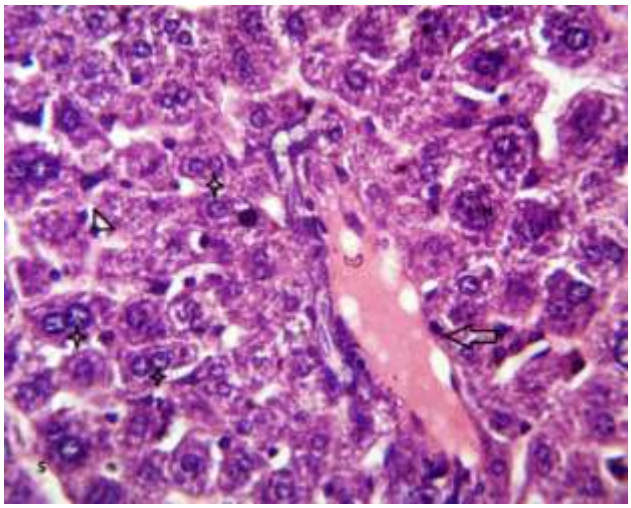

**Figure 12: X400 H & E Photomicrograph of parasitized rat administered artesunate/Vit A: congestion of the vessel (arrow), bi-nucleated hepatocytes (star) and activation of kupffer cells (arrow head). The sinusoids appear normal.**

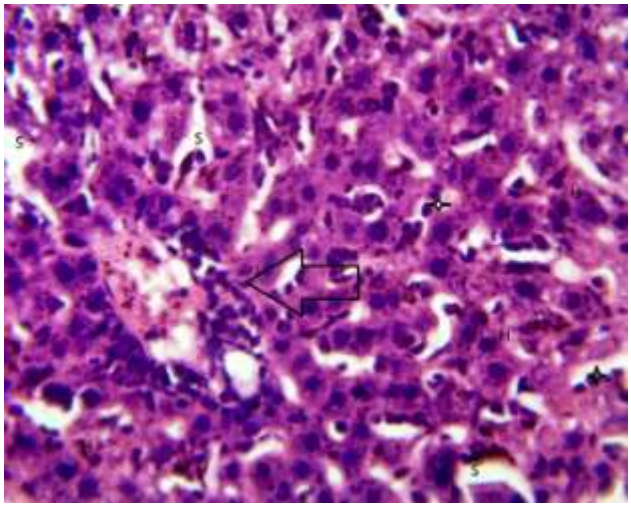

**Figure 13: X400 H & E Photomicrograph of parasitized rat administered artesunate/Vit C: marked inflammatory cells infiltrates (leukocytes) (arrow) and marked activation of kupffer cells (arrow head).**

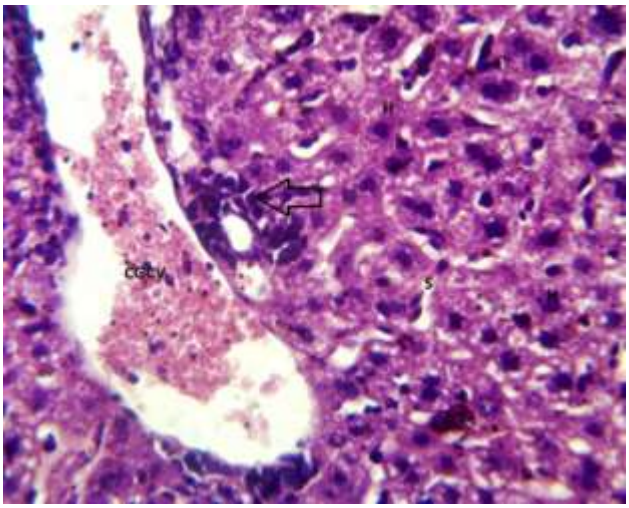


**Figure 14: X400 H & E Photomicrograph of parasitized rat treated with artesunate/Vit B: perivascular inflammatory cells infiltrates (arrow) and congestion of the central vein (CGCV). The sinusoids (S) and hepatocytes are normal.**

