Parasitemia and Antimalarial-Induced Histological Alterations and Oxidative stress in Infected Mice

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Abstract

In lieu of the reports on re-emergence of chloroquine (CQ) sensitive *Plasmodium falciparum*, the choice of the drug for the treatment of malaria especially in the endemic areas may not be ruled out. Here, we reported the anti-oxidative status and histology of some vital organs in mice infected with *P. berghei* and subsequently treated with CQ. The study was a 4 by 10 model design as follows: the control non-infected, the infected (IN) and CQ -treated infected (CqTI)/non-infected (CqTNI) mice.

The challenged animals were subjected to five days treatment after parasitemia was established using Gemsa stain. Assays were conducted on the animals following standard procedures. The results showed that parasitemia and CQ induced oxidative stress in (IN) and (CqTNI) groups. This was indicated by significant ($p<0.05$) changes in the antioxidant defence indices viz superoxide dismutase (SOD), reduced glutathione (GSH), glutathione-S-transferase (GST), catalase (CAT), malondialdehyde (MDA) and protein concentration. There were also alterations in the architectures of some vital organs such as liver, kidney and heart of the experimental groups compared with control. The subsequent treatment of the infected group with CQ (CqTI) restored some of the indices altered during the infection to a normal level. This study shows that malaria and its radical treatment *in vivo* can induce oxidative stress which in turn can cause injury to the host tissues particularly during chronic administration of CQ -an implication for prolong intake of the drug.

KEYWORDS: *Plasmodium berghei*, antimalaria, oxidative stress, organs, mice

1. Introduction

Plasmodium berghei bears similar characteristics with *P. falciparum*, a virulent human malaria parasite transmitted by *Anopheles* mosquitoes (Sherman, 2008). The parasite infects the liver after its injection into the bloodstream by a bite of an infected female *Anopheles* mosquito. After a short period (a few days) of development and multiplication (exoerythrocytic-stage infection), these parasites leave the liver and invade erythrocytes (erythrocytic-stage infection). This latter stage causes more liver apoptosis and hepatic pathological changes leading to liver dysfunction and other systemic complications (Kochar *et al*. 2003; Sand *et al*., 2005). Some of the

pathological condition and systemic complications include anaemia and damage of essential organs of the host such as lungs, liver, spleen (Janse *et al*., 2006).

The World Health Organization has advocated the use of antimalarial drugs for chemoprophylaxis, prevention and treatment of uncomplicated malaria (WHO, 1997). Chloroquine (CQ) was the first antimalarial to be widely used in endemic areas before the emergence of resistant Plasmodium species to the drug (James *et al*., 2003; Kublin *et al*., 2003; Umar *et al*., 2008). However, CQ still remains a drug of choice for treatment of malaria in the endemic areas despite the reported plasmodia resistance. For instance, CQ still remains the first-line drug to treat P. vivax malaria in Ethiopia and sub-Saharan Africa (Mekonnen *et al*., 2014). Previous literatures had described the impact of the concentration of CQ in different organs of the body following its administration during the episode of disease (Kublin *et al*., 2003; Desai *et al*., 2010). Reports have also implicated chloroquine in the disruption of a wide range of biochemical processes which include the inhibition of major metabolic enzymes like alcohol dehydrogenase, succinate dehydrogenase and glucose 6-phosphate dehydrogenase. Effect of chloroquine on antioxidant enzymes as well as its inhibitory tendency on cytochrome P-450-meditaed mixed function oxidase activities both *in vivo* and *in vitro* have been demonstrated (Thabrew & Ioannides, 1984; Magwere *et al*., 1997).

Infectious diseases such as malaria are known to activate the body phagocytes causing release of reactive oxygen species (ROS). Oxidative stress occurs when the generation of ROS in a system, exceeds the system's capability to neutralize and terminate their activity. The imbalance can result from a lack of antioxidant capacity caused by disturbance in production, distribution, or by an over-abundance of ROS from an environmental or behavioral stressor. If not properly regulated, the excess ROS can damage a cell's lipids, protein or DNA, inhibiting normal function while a more severe oxidative stress can cause cell death (Jodah *et al*., 2008).

