# Bioscene

Volume- 21 Number- 01

ISSN: 1539-2422 (P) 2055-1583 (O)

www.explorebioscene.com

# Review on Histopathological changes of Plasmodium infected mice

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***Abstract***

***Background:*** *Malaria pathogenesis is known to induce acute damage to vital organs i.e., spleen, liver, kidney, brain and bone marrow. In the present investigation, Plasmodium berghei (ANKA) was found to be lethal to Swiss albino mice, when parasitized erythrocytes were inoculated. So the present study was aimed to study the changes in these vital organs in Plasmodium berghei infected mice treated and treated mice with artemisinin-based combination drugs i.e., Artesunate + Amodiaquine (ASAQ), Artesunate + Sulfadoxine Pyrimethamine (ASSP), Artesunate + Lumefantrine (AL).* ***Methods:*** *The P. berghei course of infection was studied in infected and treated mice by observation of blood smear for the presence of malaria parasite stages under light microscope. Then the histopathological changes of the vital organs were observed by staining the sections with haematoxylin and eosin.* ***Results:*** *The histopathological changes were observed in liver, spleen, kidney, brain and bone marrow among the different experimental groups. Splenomegaly was observed in P. berghei infected group with the rise in infection. But in treated groups, the damage in the vital organs is recovered due to the antimalarial activity of the drugs.* ***Conclusion:*** *The present study established that there were no adverse effects on spleen, liver, kidney, brain and bone marrow in treated animals.*

***Key words:1.*** *Plasmodium berghei, 2.Histopathological changes, 3.Artemisinin-based combination drugs.*

## Introduction

Malaria, a disease caused by a parasitic protozoan belonging to the genus Plasmodium spp. is one of the common diseases in the tropical regions. Almost half of the world's population is at risk of the disease. About 200 million people are already infected with the disease (WHO, 2014). Malaria pathogenesis is dependent on the parasite growth, proliferation, and the potency of host immune responses (Reisinger *et al*., 2005). The malaria disease manifestations are mostly ague, sweating, and anemia and occasional cerebral symptoms, splenomegaly, and kidney problems may happen (Olsson and Johnston, 1969).

Animal models for studying malaria are useful because there are limitations on the nature and interpretation of *in vitro* studies, particularly with regard to the behavior of host immune cells and the use of animal models to understand the pathogenesis of disease. Moreover, these models have been used for pre-clinical testing of various drugs and vaccines (Sanni *et al*., 2002).

The four *Plasmodium* species that have been described in African murine rodents are *P. berghei, P. chabaudi, P. vinckei* and *P. yoelli. P. berghei* infects the liver after being injected into the blood stream by a bite of an infected female mosquito. Plasmodium species is used for the development of an effective vaccine against malaria, since it has the same effects on rodents as *P. falciparum*.

Multiplication of the parasite in the blood causes anemia and damage of essential organs of the host such as the lungs, liver and spleen. *P. berghei* infections may also affect the brain causing cerebral complications in laboratory mice with symptoms comparable to those of patients infected with *P. falciparum*. (Hall *et al*., 2005; Kooij *et al*., 2006).

Hence, the present study has been designated to evaluate the histological changes occurring in spleen, liver, kidney, brain and bone marrow of mice due to *Plasmodium berghei* infection and treatment with artemisinin-based combination drugs.

## Materials and methods

### Experimental animals:

A group of 30 male Swiss albino mice, weighing 25-30 g were divided into five groups each with 6 animals. Mice were obtained and housed in plastic cages with rice husk as beddings, provided with access to commercial pellet food and access to clean drinking water *ad libitum*. The animals were handled in accordance with the guidelines in the Guide for the care and use of laboratory animals (2011). Animal experiments were designated and approved with Ref. No. ANUCPS/IAEC/AH/Protocol/2/2014 by Institutional Animal Ethics Committee (IAEC) of ANU College of Pharmacy, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

### Parasite and Inoculation to experimental mice:

Chloroquine sensitive *P. berghei* ANKA strain parasites were maintained by intraperitoneal inoculation of $1\times10^7$ infected erythrocytes to naïve mice. A standard inoculum consisting of $1\times10^7$ parasitized erythrocytes was prepared from the infected donor mice with >25% parasitaemia, and used to infect experimental mice.