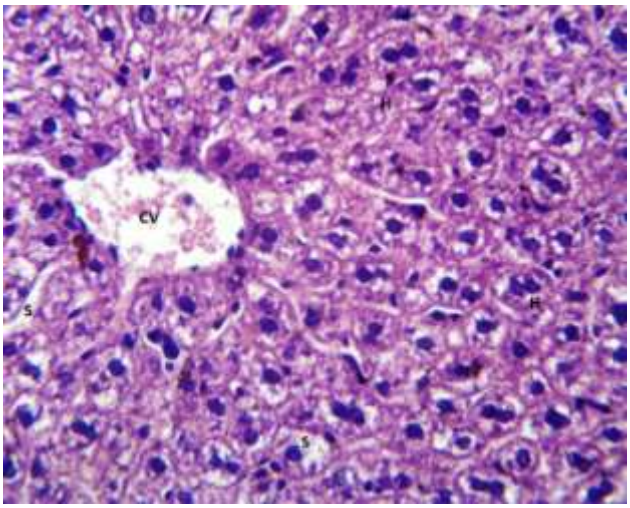


**Figure 16: X400 H & E Photomicrograph of parasitized rat treated with artesunate: section of the hepatic tissue composed of the central vein (CV), hepatocytes (H) disposed in sheet and are separated by the sinusoids that are free from collection and inflammatory cells.**

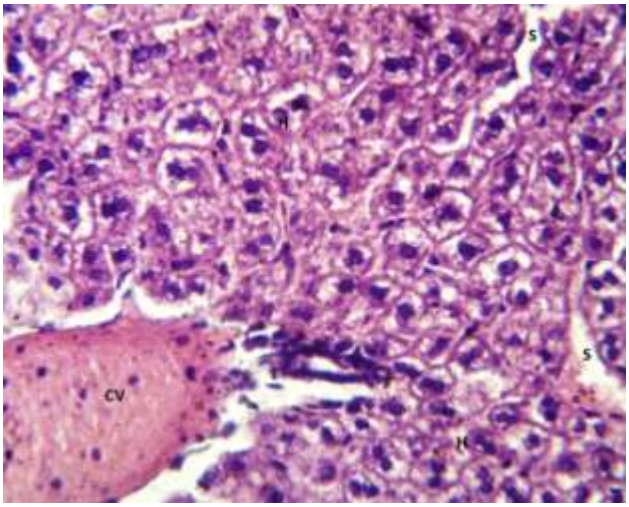


**Figure 15: X400 H & E Photomicrograph of parasitized rat treated with artesunate/Vit E: marked congestion of the central vein. The sinusoids (S) and hepatocytes (H) are not normal.**

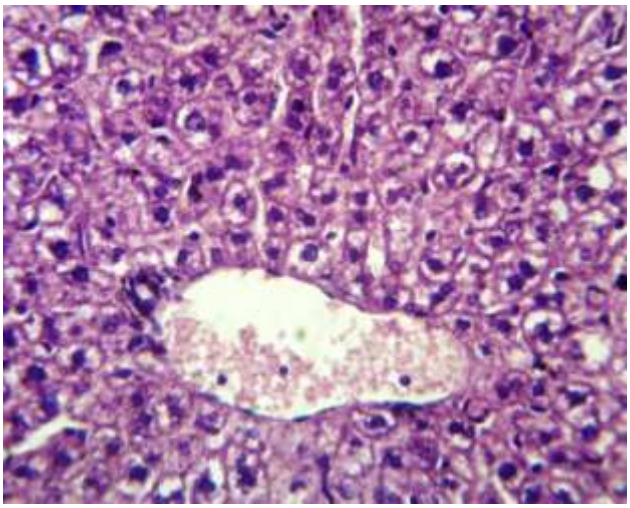

**Figure 17: X400 H & E Photomicrograph of rat not parasitized and not treated: Section appears normal.**

**BRAIN HISTOLOGY**

The histo-morphology of the brain of plasmodium-parasitized-untreated rats showed congested blood vessels and foci of fat degeneration within the neuro-parenchyma (Fig. 18). Photomicrographs of the parasitized rats administered chloroquine (Fig. 19), *P. amarus* (Fig. 20) and artesunate (Fig. 33) appeared

normal. A common histopathological lesion in parasitized rats administered antimalarial with vitamins is capillary congestion: chloroquine/Vit C (Fig 22) and artesunate/Vit C (Fig. 30). Other lesions observed include media hypertrophy of blood vessel in artesunate/Vit E (Fig 32); capillary infiltration in chloroquine/Vit B (Fig 24) and artesunate/Vit B (Fig 31); fat accumulation within neuro-parenchyma in *P. amarus*/Vit C (Fig 26) and *P. amarus*/Vit E (Fig. 28); and mild pyknosis of neurons in *P. amarus*/Vit A (Fig 25).