All cells have endogenous antioxidants (such as superoxide dismutase and glutathione) which are very important for protection against oxidative stress at all times. Glutathione (GSH) is very important as an intracellular antioxidant; GSH has been found to be low in many disease states indicating oxidative stress and inadequate antioxidant activity to "keep up" with the free radicals. This study aims at investigating the influence of *Plasmodium* infection and CQ individually and synergistically in the treatment regime on the histopathological and oxidative stress profile in mice.

2. Materials and methods

2.1. Experimental animals

Eight-week-old male albino mice bred at the animal house unit of College of Medicine, University of Lagos, Nigeria were used as experimental animals. The mice were divided into four groups of ten mice each i.e. the control non-infected, the infected (IN), and CQ-treated infected (CqTI)/non-infected (CqTNI) mice.

The animals were kept in a protected and ventilated animal house, fed *ad libitum* with commercial pellet (from Ladokun Feeds Ltd. Ibadan, Oyo State, Nigeria) and water.

2.2. Parasites

Chloroquine sensitive (NK 65) strain of *P. berghei* was obtained from the Nigerian Institute for Medical Research (NIMR) Yaba, Lagos, Nigeria. Standard inoculums of $1x10^7$ of parasitized erythrocytes obtained from the infected mouse in volume of 0.1ml was administered intraperitoneally to each experimental animal.

2.3. Drug administration and Parasitemia Test

The CqTI and CqTNI groups of mice were administered with daily oral doses of chloroquine phosphate (Sigma, USA) 25 mg/kg body weight for 3 days; 10 mg/kg on days 1&2 then 5 mg/kg on day 3 after the establishment of *P. berghei* infection. This was confirmed using thin films of blood stained with Giemsa to ascertain parasitemia levels (Aina et al., 2006).

2.4. Tissue preparation

Kidney, liver and heart tissues were obtained per animal in each group for routine histological procedures. The tissues were sectioned and stained to obtain permanent digital micrographs of the desired sections. The tissues were also prepared for the following assays as described by Iyawe & Onigbinde (2009).

2.5. Assays

Lipid peroxidation was determined followed the method described by Gutteridge & Wilkins (1982). Plasma protein estimation was determined by means of Biuret method as described by Gornall *et al*. (1949) with some modifications. The levels of SOD activity in plasma was determined by the method of Misra & Fridovich (1972). Sinha (1971) method was used to determine catalase activity. Glutathione *S*-transferase activity was determined by the method of Habig *et al.*, (1974). The total sulphydryl groups, protein bound sulphydryl groups, free sulphydryl groups (such as reduced glutathione) in biological samples was determined using Ellman's reagent (DTNB) as described by Sedlak & Lindsay (1968), and Jollow *et al.* (1974).

2.6. Statistical analysis

Statistical analysis was carried out using SPSS 15.0 for windows; SPSS Inc., Chicago, USA. The difference between mean was determined using One Way Analysis of Variance (ANOVA). Data were reported as Mean $\pm$ Standard deviation and $P < 0.05$ was considered significant. Least Significant Difference (LSD) was used to determined differences in means at 95% confidence interval as post hoc analytical method.

3. Results

3.1. Antioxidative status

In CQ-treated infected (CqTI) mice, there was significant ($F_{3, 20}=4.279$, $P<0.05$) decrease in SOD activity compared to the control and other experimental groups (Fig 1). The activity of the catalase was increased in all the groups except in the infected (IN) mice where it was significantly ($F_{3, 20}=5.340$, $P<0.05$) low (Table1). Glutathione transferase in all the experimental

groups was maintained at relatively the same level although low compared to the control but no remarkable significant ($F_{3, 24}$=1.241, P>0.05) difference. There was also no significant ($F_{3, 24}$=1.241, P>0.05) difference in GSH level among all the groups but the level was high in the infected (IN) mice (Table1). Malondialdehyde **(**MDA) level in control group was low compared to the experimental groups and was significantly ($F_{3, 21}$=5.967, P<0.05) increased in infected (IN) mice (Fig.2). The erythrocyte protein concentration level was significantly ($F_{3, 26}$=21.284, P<0.05) high in CQ-treated non-infected (CqTNI) mice compared to the other experimental groups (Fig.3). However, the difference was not significantly (P>0.05) different from the control.

