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Biochemical and Histological Studies on the effects of Lonart Ds on *Plasmodium berghei* Infected Mice.

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ABSTRACT

Biochemical and histological studies on the effects of Lonart Ds on *Plasmodium berghei* infected mice was investigated. Fifty mice were divided into five study groups of ten mice each. The groups were positive control, negative control, plasmodium with half therapeutic dose, plasmodium with therapeutic dose and plasmodium with double therapeutic dose respectively. Serum ALT, AST and ALP activities were significantly increased in negative control mice when compared with other groups, while parasitized mice with half therapeutic dose and double therapeutic doses showed significant increase in serum enzyme level when compared with positive control at ($P < 0.05$). Parasitized mice with therapeutic dose showed a significant decrease in AST and ALT levels and no significant difference was observed in ALP level when compared with positive control. This result was also supported by histological examination of the liver of parasitized mice treated with therapeutic dose showing marked improvement in renal epithelium and reduction in hepatic macrophage.

Keywords: *Aspartate transaminase, Alanine transaminase, Alkaline phosphatase and Liver tissue.*

INTRODUCTION

Malaria is a disease of global public health importance. Its social and economic burden is a major obstacle to human development in many of the world's poorest countries. In heavily affected countries, malaria alone accounts for 40% of public health expenditure, 30% to 50% of hospital admissions, and up to 60% of outpatient visits (WHO, 2007). Malaria is transmitted from person to person by the bite of mosquitoes infected with the protozoan parasite *Plasmodium*. Four *Plasmodium* species are capable of causing malaria in humans: *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae* and *Plasmodium ovale*. Of these *Plasmodium falciparum* is responsible for over 90% of cases and almost all of the malaria deaths worldwide (WHO, 2008b).

Artemisinin derivatives have been shown to produce faster relief of clinical symptoms and faster clearance of parasites from the blood than other antimalarial drugs (McIntosh and Olliaro, 1999; Adjuik *et al*., 2004 and WHO, 2006). A mosquito infects a person with sporozoites in the process of taking a blood meal. The sporozoites then enter the bloodstream and migrate to the liver. In the liver, they multiply into merozoites which infect and rupture the liver cells in an attempt to escape back into the bloodstream where infection continues. The invasion of liver cells by the sporozoite form of the malarial parasites can cause organ congestion, sinusoidal blockage and cellular inflammation (Jarikre *et al*., 2002). These changes in hepatocytes can lead to the leakage of parenchymal (transaminases) and membranous (alkaline phosphatase) enzymes of the liver into the circulatory system (Burtis *et al*., 2001). Hence the increase in liver enzymes (AST, ALT and ALP) which have been observed among malarial infected patients. Maegraith, 1981, Onyesom and Onyemakonor, 2011 also demonstrated that the various liver enzyme

(AST, ALT and ALP) activities in serum increased with increase in malarial parasite density and confirmed that the hepatic (liver) stage of the parasite's life cycle in its human host is accompanied by significant perturbation in the hepatocyte's parenchyma and membrane, leading to leakage of the liver enzymes into the general circulation. Artemether and lumenfantrine combined drug is currently receiving global attention as the most potent therapy for malaria infection. The treatment protocol is simple and there is no incident of drug resistance. However, there is a paucity of data on the biochemical studies evaluating the safety or toxic risk potentials associated with this synergistic drug.

MATERIAL AND METHODS

Animal treatment

Animals were grouped for experimental studies and were treated with Lonart DS following plasmodium parasite inoculation. The mice were weighed at the start of the experiment and randomly assigned on the basis of their weight into five study groups of ten mice each. Group A (positive control) received normal diet, Group B (negative control inoculated with plasmodium) received normal diet while groups C, D and E were administered half therapeutic dose, therapeutic dose and double therapeutic dose of Lonart DS respectively.

Parasite inoculation

The malaria parasite used was a chloroquine-sensitive strain of *Plasmodium berghei* (NK-65), obtained from the National Institute for Medical Research (NIMR), Ibadan, Nigeria and kept at the Department of Biochemistry Joseph Ayo Babalola University, Ikeji-Arakeji, Nigeria. The parasites were maintained by serial blood passage in mice. Parasitized erythrocytes were obtained from a donor-infected mouse by cardiac puncture in heparin and made up to 20ml with normal saline. Animals were inoculated intraperitoneally with infected blood suspension (0.2 ml) containing 1x 10^7 of parasitized erythrocytes on day zero. Parasitaemia was assessed by thin blood film made by collecting blood from the cut tip of the tail and this was stained with Geimisia stain (WHO, 2000). Infected mice with parasitaemia of 5-7% were allocated into four study groups of ten mice each (Builders *et al*., 2011).