The infection of the recipient mice were initiated by needle passage of the parasite preparation from the donor to healthy test animals via the intraperitoneal route as described previously (David *et al.,* 2004). The day of inoculation was defined as Day 0 and subsequent days as Day 1, Day 2, and Day 3 up to Day 28.

### Drugs and Dosage Regimens:

In the present work, three Artemisinin-based combination drugs were used namely Atesunate + Amodiaquine (AS+AQ), Artesunate + Sulphadoxine Pyrimethamine (AS+SP), Artemether + Lumefantrine (AL). All the drug dosages were given according to the body weight of mouse by following standards of World Health Organization (WHO). These combination drugs manufactured from IPCA Laboratories Limited, Mumbai, India were procured for the present study.

- **Artesunate+Amodiaquine (ASAQ):** The WHO dosage regimen is Artesunate 4 mg/kg + Amodiaquine 10 mg/kg once a day for 3 days.
- **Artesunate + Sulphadoxine Pyrimethamine (ASSP):** The WHO dosage regimen is Artesunate 4 mg/kg once daily for 3 days and Sulphadoxine + Pyrimethamine as single dose of 25 mg/kg + 1.25 mg/kg on Day 1 administered orally.
- **Artemether+Lumefantrine (AL):** The WHO dosage regimen is Artemether 1.5 mg/kg and Lumefantrine 9 mg/kg at 0, 8, 24, 36, 48 and 60 hour. The same WHO regimen was followed and 6 doses were given on 3 consecutive days.

So in the present experiment, the same WHO recommended dosage regimens were followed and administered to the infected mice for 3 days by oral gavage according to the body weight.

**Animal Groups:**

The mice were divided into following 5 groups with 6 mice (n = 6) in each group:

1. Group 1 (Control uninfected): The mice were given only distilled water.
2. Group 2 (Infected Non-treated): The mice were infected with *P. berghei* antigen.
3. Group 3 (Infected + ASAQ): The mice were first infected with *P. berghei* antigen and then treated with Artesunate+Amodiaquine combination.
4. Group 4 (Infected +ASSP): The mice were first infected with *P. berghei* antigen and then treated with Artesunate + Sulphadoxine Pyrimethamine combination.
5. Group 5 (Infected + AL): The mice initially were parasitized with *P. berghei* and then treated with Artemether + Lumefantrine combination.

**Study of the course of infection in *Plasmodium berghei* infected mice:**

Thin blood films were prepared on clean slides, initially fixed with methanol. These blood smears were stained with Giemsa stain for 5-8 min. A minimum of 1000 RBCs were counted and among those, number of infected RBCs will be recorded. The percent of infected RBCs (parasitaemia) was determined by enumerating the number of infected RBCs in relation to the number of uninfected RBCs (Oyewole *et al.,* 2010) as follows.

Percentage (%) of Parasitaemia = $\frac{\text{No. of infected RBCs}}{\text{No. of RBCs counted}} \times 100$

**Histopathological studies:**

For histopathological studies; control mice, *P. berghei* infected mice and drug treated mice were sacrificed at the end of the experiment i.e., on 7$^{th}$ day of the experimental period. The mice were sacrificed under chloroform anaesthesia and the liver, spleen, kidney, brain and bone marrow were excised from the animals and preserved in 10% formaldehyde. These were dehydrated in with ethyl alcohol and embedded in molten paraffin wax. Paraffin embedded tissues were cut at a thickness of 5 µm, were fixed in Bouin's fixative and stained with Haematoxylin and eosin (H & E) (Baker, 1946). The sections were mounted in DPX and the photomicrographs of the relevant stained sections were taken with the aid of a light microscope (Nanji *et al*., 2001).

