Analysis of Liver Function Biomarkers and Histopathology in Plasmodium Berghei-Infected Albino Mice Treated with Sodium Bicarbonate

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Malaria still remains an endemic disease especially in Sub-saharan Africa. The current study was aimed at evaluating liver function biomarkers and histology in albino mice following their infection with *Plasmodium berghei* and treated with Sodium Bicarbonate. Twenty mice were divided into five groups of four each. Groups 1; normal control, group 2; infected with *P. berghei,* untreated, groups 3, 4, 5; infected, treated 84mg/kg $NaHCO_3$ once, twice and thrice respectively. Blood samples and liver were collected for analysis of liver function biomarkers and histopathology by standard procedures. AST was significantly ($p<0.05$) higher in group 5 (13.33±0.707) when compared to the control (11.33±0.707). ALP activity increased significantly ($p<0.05$) in group 5 (11.76±0.707) when compared to the control (10.29±0.707). Total protein increased significantly ($p<0.05$) in all the test groups; 2 (4.29±0.007), 3 (4.09±0.007), 4 (4.46±0.007) and 5 (4.65±0.007) when compared to the

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control (4.05±0.007). Albumin increased significantly ($p<0.05$) in all the test groups; 2 (3.58±0.007), 3 (3.76±0.007), 4 (3.61±0.007) and 5 (3.58±0.007) compared to the control (3.57±0.007). Total bilirubin concentration significantly ($p<0.05$) decreased in groups 3 (0.42±0.007), 4 (0.47±0.007) and 5 (0.48±0.007) compared to the control. Direct bilirubin concentration was significantly ($p<0.05$) higher in groups 4 (0.20±.007) and 5 (0.22±.007) compared to the control (0.15±.007). Photomicrograph images showed inflammation in group 2; infected, not treated. Sodium bicarbonate did not play ameliorative role against *plasmodium berghei* infected liver.

Keywords: Malaria; liver biomarkers; histopathology; alkalinization; acidic pH.

1. INTRODUCTION

The parasitic organisms, 'Plasmodium spp' cause malaria, the parasite on getting access into the blood via a vector thrives by feeding on the host cell hemoglobin using cytostome-dependent invaginations. The hemoglobin is transferred to an acidic digestive vacuole (DV) and degraded by proteases providing a source of amino acids and of osmolytes [1] and generating space for growth [2]. Part of the life cycle of the parasite takes place in the liver, hence an accumulated parasite number could endanger the hepatocytes.The DV pH has long been thought to play a major role in the degradation of hemoglobin, the detoxification of heme (a toxic waste product of hemoglobin degradation) and in the event of antimalarial drug action and resistance [3]. Several proteolytic enzymes of the parasite such as aspartic and cysteine proteases involved in hemoglobin degradation have pH optima in the range 4.5-5.0 suggesting that the DV maintains an acidic environment [1]. The vacuolar-type proton-pumping ATPase (V-type H+-ATPase) is thought to be responsible for maintaining an acidic DV [4]. There is evidence for the presence of this proton pump on the parasite plasma membrane, DV and small vesicle compartments [5].The death of cells have been shown to correlates with acidosis in some cases and intracellular pH rise after chemotherapy may reflect response to chemotherapy [6]. It has been suggested that inducing metabolic alkalosis may be useful in enhancing some treatment regimens by using sodium bicarbonate, carbicab, and furosemide [7]. Extracellular alkalinization by using bicarbonate may result in improvements in therapeutic effectiveness [8].

Sodium bicarbonate is widely available in the form of baking soda and combination products. It reacts almost instantaneously to neutralize HCl to produce CO_2 and NaCl. The formation of CO_2 results in belching and gastric distention. Sodium bicarbonate is often referred to as a "systemic" antacid because the unreacted fraction is readily absorbed into the general circulation and may alter systemic pH. The potential for Na^+ overload and systemic alkalosis limits its use to short-term relief of indigestion. Na^+ overload resulting from repeated use of large doses may contribute to fluid retention, edema, hypertension, congestive heart failure, and renal failure. Sodium bicarbonate is contraindicated in patients on a low-salt diet. According to [9], Sodium bicarbonate administered to albino mice infected with *P. berghei* caused the elevation of WBC, leucocytes and Eosinophils count. These are cells usually mobilized during an immune response, hence the suggestion that sodium bicarbonate may favour immunomodulation by posing an unfavorable pH condition to some parasites that prefer to thrive in acidic pH because it is alkaline in nature.