Curative (established infection or rane test)

The curative potential of the drug was done employing the method described by (Ryley and Peters, 1970). The mice were injected intraperitoneally with standard inoculum of 10^7 *P. berghei* NK 65 infected erythrocytes on the first day (day 0). After 72 hours and following confirmation of parasitemia, the mice were divided into 4 groups of ten mice each.

Sample collection and biochemical assays

24 hours after the 3 days experimental period all mice were sacrificed by suffocation in chloroform vapor and dissected. Blood was obtained by cardiac puncture using sterile syringe and needle, the blood sample was allowed to stand for 30minutes to clot at room temperature and further spun at 2000rpm for 15 minutes in an MSE table top centrifuge. The serum removed with sterile needle was then used for biochemical investigations.

Assay for alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferases (AST) activities.

Alkaline phosphatase (ALP) (E.C 3.1.3.1) activity was assayed according to the method described by (Bassey *et al*., 1946) as modified by (Wright and Plummer, 1974). The procedure as described by Pratt and Kaplan (2000) and Lee *et al* (2008) were employed for the assay of aspartate aminotransferase (AST) (E.C 2.6.1.1) and Alanine aminotransferase (ALT) (E.C 2.6.1.2) activities respectively. All measurements were done using campsec

spectrophotometer.

Histological study

For light microscopic examination, liver tissues from each group were fixed with 10% buffered formalin, embedded with paraffin. After routine processing, paraffin sections of each tissue were cut into 5m thickness and stained with haematoxylin and eosin (Drury et al., 1967). The photomicrographs of the relevant stained sections were taken with the aid of a light microscope.

Statistical analysis

All data collected were summarized as mean ± SEM. Significant differences were determined using a Student's t- test and the differences were considered significant if $p < 0.05$.

RESULTS

Effect of Plasmodium and lonart DS treatment on serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) of mice.

This study was aimed at assessing the extent to which plasmodium affected serum enzyme levels in mice and how treatment with varying doses of Lonart DS ameliorated the conditions of the plasmodium infected mice.

Table 1: Effect of plasmodium and Lonart DS treatment on serum enzyme activities

TREATMENT GROUP	AST (U/L)	ALT (U/L)	ALP (U/L)
GROUP A Control	118.0 ± 0.27	56.9 ± 0.25	49.5 ± 0.28
GROUP B Plasmodium treated	136.9 ± 0.25	131.6 ± 0.23	180.4 ± 0.17
GROUP C Half therapeutic dose	a, b 130.8 ± 0.31	a, b 60.2 ± 0.21	a, b 61.9 ± 0.25
GROUP D Therapeutic dose	a, b 116.7 ± 0.35	a, b 48.8 ± 0.89	a, b 49.3 ± 0.22
GROUP E Double therapeutic dose	a, b 135.4 ± 0.23	a, b 65.8 ± 0.31	a, b 59.7 ± 0.39

Values are mean ± SEM, n = 9

a; indicate significant difference in results of the different therapeutic doses in mice compared with control at 0.05 level of significance.

b; indicate significant difference in results of the different therapeutic doses in mice compared with the plasmodium treated group at 0.05 level of significance.

Effects of plasmodium and Lonart DS on histopathology of liver

The histopathological effect of plasmodium and Lonart DS (at different doses) were done on the liver sections of the experimental mice. Microscopic observation indicated chronic infiltrations of polymorphs, marked degenerating features in hepatocytes of plasmodium treated mice when compared with control, but the mice treated with half therapeutic dose showed perivascular inflammatory cells and vascular congestion when compared with control while mice treated with normal therapeutic dose showed reduction in hepatic macrophage and the mice treated with double therapeutic dose showed the presence of mild perhepatic inflammatory cels