**Statistical analysis:**

MS Excel was used for the analysis of the data and for drawing graphs.

**Results**

**1. Course of infection to *P. berghei* in experimental mice:**

**(i)** ***P. berghei* Infected Non-treated group:** During the study of course of infection, *P. berghei* parasite was given to the experimental mice on Day 0. After inoculation the parasitaemia was first appeared on Day 3 (72 hours). Then the parasitaemia was gradually increased up to the peak level on Day 7. On Day 3, initial parasitaemia was 19%, on Day 4 with 23%, on Day 5 with 27%, on Day 6 with 32% and on Day 7 with 36% of parasitaemia. High rate of parasitaemia was observed on 7$^{th}$ day post inoculation after which all the mice died due to heavy infection by Day 8 (Figure 1).

**(ii)** ***P. berghei* Infected + ASAQ treated:** In this group, initial parasitaemia was 20% on Day 3. On Day 3, Day 4 and Day 5; the therapeutic dose of ASAQ combination drug was administered orally.

Then the parasitaemia was decreased to 8% on Day 4. On Day 5, the parasitaemia was 0% and so the parasite clearance occurred within 48 hours. No recrudescence was observed during the follow-up of 28 days. Hence, the survival rate was 100% and parasite clearance time (PCT) in ASAQ treated mice was 2 days (48 hours) (Figure 1).

**(iii)** ***P. berghei*** **Infected + ASSP treated:** In this group, the initial parasitaemia was 21% on Day 3. On Day 3, Day 4 and Day 5; the therapeutic dose of ASSP drug was administered orally. Then the parasitaemia decreased to 10% on Day 4. On Day 5, the parasitaemia was 2% and on Day 6 with 0%. No recrudescence was observed during follow-up of 28 days. Hence, the survival rate was 100% and parasite clearance time of (PCT) in ASSP treated mice was 3 days (72 hours) (Figure 1).

**(iv)** ***P. berghei*** **Infected + AL treated:** In this group, the initial parasitaemia was 19% on Day 3. Then the mice were treated with AL combination drug for 3 consecutive days orally on Day 3, Day 4 and Day 5. On Day 4 the parasitaemia was 11%, on Day 5 parasitaemia decreased to 3% and on Day 6 no parasitaemia was observed. Also no recrudescence was observed during the follow-up of 28 days. Hence, the survival rate was 100% and parasite clearance time (PCT) in AL treated mice was 3 days (72 hours) (Figure 1).

**1. Histopathological changes in experimental mice**

Tissues of spleen, liver, kidney, brain and bone marrow from control and experimental mice were fixed in Bouin's solution and stained with for haematoxylin and eosin for histopathological studies.

**(i) Histopathology of Spleen:** And *P. berghei* infected spleen section shows marked congestion of red pulp. Whereas ASSQ treated section studies are normal. ASSP treated section shows foci of extramedullary haematopoiesis. AL treated section, shows congestion of red pulp and prominent extramedullary haematopoiesis (Plate 1).

**(ii) Histopathology of Liver:** *Plasmodium berghei* infected liver section studies show normal liver parenchymal architecture and mild sinusoidal as well as hepatic vein congestion. But ASAQ treated section shows normal parenchymal architecture. sinusoidal congestion. AE treated, shows normal liver architecture. ASSP treated section show normal parenchymal architecture and sinusoidal congestion. And AL treated section show normal parenchymal architecture with marked sinusoidal congestion (Plate 2).

**(iii) Histopathology of Kidney:** *Plasmodium berghei* infected kidney sections are normal with mild interstitial congestion. AS treated section studies are normal. But ASAQ treated section are normal. ASSP treated section studies are normal except for mild interstitial congestion. And AL treated section studies show interstitial congestion (Plate 3).