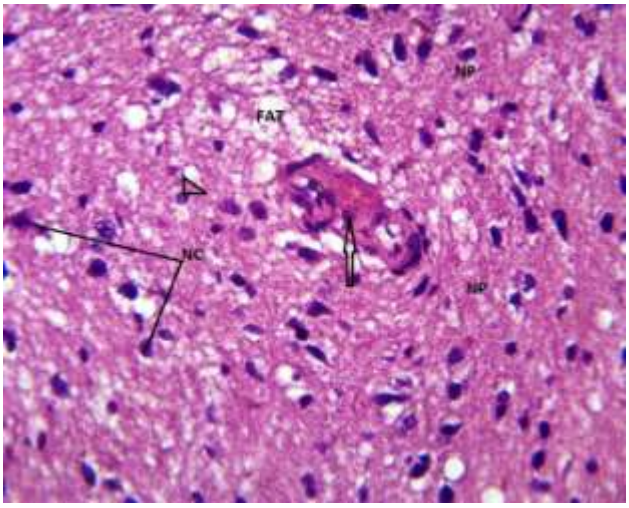


**Figure 18: X400 H & E Plasmodium untreated section: the cerebral cortex composed neuronal cells (NC), neuroglia (arrow head). The blood vessel (arrow) is congested. There are foci of fat degeneration (FAT) within the neuro-parenchyma (NP)**

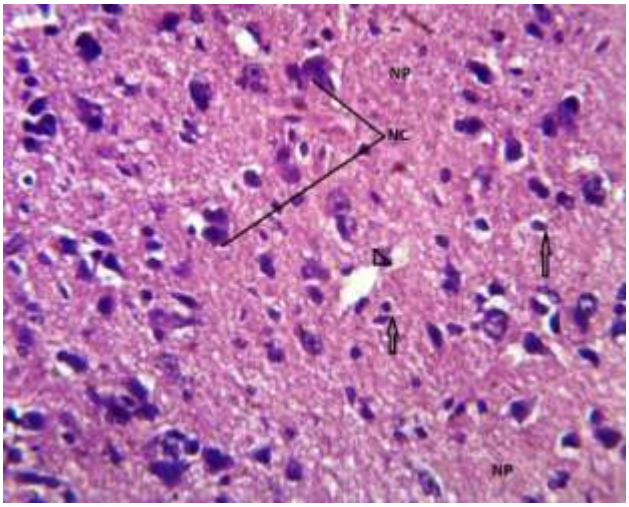


**Figure 19: X400 H & E Photomicrograph of rat administered chloroquine: cerebrum containing clusters of nerve cells (NC) (arrow), neuro-parenchyma (NP) and capillaries appearing normal.**

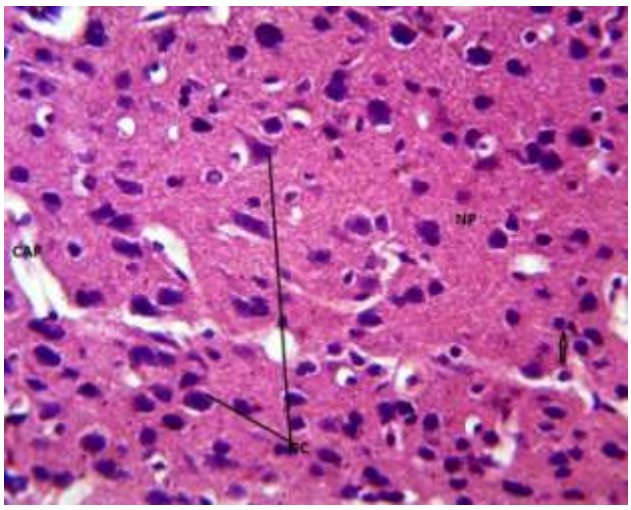


**Figure 20: X400 H & E Photomicrograph of rat administered *P. amarus*: cerebrum containing clusters of nerve cells (NC) (arrow), neuro-parenchyma (NP) and capillaries (CAP) appearing normal.**