3.2. Histological observations

Histological examinations of the stained sections of the liver, kidney and heart showed marked hepatocytes necrosis in the liver ((Fig. 4d), mild vascular congestion in the kidney (Fig. 4e) and marked congestion in the heart (Fig. 4f) of the infected (IN) mice. In CQ-treated non-infected (CqTNI) mice, the liver showed binucleate hepatocytes with eosinophilic cytoplasm, severe vascular congestion and marked periportal infiltrate with focal hepatocyte necrosis (Fig.4g) while the kidney and heart indicated moderate vascular congestion with no trace of abnormalities (Fig 4h & i). CQ-treated infected (CqTI) mice showed normal hepatocytes in the liver (Fig 4j), mild congestion and normal glomerulus & tubules in the kidney (Fig 4k) and mild congestion and normal myocytes in the heart (Fig 4 l). The tissue structures in the later group were similar to that in non-infected control whereby sections of organs (liver, kidney and heart) all appeared normal (Fig 4a, b, c).

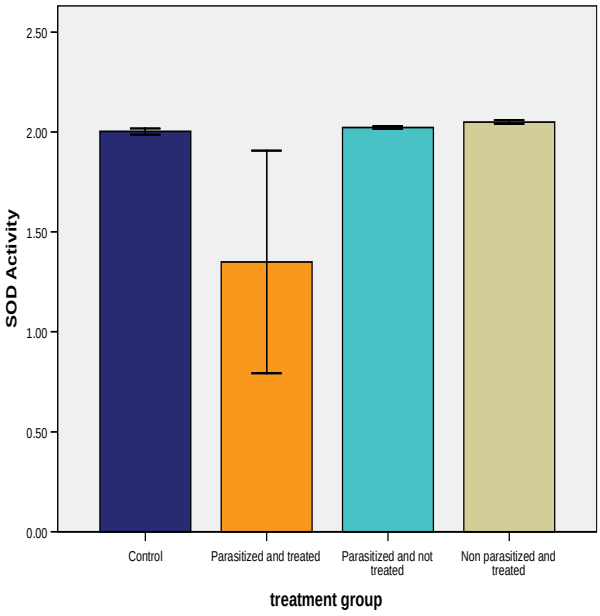


Fig. 1: Superoxide Dismutase activity in control and experimental groups: CqTI, IN and CqTNI.

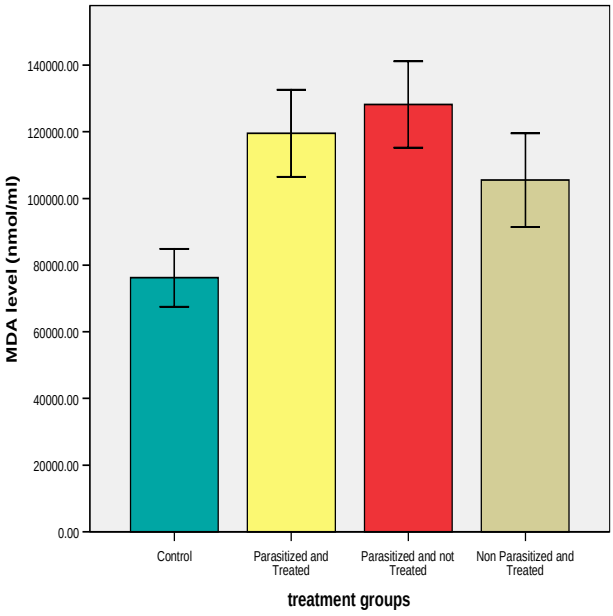


Fig. 2: MDA levels in control and experimental groups: CqTI, IN and CqTNI.