**(iv) Histopathology of Brain:** *Plasmodium berghei* infected section studies show mild congestion. Whereas ASAQ treated and ASSP treated section studies are normal. But AL treated section show congestion (Plate 4).

**(v) Histopathology of Bone marrow:** *Plasmodium berghei* infected section studies show skeletal muscle, bony trabeculae with marrow spaces showing normocellular marrow, normal erythropoiesis, myelopoiesis and megakaryopoiesis. But ASAQ treated section show normocellular marrow with normal erythropoiesis, myelopoiesis and megakaryopoiesis. ASSP treated section show normocellular marrow with normal erythropoiesis, myelopoiesis and megakaropoiesis. Whereas AL treated section show occasional marrow space showing normocellular marrow, normal erythropoiesis, myelopoiesis and megakaryopoiesis (Plate 5).

**Discussion**

Thus the histopathological disturbances are more observed during *P. berghei* infection in different organs of experimental mice. But these changes and disturbances are rectified after artemisinin-based combination therapy with ASASQ, ASSP and AL. Among these ASAQ effectively eliminated the parasite and restored the histopathological changes when compared to ASSP and AL. More histopathological disturbance was observed with AL in spite of clearance of parasite because of growing resistance of antimalarials. Hence much attention is needed to formulate new Artemisinin-based combination drugs.

The spleen is complex, secondary important lymphoid organ that is perfectly modified to selectively filtering and destroying senescent red blood cells (RBCs), infectious microorganisms and Plasmodium - parasitized RBCs. Artemisinin-based combination drugs used might have caused changes in spleen and liver size and color by reducing or treating spleen and liver damage. The spleen plays important role in human immunity, especially in the process of phagocytosis of the parasite of the malaria parasites.(Chotivanich *et al*., 2002; Engwerda *et al*., 2010).

In our study, the spleen section shows congestion of red pulp during *P. berghei* infection at peak level. But in normal spleen red and white pulp areas are clearly seen. When treated with ACTs, better histological restoration was observed. In ASAQ treated mice, more restoration was observed when compared to ASSP and AL treated mice. In ASSP and AL treated mice, extramedullary haematopoiesis was observed.

Other studied reported that spleen of post infection mice with *P. berghei* (NK-65), exhibited disturbed splenic architecture, enlarged white pulp area, infected cells, sinusoidal dilation, haemozoin deposition and transient loss of marginal zone due to *P. berghei* infection (Kumar and Bagai, 2014). Expansion of white pulp, marginal zone and red pulp were the major changes that occur in the spleen during murine malaria infection which resulted in haematopoiesis and similar observations were reported by Helmby *et al*. (2000), Stevenson and Krall (1989), Alves *et al.* (1996) and Freeman and Parish (1978). Accumulation of haemozoin pigment has in spleen is due to the protein degradation by parasite (Achtman *et al*., 2003). The present finding also indicates accumulation of haemozoin and infiltration of red blood cells in sinusoidal spaces in infected spleen.

The photomicrographs of the present study revealed that the histoarchitecture of the liver is normal with radially channeled sinusoids towards the central vein was observed in control mice. There were few Kupffer cells within the sinusoids and endothelium of the central vein was clearly distinctive from the membranes of the hepatocytes surrounding it. There was also no endemic inflammation of hepatocytes. The hepatocytes were normal, and were positioned wall-to-wall and membrane with one another with sinusoids between them. Hepatic dysfunction in severe malaria infection was due to failure of bilirubin excretion from heavy parasitaemia, ischemia and acidosis (Bhalla *et al.*, 2006).