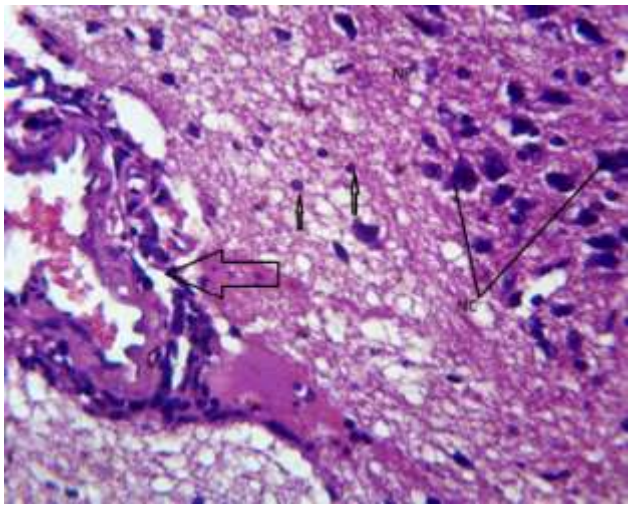


**Figure 21: X400 H & E Photomicrograph of rat administered chloroquine/Vit A: section of the cerebrum with perivascular infiltrates and intra-parenchyma collection. The neuronal cells (NC) (Arrow) and neuro-parenchyma appear normal.**

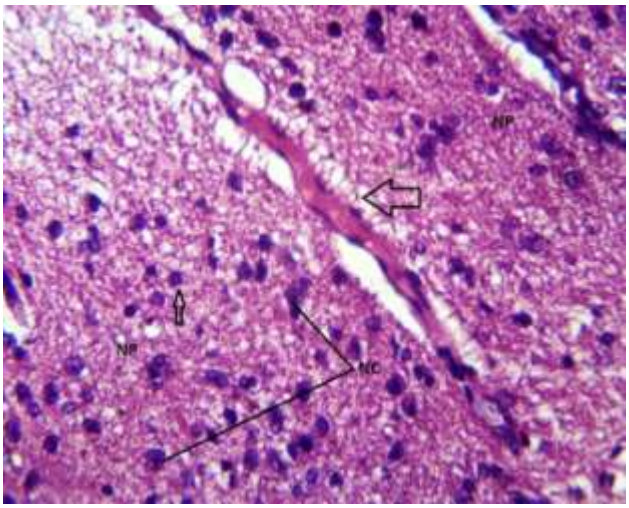


**Figure 22: X400 H & E Photomicrograph of rat administered chloroquine/vit C: section of the cerebrum with marked capillary congestion.**

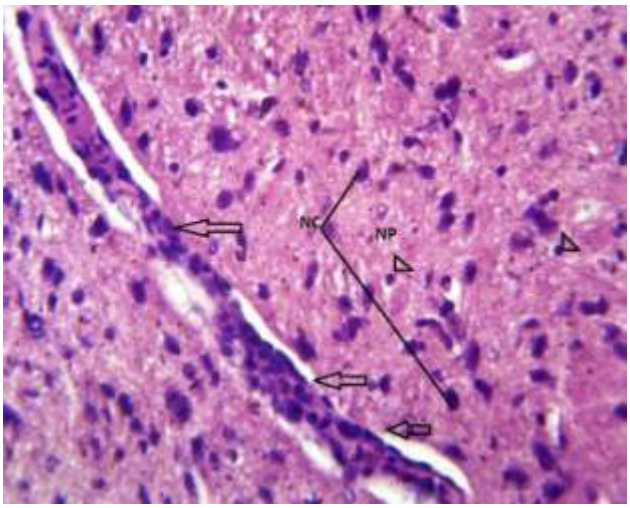


**Figure 24: X400 H & E Photomicrograph of rat administered chloroquine/Vit B: section of the cerebrum with marked peri-capillary (arrow) infiltrates. The neuronal cells (NC), (Arrow) and neuro-parenchyma appear normal.**

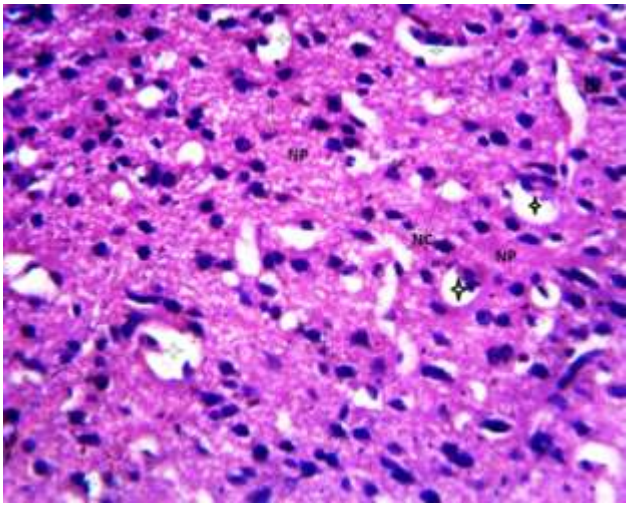


**Figure 23: X400 H & E Photomicrograph of rat administered chloroquine/Vit E: section of the cerebrum composed of nerve cells (NC), (arrow) and capillary that are essentially normal.**