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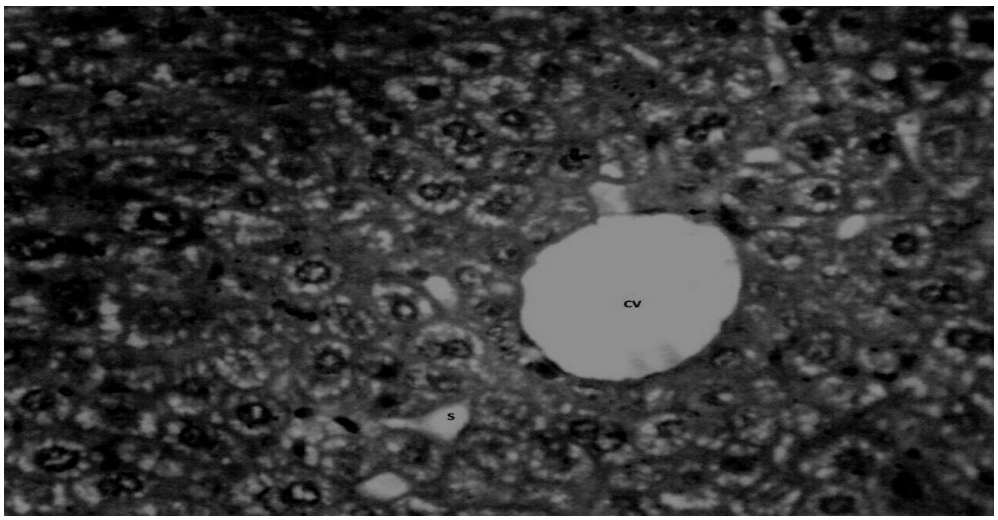

Plate 1.1: Photomicrograph of liver section of control mouse showing hepatic tissue composing of hepatocytes disposed in sheet, the central vein (CV) and sinusoids (s) are well outlined and free of congestion, inflammatory cells and interstitial collections. Cells appear essentially normal. (H&E ×400).

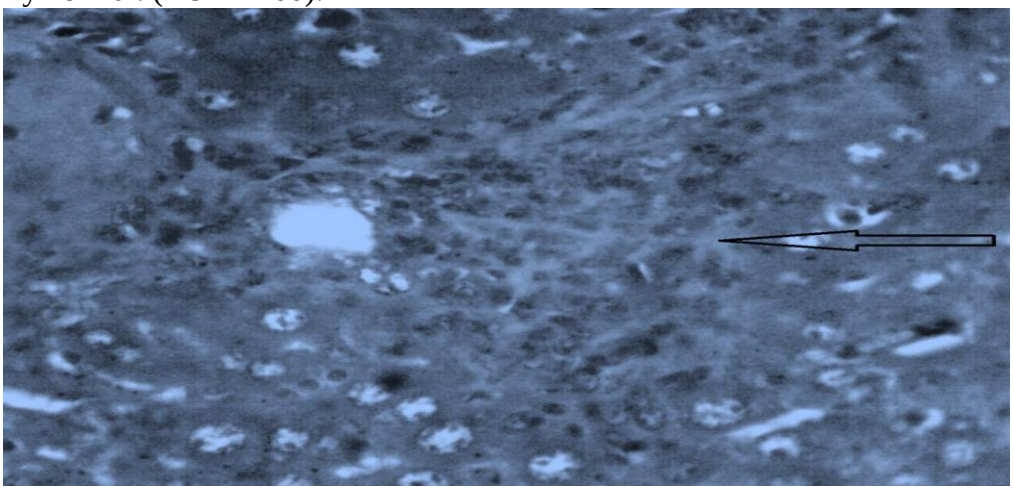

Plate 1.2: Photomicrograph of liver section of plasmodium treated mouse showing chronic infiltrations of polymorphs, marked degenerating features in hepatocytes. There is heavy presence of hepatic microphages. (H&E ×400).