In *P. berghei* infected mice at peak level of infection, liver section shows hyperplastic Kupffer cells were observed in our study in accordance with Whitten et al. (2011). Portal tract inflammation was revealed by the presence of inflammatory lymphocytes surrounding the portal triad, as previously reported by Chawla *et al*. (1989). Our study has shown derangement in the radial channeling of the sinusoids towards the portal which corroborates the study of Rupani and Amarapurkar (2009). This was evident in *P. berghei* infected mice, where disturbance in the pattern of sinusoid arrangement and hyperplastic Kupffer cells were noticed. In our study, when these infected were treated with Artemisinin-based combination drugs, there appeared histological restoration in experimental mice. We observed no congestion of sinusoids and portal vein, no derangement of sinusoids, hyperplasia of Kupffer cells and necrosis in ASAQ and ASSP treated mice except for the congestion of sinusoids in liver of AL treated mice.

Haemozoin is a byproduct formed from the digestion of erythrocytes by malaria parasites, and it is observed in either cytoplasm or outside hepatocyte and Kupffer cells as black or brownish granules. On the hand , haemosiderin is a granular brown substance composed of ferric oxide ($Fe_2O_3$) left from the breakdown of haemoglobin. It is usually observed in the cytoplasm of Kupffer cells. Haemosiderosis is a form of iron overload disorder resulting in the accumulation of haemosiderin. Both haemosiderosis and haemozoin were observed in the liver, but haemozoin was more in the spleen (Soniran *et al*., 2012). Soniran *et al*. studied the effects of Chloroquine and Artesunate on histopathological damages caused by *P. berghei* in liver of infected albino mice. The histopathological examination revealed the absence of accumulation of iron (haemosiderosis) in the liver of artesunate treated group and absence of megakaryoblast hyperplasia in spleen of chloroquine treated group which is in correlation with our findings.

Similar study was made by Olayode *et al.* (2015) who studied the histomorphological disturbance in liver of *P. berghei* infected albino mice. Whereas treated mice with leaf extract of *Mangifera indica* (Linn.) a dose-dependent ameliorative changes in the organization of histoarchitecture such as reduction of collagen fibers, reticular fibers and haemozoins. Vafaei *et al.* (2018) studied the histopathological changes in liver, spleen and kidney of *P. berghei* infected mice after treatment with sulfadoxine-pyrimethamine, pyrimethamine, and hydroxychloroquine sulfate. The highest rate of histopathological disturbances was observed in all these organs during *P. beghei* infection which is in accordance with our study. But except for the spleen, hydoxychloroquine sulfate and sulfadoxine pyrimethamine performance was good as compared to Pyrimethamine. Udonkang *et al*. (2018) reported the liver histopathological changes due to the effect of *Artocarpus altilis* in *P. berghei* infected mice. The *Artocarpus atilis* has shown antimalarial activity on only the erythrocytes in the peripheral blood circulation but not on the liver merozoites. Thus mild hepatic changes such as mild sinusoidal dilatation, hypochromatic staining of hepatocytes nuclei and cytoplasm suggesting hepatic necrosis, and sinusoidal congestion with Plasmodium merozoites similar to the *P. berghei* infected mice were observed which are in correlation with our study.

In the present study, histopathological study of normal kidney of mice revealed the presence of outer cortex and inner medulla with Bowman's capsule, glomeruli and renal tubules. But in *P. berghei* infected mice, necrotic changes in epithelium of renal capsule and renal tubules and proliferation of mesangial cells between loops, and shrinkage of renal corpuscles was evident. Similarly proliferative changes in the glomeruli have been reported in *P. falciparum* in humans (Berger *et al*., 1967). Similar changes were observed in the study of Kalia *et al.* (2015) during *P. berghei* infection in mice which correlate with our findings. However in our study, after treatment with ASAQ and ASSP, renal morphology was restored but mild interstitial congestion was observed in AL treated mice. Thus our study confirms the report of Kalia *et al.* (2015) that treatment with chloroquine caused slight changes in renal morphology and haemozoin deposition and glomerular infiltration in kidney were observed after treatment with ethanolic bark extract of *Albizia lebbeck*.