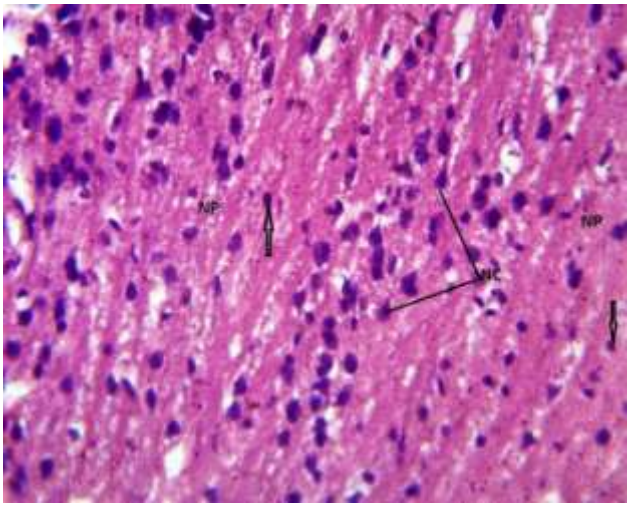


**Figure 25: X400 H & E Photomicrograph of rat administered *P. amarus*/vit A: cerebrum containing clusters of nerve cells (NC) (arrow), neuro-parenchyma/neutrophil (NP) and capillaries appearing normal.**

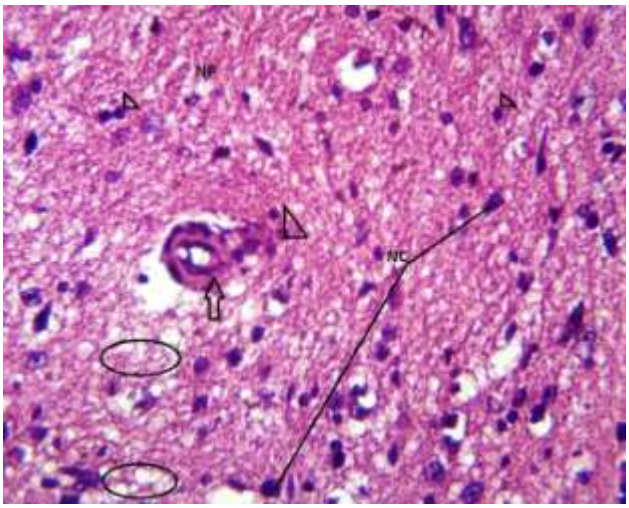


**Figure 26: X400 H & E Photomicrograph of rat administered *P. amarus*/vit C: section of the cerebrum with fat accumulation within the neuro-parenchyma (Circle).**

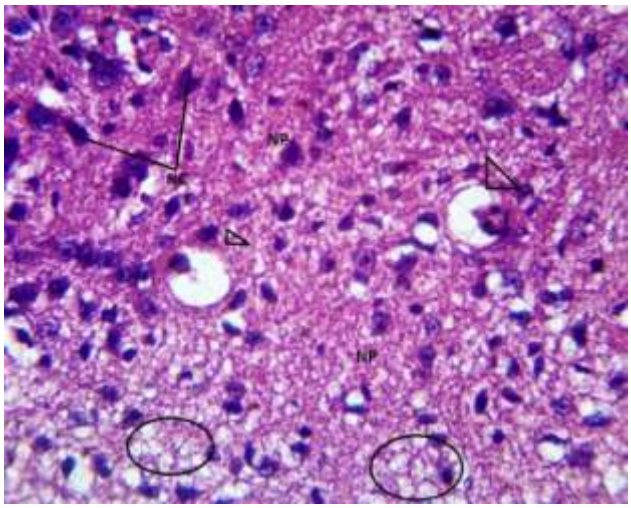


**Figure 28: X400 H & E Photomicrograph of rat administered *P. amarus*/vit E: section of the cerebrum with fat accumulation within the neuro-parenchyma (Circle).**