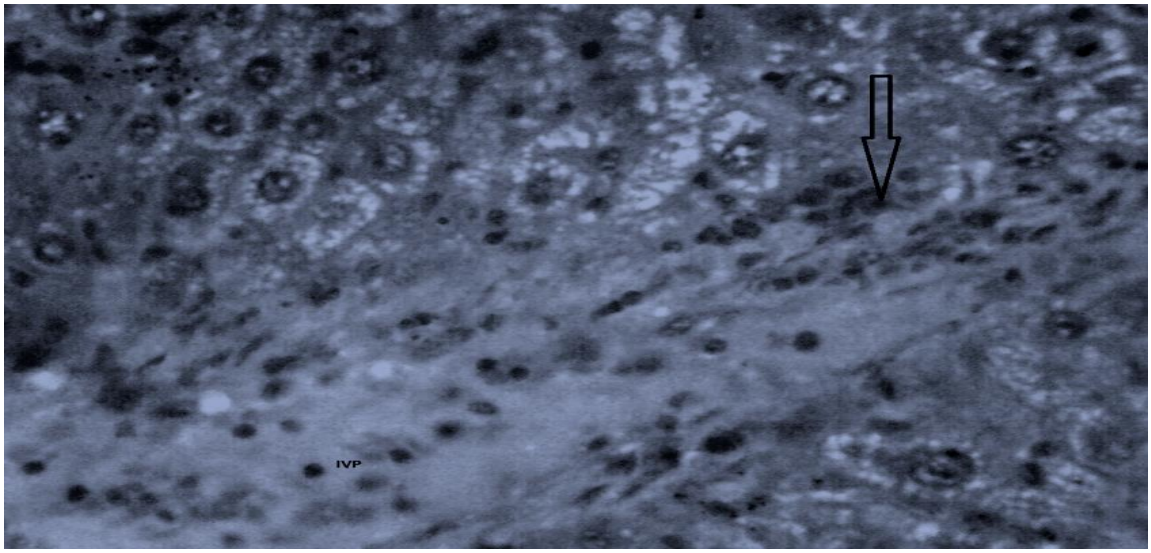

Plate 1.3: Photomicrograph of liver section of mouse treated with half therapeutic dose shows heavy presence of intravascular polymorphs (IVP), perivascular inflammatory cells (arrow), vascular congestion, the hepatocytes appear essentially unremarkable. (H&E ×400)

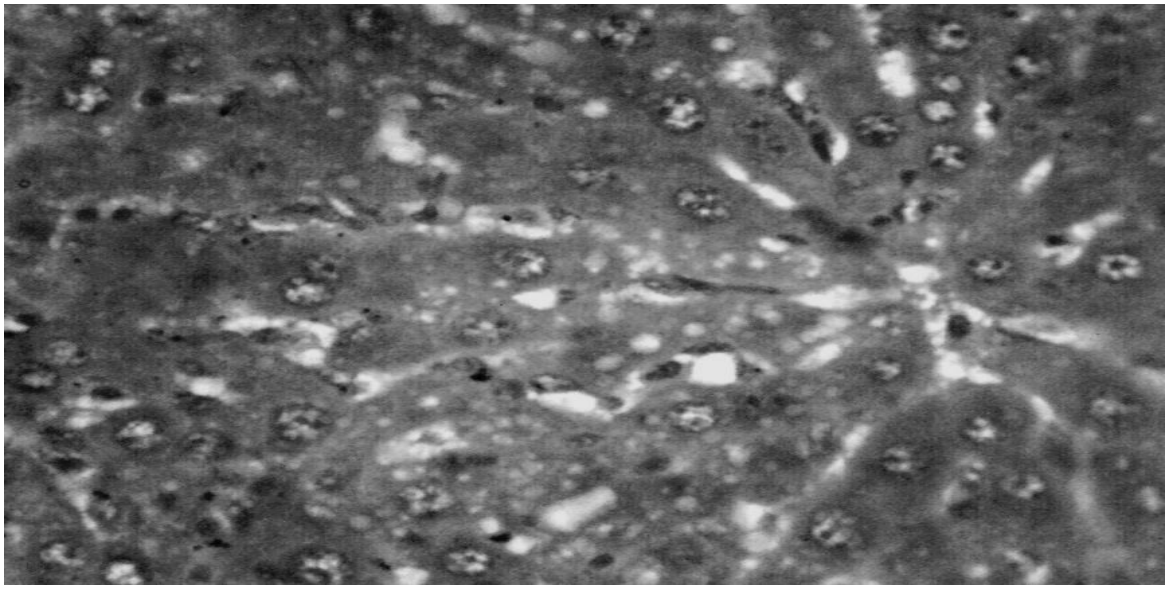

Plate 1.4: Photomicrograph of liver section of mouse treated with normal therapeutic dose showing mild dilation of the sinusoids, reduction in hepatic macrophage, the vascular channels are free of inflammatory cells and congestion (H&E ×400).