Soniran *et al*. (2012) studied the effects of Chloroquine and Artesunate on histopathological damages caused by *P. berghei* in kidney of infected albino mice. He observed marked damage of the nephron in the kidney (tubular nephrosis) in infected mice which is in accordance with our study. But chloroquine and artesunate treatment totally prevented the infiltration of perivascular interstitial mononuclear cells which are located between tubules of the nephron (tubular nephrosis) by the malaria parasites in the kidney of treated mice which is in agreement with our findings where artemisinin-based combination therapy restored the kidney morphology.

On the other hand, Sibiya *et al.* (2017) reported the injured glomeruli in kidney during *P. berghei* infection. But after chloroquine treatment, they observed thickened glomerular basement membrane and Bowman's capsule in treated mice. But these features were attenuated when mice were treated with *Syzygium aromaticum* derived Oleic acid.

The present study revealed normal brain architecture of control mice. But in *P. berghei* infected mice, the brain section study has shown congestion at peak level of infection. After ASAQ, ASSP and AL treatment in experimental mice, the brain section studies were normal without any considerable changes.

Onyesom *et al*. (2015) reported histopathological changes in brain due to *P. berghei* infection in experimental mice. The brain section has shown mild loosening of brain tissues together with mild vascular congestion and clogging of vessels with the parasite which is in accordance with our study. However treatment with *Nauclea latifolia* restored the disturbances in brain tissue except for negligible tissue loosening. So also in our study, the artemisinin-based combination therapy restored the brain architecture.

Junaid *et al*. (2017) studied the pathogenesis of *Plasmodium berghei* ANKA infection in gerbils (*Meriones unguiculatus*). Their data (haematological and cytokines assessment) do not support the conclusion that gerbils die as a result of cerebral malaria (CM). This is due to the fact that none of the gerbils showed neurological symptoms which induce ataxia, convulsion and deviation of the head. This study support our finding that *P. berghei* infected mice died on 8$^{th}$ day not because of cerebral malaria. A report by Amani *et al*. (1998) had shown that the genetic background of the mouse affects the disease outcome of *P. berghei* ANKA infections of , but also that the cloned lines of *P. berghei* ANKA differ in their ability to induce experimental cerebral malaria.

Lou *et al*. (2001) also studied the pathogenesis of cerebral of malaria due to different malaria parasites in different mouse models. The histopathological of experimental cerebral malaria (ECM) varies according to parasite-host combinations. The differential pathological changes in animal models were found to be related to different malaria parasites. For example CBA mice clearly exhibit a brain vascular pathology when infected with *P. berghei* ANKA (Grau *et al*., 1987). *P. vinckei* causes other features of severe falciparum malaria. *P. yoelli* 17XL- infected Swiss albino mice show a significant sequestration of pRBC (Yoelli *et al*., 1975). The earlier reports have shown that *P. berghei* ANKA infected CBA mice develop a fatal cerebral malaria (Neil and Hunt, 1992; Rest, 1983) whereas DBA/2 mice develop a nonfatal cerebral syndrome (Neil et al., 1993). And BALB/c mice do not develop any cerebral pathology (Grau *et al*., 1987) which supports our study that albino mice of BALB/c strain infected with *P. berghei* ANKA were not affected with cerebral malaria.

Strangward *et al*. (2017) studied the experimental cerebral malaria pathology of *P. berghei* ANKA and *P. berghei* NK65 in C57BL/6 mice. They reported significantly higher accumulation of pRBCs, intracapillary trophozoites and schizonts in brain smears in all assessed brain regions during *P. berghei* ANKA infection compared with *P. berghei* NK65. And C57BL/6 mice infected with *P. berghei* ANKA typically developed signs of late stage experimental cerebral malaria (ECM); including ataxia, convulsions, paralysis and/or coma, hyperparasitemia on day 7. But these symptoms were not observed in our study, may be because *P. berghei* ANKA has not caused cerebral malaria in the experimental BALB/c mice (Grau *et al*., 1987) but developed peak parasitaemia on 7$^{th}$ day (p.i) during our study.