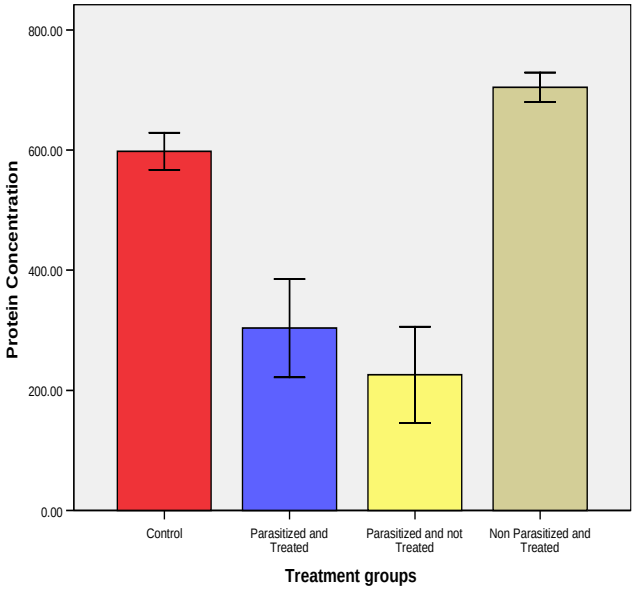


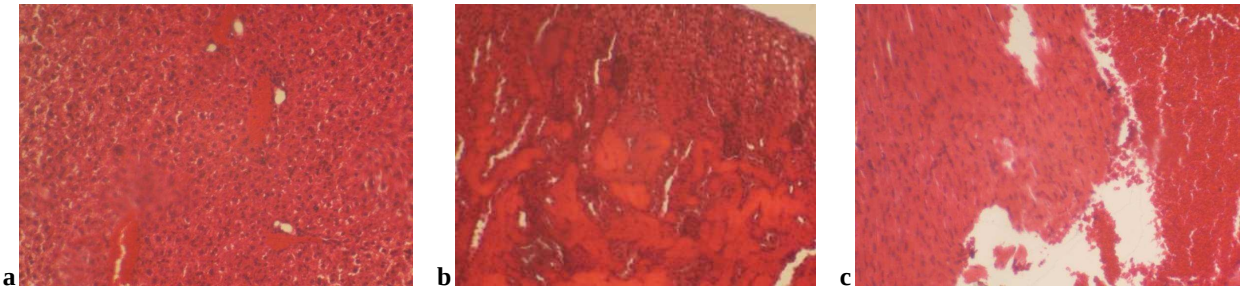
Figure 3: Protein concentration in control and experimental groups: CqTI, IN and CqTNI

(Values were derived from unit/mg protein and expressed as Mean ±S.D)

Table 1: The activities of GSH, Catalase and GST in Control and experimental groups

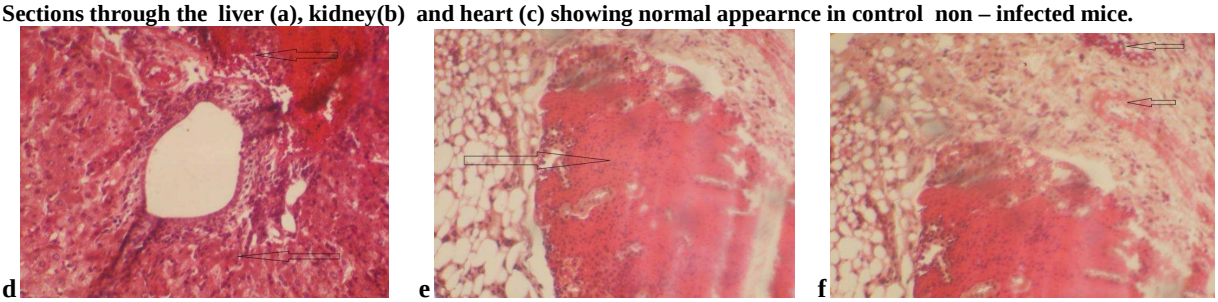
Parameter	**Control**	**CqTI**	**IN**	**CqTNI**
GSH(ug/mg)	19.62± 0.23	19.57± 0.24	19.63 ± 0.16	19.53± 0.18
Catalase (U/L	4.78±3.15	5.37±2.28	1.14±7.71 *	4.47±4.24
GST(ug/mg)	4.45±1.32*	3.13±2.37	3.23±2.42	3.56±1.27

Values were expressed as Mean ±S.D., CqTI, IN and CqTNI*; n=10 in each group.*Significantly different from other groups at <p0.05*



a b c

Sections through the liver (a), kidney(b) and heart (c) showing normal appearnce in control non – infected mice.