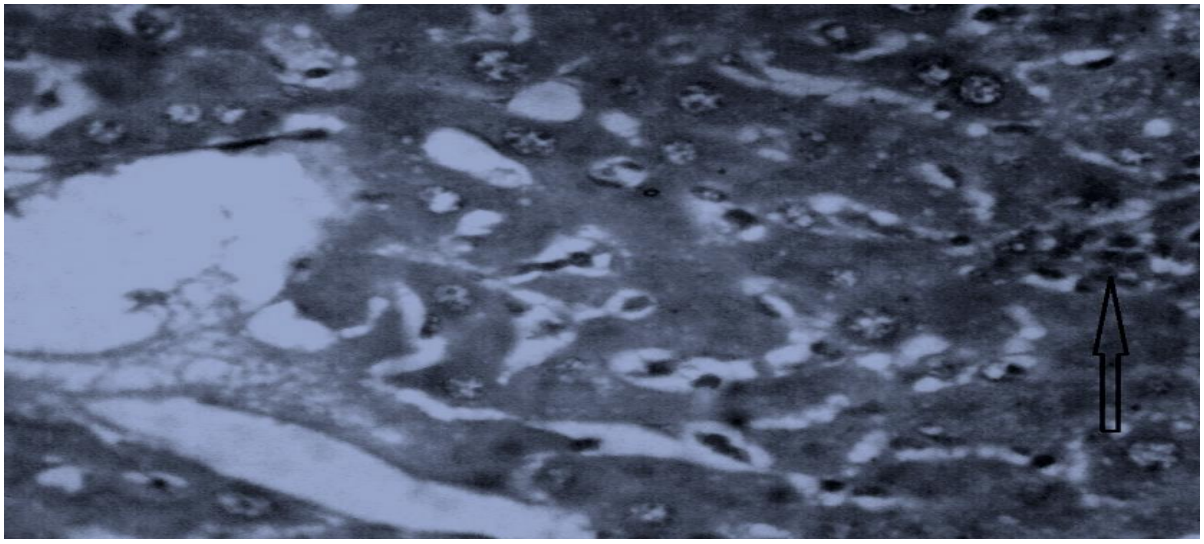

Plate 1.5: Photomicrograph of liver section of mouse treated with double therapeutic dose showing increased sinusoidal spaces, reduced microphage, presence of mild perihepatic inflammatory cells, portal vessels are free of congestion and inflammatory cells (H&E ×400).

DISCUSSION

Liver destruction can affect the metabolic processes in the body due to the role of liver in general metabolism of the organism. Enzymes are necessary for normal cellular metabolism including that of the liver (Rajamanickam and Muthuswamy, 2008). This study investigated the possible effect of Lonart DS on this cellular damage. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are considered indicators of hepatocellular health (Yang and Chen, 2003), (Vozarova *et al*., 2002).

A significant increase in the activities of ALT, AST and ALP in the blood and liver of control parasitized mice as compared with all the other groups was observed. This increase in enzyme activities may be as a result of liver injury caused by the *Plasmodium* infection and the consequent release of the enzymes into the blood stream. This is shown in the histopathological examination of the liver of the parasitized mouse which possesses marked chronic infiltrations of polymorphs, marked degenerating features in hepatocytes. The level of these enzymes in serum reflects pathological and physiological state of the liver and hence where Lonart DS exhibit its effects most. The distortion of tissues which occurs with subsequent passage of enzymes into the blood stream depends on the severity of cellular damage hence the need to monitor these enzymes (AST, ALT and ALP) to ascertain the physiological state of the tissues of mice administered half therapeutic, therapeutic and double therapeutic doses of Lonart DS.

The liver plays a central role in transforming and clearing chemicals and is susceptibility to toxicity from these agents. This is primarily because of its unique metabolic responsibility and close relationship with the GIT. Previous studies have reported that some antimalarial agents such as chloroquine (Pari and Amail, 2005) and amodiaquine (Farombi *et al*., 2000) can induce hepatic damage. Previous authors however, did not agree on the capacity of ACT to induce liver injuries, indicating a slight decrease in the activities of AST, ALT and ALP when compared to the normal control suggest that this drug does not disrupt or induce hepatic injury similar to those suggested by Georgewill and Ebong, 201). They reported normal hepatic cells in mice administered with ACT. Whereas Adaramoye *et al*., (2008) reported increased liver damage in rats administered

with ACT, in the present study it was observed that administration of Lonart DS caused a significant but dose dependent increase in activities of serum enzymes AST, ALT and ALP. This may suggest the ability of the drug to predispose to hepatic toxicity (Vahdati-Mashhadian and Rakhshandeh,(2005); (Ewaraiah and Satyanarayana, 2010) .

A significant decrease was observed in enzyme activities of groups treated with half therapeutic, therapeutic and double therapeutic doses of Lonart DS when compared with the plasmodium treated group indicating that the drug has reduced the sudden increase in enzyme activity.

The pharmacokinetics of the ACTs has also shown that their primary site of metabolism is the liver. Thus it would be expected that the liver would be susceptible to injury from these agents. However, results from this current study shows a mild presence of inflammatory cells in plasmodium treated mice, mice treated with half therapeutic and double therapeutic doses when compared with the control, but there was a marked improvement in the liver section of mice treated with normal therapeutic dose. Plasmodia infection caused marked congestion, marked hepatocytes necrosis and mild vascular congestion in the 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) and Saroj and Bhabani, (2008).

CONCLUSION

From this study it can be concluded that Lonart DS has anti- plasmodial effect on *Plasmodium berghei* infected mice in varying degrees and in a dose dependent manner, but may cause marked renal and hepatic toxicity when administered at half and double therapeutic doses as such caution should be taken in administering the drug beyond the therapeutic dose.

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