In the present study, the bone marrow section of *P. berghei* infected mice has shown histopathological disturbances when compared to the normal mice. In *P. berghei* infected mice, a dramatic decreases in bone marrow cellularity and erythroblasts was observed at peak level of infection. But when these mice were treated with artemisinin-based combination therapy the bone marrow histological architecture was restored.

Pathak and Ghosh (2016) made a review study on bone marrow pathology due to malaria in patients, mouse models and *in vitro* cultures reporting erythropoietic changes. Their study reported decreased production of erythroid cells which was almost always found to be associated with dyserythropoiesis, that is the production of morphologically defective cells which in functional terms

results in ineffective erythropoiesis (Philips and Paswol, 1992). In many studies, it was observed that bone marrow inhibition was found to be correlated with the degree of parasitemia and could be reversed after clearance of parasites from blood (Srichaikul *et al*., 1976). This phenomena is supporting our findings that during peak of *P. berghei* infection at 7$^{th}$ day, the cellular component of hematopoietic area was decreased and replaced with adipose or fat cells.

A review study was conducted by De Niz *et al*. (2018) who reported *P. berghei* ANKA gametocytes homing and vascular transmigration in the bone marrow of different mouse models. During the acute phase of infection, they observed vascular leakage resulting in further parasite accumulation in this environment. Mature gametocytes showed high deformability and were found entering and exiting the intact vascular barrier and suggested that this is essential for gameto-cytogenesis and transmission of Plasmodium to the mosquito. Another study in humanized mice demonstrated some *Plasmodium falciparum* gametocyte enrichment in bone marrow of humanized mouse model (Duffer *et al*., 2016). But in our study we observed no such changes in bone marrow except for the decrease in the cellular component of erythropoietic area.

Haltalli *et al.* (2018) studied malaria-induced remodelling of the bone marrow microenvironment mediates loss of haematopoietic stem cell (HSC) function. They revealed that during *P. berghei* infection in C57BL/6 mice, the HSC component turns over significantly faster than in steady state, and that a global interferon response and loss of functional HSCs are linked to changes in bone marrow endothelium function and osteoblasts number. This supports our study that during *P. berghei* infection decrease in haematopietic area in bone marrow was observed. But after treatment with ASAQ and ASSP, more recovery of the haematopietic cellular area was observed than AL treatment.

It was reported that both Plasmodium infection and sepsis affect bone biology (Lee *et al*., 2017) and increasing attention is given to a potential role of the bone marrow microenvironment in maintaining a reservoir of Plasmodium parasites in human malaria (Joice *et al*., 2014; De Niz *et al*., 2018). In support of this a recent study by Rastogi and Rehman (2018) studied the changes in bone marrow during *P. vivax* malaria they observed presence of trophozoites in bone marrow showing normoblastic and megaloblastic hyperplasia, presence of haemophagocytosis and increase in plasma cells in bone marrow aspiration studies of 47 cases.

Previous studies have shown that invasion of *P. berghei* ANKA infected red blood cells (iRBCs) can be found in organs such as the spleen, liver, kidneys, lungs and adipose tissues in different mouse models (Amante *et al*., 2007; Franke-Fayard *et al*., 2005; Martins *et al.*, 2009; Neill and Hunt, 1992). These reports are correlating with the present findings with the Swiss albino mice where *P. berghei* ANKA infection caused histopathological changes in different organs such as spleen, liver, kidney, brain and bone marrow.

## Conclusion

Considering the higher efficacy rates of Artesunate+Amodiaquine (ASAQ) when compared with Artesunate+Sulfadoxine Pyrimethamine (ASSP) and Artemether+Lumefantrine (AL), we conclude that ASAQ is clinically more effective than ASSP and AE. No adverse effects were observed on histological organs when animals were treated with ASAQ, ASSP and AL respectively. We observed rapid parasite clearance and restoration of histological architecture when mice were treated with artemisinin-based combination therapy.

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