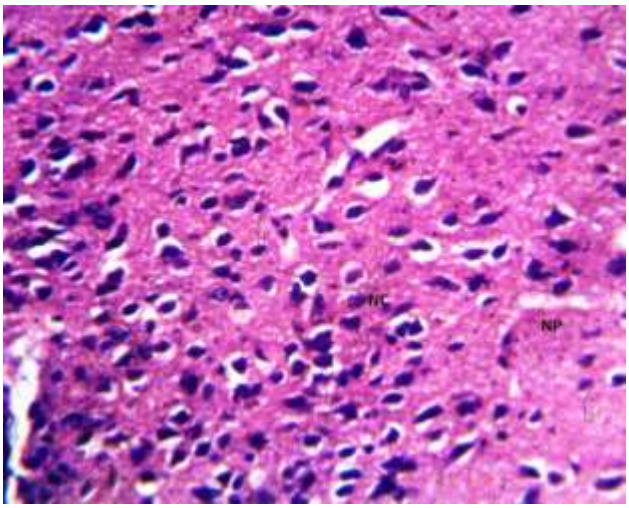


**Figure 27: X400 H & E Photomicrograph of rat administered *P. amarus*/Vit B: section of the cerebrum with mild pyknosis of neurons (NC) compared with that of control. The neuro-parenchyma (NP) appears normal.**

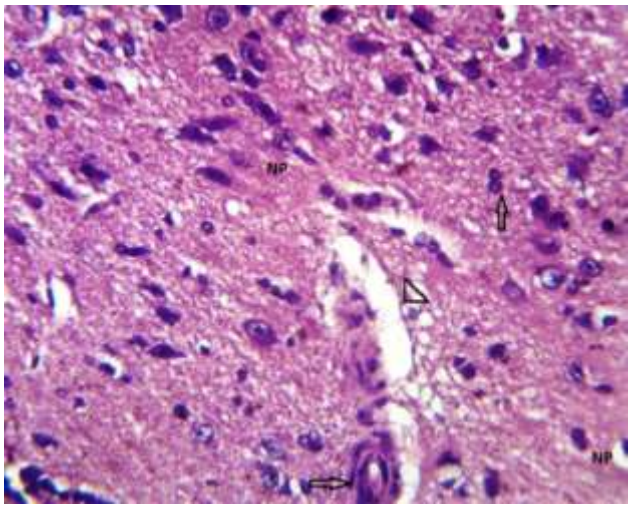


**Figure 29: X400 H & E Photomicrograph of rat administered artesunate/Vit A: cerebrum containing clusters of nerve cells (NC) (arrow), neuro-parenchyma (NP) and blood vessel (arrow) appear normal.**

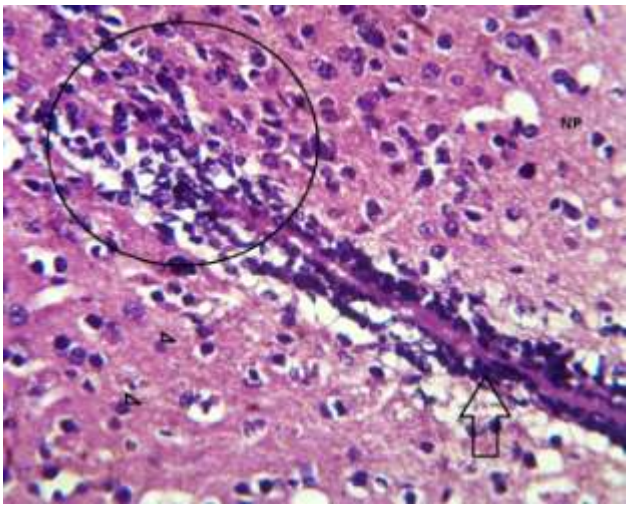


**Figure 30: X400 H & E Photomicrograph of rat administered artesunate/Vit C: section of the cerebrum composed of clusters of nerve cells (circle), neuro-parenchyma (NP), that are essentially normal. However, there is capillary congestion and peri capillary infiltrates.**