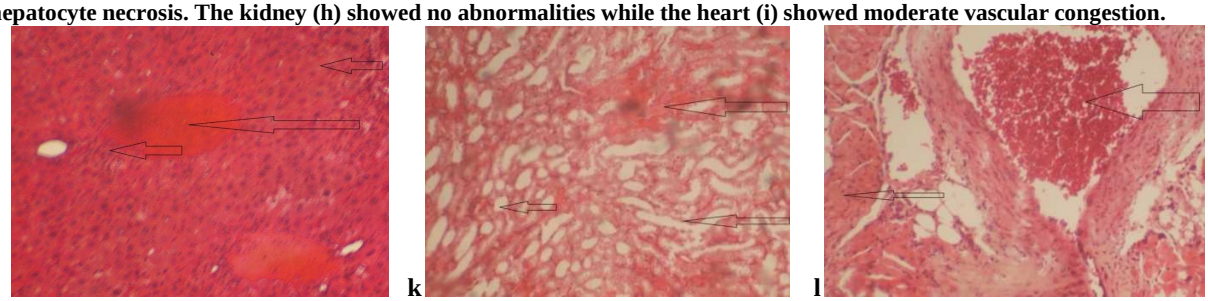


d e f

Sections through the liver (d), kidney(e) and heart (f) in infected (IN) mice. The arrow (up, right) in (d)indicates interlobular neutrophilic infilterate and marked vascular congestion. The arrow (down,right) indicates marked hepatocytes necrosis (interlobular) and piece meal necrosis. In (e), the arrow (left) indicates mild vascular congestion. In (f), the arrows (right) indicates marked congestion.

g h i

Sections through the liver (g), kidney(h) and heart (i) in chloroquine-treated non-infected (CqTNI) mice. The arrow (up, right) in (g) indicates binucleate hepatocytes with granular eosinophilic, arrow(middle) indicates severe vascular congestion in cytoplasm and arrow (down) indicates marked periportal and interlobular neutrophilic infilterate with focal hepatocyte necrosis. The kidney (h) showed no abnormalities while the heart (i) showed moderate vascular congestion.



j k l

Section through the chloroquine-treated infected (CqTI) mice, liver (j) with normal hepatocyte tract and central vein. Kidney(k) with arrows(up, right) and (down, left and right) showing mild congestion respectively with normal glomerulus & tubules.The heart(l), arrow (up,right) showed mildportal congestion and arrow (down, left) showed normal myocytes.

Fig 4: Sections of stained organs (liver, kidney and heart) prepared from control mice (a, b & c), *P. berghei* infected mice (d, e & f), CQ **–treated non-infected (g,h &i) and treated infected mice (j, k & l),**

4. Discussion

Alteration in certain conditions and chemical application can lead to excessive production of reactive oxygen species (ROS) (Farombi *et al*., 2003). These ROS are important mediators of tissue injury either during the infection or the application of the drug. The sensitivity of malaria parasites to oxidative damage has been proved by the efficacy of some antimalarial drugs that are known to act via generation of ROS. In the present study, malaria infection causes a significant increase in antioxidant defence indices viz., superoxide dismutase (SOD), reduced glutathione (GSH), Glutathione *S*-transferase (GST) and plasma lipid peroxidation (MDA) while catalase showed a significant decrease. The increase in SOD activity here is attributed to parasitemia and it could be due to mobilization of immune system in infection state whereby free radicals were generated against xenobiotics as been asserted in the earlier report (Iyawe & Onigbinde, 2012). Previous investigation has also reported decrease in catalase activity particularly in *Plasmodium berghei* and *P. vivax* infection (Erel *et al*., 1997; Seth *et al*., 1985). ROS such as superoxide anion has been shown to inhibit catalase by converting it to ferroxy and ferryl states, the inactive forms of enzyme (Areekul *et al*., 1986). This situation will lead to the accumulation of H_2O_2 and increase the burden of H_2O_2-induced oxidative stress (Aniya & Naito, 1983) in the *Plasmodium* infected erythrocytes. However, increased GSH will eliminate hydrogen peroxide and this may reduce the burden of H_2O_2-induced oxidative stress in the infected erythrocytes. Although, previous report has demonstrated the potential of glutathione peroxide to eliminates H_2O_2 at low concentration (Casado *et al*., 1995). Similar reports of raised GST levels have been documented (Farombi & Emerole, 1998) and this could be due to its induction in an attempt to counter the effect of increased oxidative stress (Status of lipid peroxidation, glutathione, ascorbic acid, vitamin E and antioxidant enzymes in patients with osteoarthritis (Surapaneni & Venkataramana, 2007). During the schistosome parasite infection, GSTPi was reported as one of the proteins upregulated (Harvie *et al*., 2007). Also, GSTPi mRNA has been shown to be upregulated in blood of *P. vivax*-infected subjects as compared to healthy ones (Sohail *et al*., 2010). Earlier investigation showed MDA level as an index for lipid peroxidation and this increased during *P. berghei* infection in mice (Siddiqi *et al*., 1999). The elevation in lipid peroxidation in the plasma of infected mice may point to lipid peroxide level as a reflection of the severity of a disease progrss (Das *et al*., 1993).