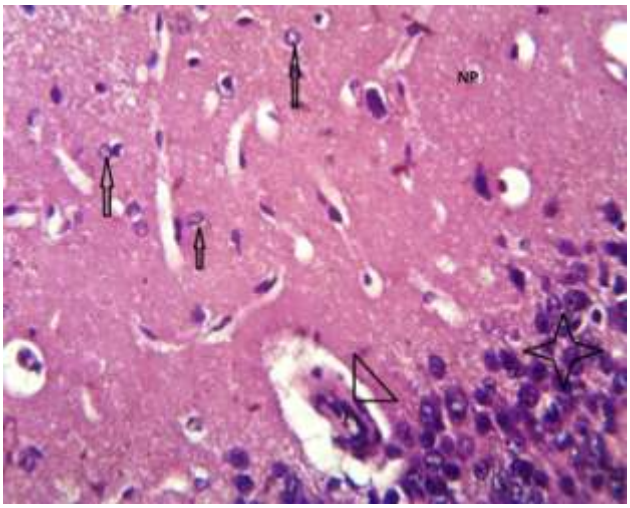


**Figure 32: X400 H & E Photomicrograph of rat administered artesunate/Vit E: section of the cerebrum composed of clusters of nerve cells (star), neuro-parenchyma (NP), that are essentially normal. However the blood vessel exhibited media hypertrophy.**

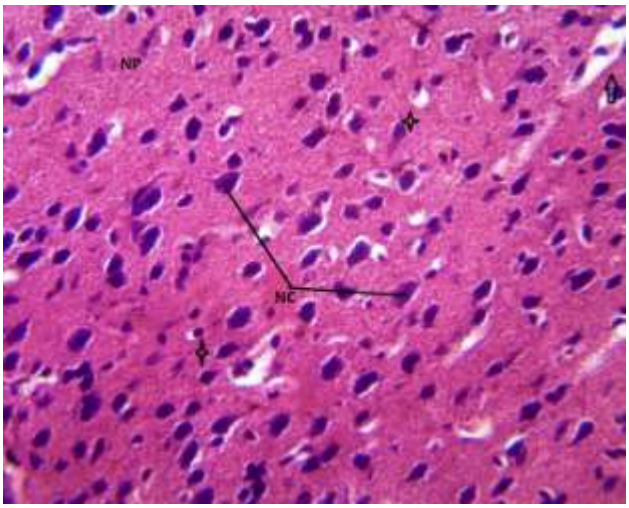


**Figure 31: X400 H& E Photomicrograph of rat administered artesunate /vit B: cerebrum containing clusters of nerve cells (NC) (star), neuro-parenchyma (NP) and capillaries (Arrow) appearing normal.**

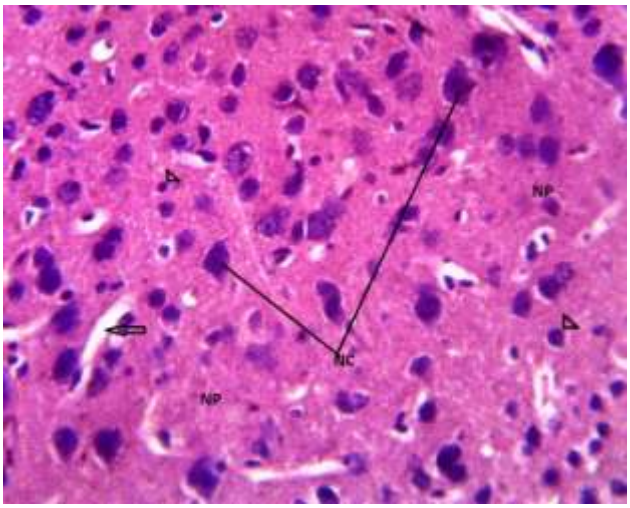


**Figure 33: X400 H & E Photomicrograph of rat administered artesunate: cerebrum containing clusters of nerve cells (NC). The neuro- parenchyma (NP) and capillaries (arrow) appear normal.**

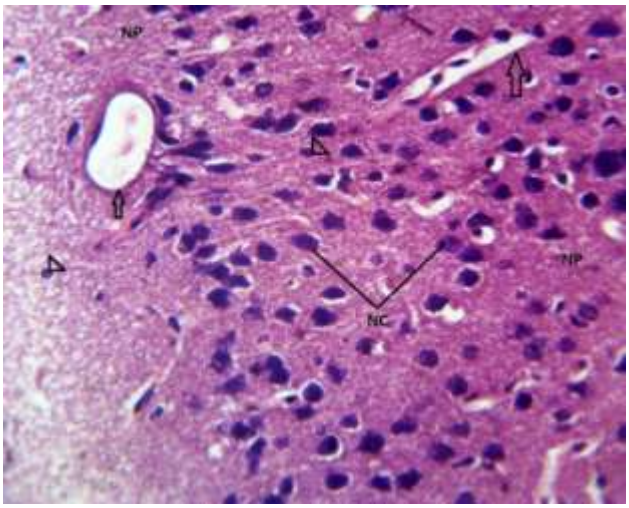


**Figure 34: X400 H & E Photomicrograph of rat non-parasitized/untreated: cerebrum containing clusters of nerve cells (NC). The neuro- parenchyma (NP) and capillaries (arrow) appear normal.**

## DISCUSSION

The liver is a major organ involved in the transverse of the malaria parasite in the human body during its life cycle. It is therefore not uncommon that pathology of the liver, in severe malaria infection in human, is correlated with morbidity and mortality.[15] This sometimes manifests as jaundice as the capacity of the liver is overwhelmed in the massive haemolysis resulting from one or combination of factors such as G6PD deficiency, parasitised RBCs (PRBCs) or antimalarials.[16] In experimental animals, pathologic apoptotic hepatic changes have been documented in Kupffer cells (hepatic phase) and hepatocytes (erythrocyte phase) [17, 18]; regulated via death-receptor and mitochondrial pathways. In light of this, the observed lesions in hepatic histomorphology observed some groups of experimental rats in the present study could be attributed to the effect of the parasite, *Plasmodium berghei*.

In the present study, marked Kupffer cells activation and inflammatory cells infiltration in the untreated and some treatment groups are similar to those of previous studies. Hepatic histopathological lesions in previous studies include minute PRBCs sequestration, reactive/hyperplastic Kupffer cells and withholding of haemozoin pigment.[19, 20] Others include copious inflammatory cell response, fatty degenerative changes and sometimes necrosis of the hepatic cells.[21]

Hyperplasia is an aftermath of the phagocytic activities of the Kupffer cells.[22] It could be inferred that one of the causes of hypertrophy could be increased activity of the Kupffer cells in processing a number of RBCs in massive RBC destruction as can be observed in high plasmodium parasitaemia. High plasmodium burden has been linked to increased total bilirubin which has demonstrated to be positively correlated with the occurrence of apoptosis in human plasmodium parasitaemia.[23] It could be deduced that observed increased Kupffer cells activity in the groups co-administered vitamins (especially those notable for antioxidant effect) and artesunate was due to ineffectiveness in reducing or eradicating parasitaemia. Artesunate has been observed to act by generating reactive oxygen species (ROS) in the parasite.[24] Endogenous supplements of antioxidant have been established as a reason for treatment failure when co-administered with artesunate.[12] It stands to reason, therefore, that the hypertrophic Kupffer cells in the treatment groups in the present study, similar to the untreated group, could be as a result of treatment failure. Since the same trend was observed for *P. amarus*, it could be deduced that the plant extract has a similar antiplasmodial mechanism of action as artesunate. Furthermore, if left unchecked, the treatment failure could lead to increased overwhelming total bilirubin beyond hepatic capacity which could lead to pathologic apoptosis. Presence of inflammatory cells in the untreated groups and some treatment groups may be an indication of the onset of hepatic necrosis as observed in a previous study.[21] From the foregoing, hepatic necrosis and pathologic apoptosis could be the aftermath of plasmodium parasitaemia in untreated cases and treatment failure.

Cerebral malaria is a most severe neurological complication of Plasmodiasis affecting more of children in sub-Saharan Africa.[25] Management has been shown to be difficult and survivors are prone to cognitive and behavioural disorders.[26] The pathogenesis of cerebral malaria remains poorly understood. However, sequestration of infected erythrocyte in brain vasculature which includes accumulation of pro-inflammatory cells has been projected as a likely underlying mechanism [27, 28] following a compromise of the blood-brain barrier. A sequel of this in some instances is haemorrhage. The observed histopathological lesions in some of the treatment groups like capillary congestion and vascular media hypertrophy may suggest preceding events which may culminate in haemorrhage; futher study to confirm this is suggested. Cellular infiltration observed in this study is similar to that observed in previous studies on cerebral malaria in mice. [29, 30]

**CONCLUSION**

Since the histopathological lesions (brain and liver) are confined to the groups co-administered antimalarial and vitamins, it could be concluded that some combination therapy with antimalarial may affect the histo-archicture of the organs and ultimately their functions.

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