Following administration of CQ to un-infected mice, we noticed increase in all the parameters considered in this study except reduced GSH. The increased SOD levels could be due to the response to an increased production of ROS as a result of exposure to chloroquine or its metabolites (Farombi *et al*., 2003). The increase in the activity of SOD supports the earlier report which shows the effect of CQ on experimental animals (Magwere *et al*., 1997). In other investigation, amodiaquine, another 4-aminoquinoline was reported to have similar effect on rats (Farombi, 2000). The increased activity of GST may have resulted in response of GST to detoxify the CQ (Glatt *et al*., 1983, Anyasor *et al*., 2012). High level of MDA **(**Malondialdehyde) might be due to the formation of hydroxyl radicals that perturbed the membrane to increase the formation of malondialdehyde as reported by Iyawe & Onigbinde (2009). The low level of GSH may arise from CQ reaction with other molecules making it to inhibit these antioxidants with consequence reduction in their activities followed by the utilization of cellular reduced glutathione. Reduced Glutathione (GSH) serves as a sulfhydryl

buffer which protects the –SH groups of proteins from the damaging effects of reactive oxygen species (Akkus, 1995; Kidd, 1997).

Treatment of infected mice with CQ resulted in decrease of parasitemia and restoration of all oxidative stress and antioxidant defence indices to normal levels. Decreased SOD activity may indicates less production of H_2O_2 while increased catalase activity my mean low level of ROS such as superoxide anion generated since this could not inhibit catalase. However, catalase could have catalyzed available hydrogen peroxide to molecule oxygen and water thereby mopping up the non radical hydrogen peroxides (Mishra *et al*., 1994, Ueda *et al*., 2005). This situation will lead to decrease in the burden of H_2O_2-induced oxidative stress. Previous report has also demonstrated the potential of GPX to eliminate H_2O_2 at low concentration (Casado *et al*., 1995). Increased MDA **(**Malondialdehyde) as reported here would trigger the induction of endogeneous antioxidant defense system to scavenge reactive species (Pham- Huy *et al*., 2008). Decrease in the level of GSH was observed; its alteration under conditions of oxidative stress (Stocker *et al*., 1986) in malaria infected subjects treated with CQ has been reported. Such decrease has been linked with an attempt to increase defense against oxidant damage.

Plasmodia infection caused marked congestion, marked hepatocytes necrosis and mild vascular congestion in the heart, liver and kidney respectively. The presence of the parasite might have induced the cells of these organs causing the observed damages. Heavy parasitemia have been implicated in the occurrence of tubulointerstitial damages as well as glomerulonephritis and renal failure in the kidney of the infected patients (Mahakur *et al*., 1983; Rajapurkar, 1994; Saroj & Bhabani, 2008). In CQ-treated non-infected (CqTNI), liver showed binucleate with granular eosinophilic cytoplasm, severe vascular congestion and hepatocyte necrosis. Previous studies showed lisosomotrophic properties of CQ where relatively loose arrangement of hepatocytes was observed in the liver (Patel *et al*., 2005). This was attributed to the activation of lysosome enzymes leading to cell damage and /or necrosis (Maniam *et al*., 2012). The CQ -treated infected (CqTI) mice showed normal conditions in all the organs tested with mild congestions in the heart and kidney In other studies, liver histology was found to be restored to normal after administration of CQ for four days following the infection (Maniam *et al*., 2012). Here, the ability of the parasite to digest the haemoglobin was interrupted by CQ (Sharrock *et al*., 2008). Hence, the free haem monomers produced might have caused oxidative damage to *Plasmodium* parasite (Patel *et al*., 2005).

5. Conclusion

This study implicates the dual action of malaria parasite and antimalaria in altering antioxidant status *in vivo* leading to oxidative damage. The resulting injuries contribute to pathophysiological effects of the disease and administration of the antimalaria to un-infected animals. The administration of antimalaria to infected animals restored the oxidative stress and pathological conditions of the affected organs. However, caution should be taken in prolong administration of the drug.

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