Sodium bicarbonate ($NaHCO_3$) solutions are sometimes administered to patients with metabolic acidosis who have both a low plasma HCO_3^- concentration, and a low plasma pH (< 7.2). Since this salt is for the most part completely dissociated in aqueous solution, Na^+, HCO_3^-, and H_2O are effectively added to the extracellular fluid (ECF) compartment. Since Na^+ molecules are being added without Cl^-, and since HCO_3^- has a tendency to displace Cl^- from the ECF compartment, both effects contribute to increase the "strong ion difference" (SID), thus causing 'alkalinization'. In addition, added HCO_3^- acts as a buffer to accept protons, and generate CO_2 and H_2O. Although the Pco_2 rises by about 0.5 mmHg for each mEq/L increase in the plasma HCO_3^- concentration, assuming the lungs are normal, this excess CO_2 should stimulate ventilatory drive, and thus expiration. If a $NaHCO_3$ solution is administered rapidly, however, ventilatory drive may not be appropriately regulated. In this study, we leveraged on the claims in various literatures that the pathogenicity of plasmodium is favoured

under extracellular and intracellular acidic pH environment hence we tried to alkalinize the host system with sodium bicarbonate amidst *P. berghei* and monitored the ravaging or otherwise effects of the parasite on the liver by evaluating the liver function parameters and conducting a histopathological studies of the liver.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Experimental animals

Healthy albino mice of both sexes weighing between 20-33g each were used for the experiment. The animals were obtained from the zoology Department, University of Jos Nigeria and transported to the Animal House, Department of Biochemistry and Molecular Biology, Nasarawa State University, Keffi, Nigeria and acclimatized for seven days before commencement of the experiment, they were fed with standard feed and water *ad libitum*. They were also maintained under standard conditions of humidity, temperature and 12 hours light/darkness cycle. Experiments were conducted in strict compliance with internationally accepted principle for laboratory animal use and care as contained in the Canadian council on animal care guidelines and protocol review [10].

2.1.2 *Plasmodium berghei*

The plasmodium parasite; *P. berghei* NK 65 was used for the study. It was bought from National Vertinary Research Institute, Vom, Plateau State, Nigeria and kept alive by continuous intra peritoneal passage in mice every four days at the Department of Biochemistry and Molecular Biology, Nasarawa State University, Keffi, Nigeria according to Jigam [11].

2.1.3 Sodium Bicarbonate

Sodium Bicarbonate injection (Pauco Sodium Bicarbonate; 8.4%w/v) was bought from a pharmaceutical shop in Keffi town, Nasarawa State, Nigeria and stored at between 8-25 ^{0}C in the laboratory at the Department of Biochemistry and Molecular Biology, Nasarawa State University, Keffi until commencement of the experiment.

2.2 Methods

2.2.1 Experimental design

A Simple randomized designed was adopted where twenty mice were randomly divided into five groups of four mice per group. Groups 1 served as the normal control; not infected with the parasite and not treated with sodium bicarbonate and group 2 served as the positive control; it was infected with *P. berghei* but not treated with sodium bicarbonate while three other groups (3, 4 and 5) were assigned as test groups and administered 84mg/kg b.w of sodium bicarbonate injection once, twice and thrice per day respectively for three days.

2.2.1 Parasite inoculation

The method described by Kabiru [12] was used for the inoculation of parasite into experimental animals. The inoculums consisted of 5x107 of *P. berghei* parasitized erythrocytes per ml. This was done by first determining the percentage parasitaemia and then the erythrocytes count of the donor mouse. The blood was diluted with phosphate buffer saline in proportions indicated by both determinations. The Albino mice were inoculated intraperitoneally, with 0.2 ml of the already infected blood.

2.2.2 Determination of percentage parasitaemia

To obtain the percentage parasitaemia, thin blood smears were made from the tail of each mouse, fixed with methanol and stained with 10% Giemsa stain. The parasitaemia level was determined by counting the number of parasitized erythrocytes out of 100 erythrocytes in random 8 fields of microscope. Parasitaemia was calculated by light microscopy by using the 100× objective lens and the following equation was used

$$\% \text{ Parasitemia} = \frac{\text{Number of Parasitized RBC}}{\text{Total Number of RBC Counted}} \times 100$$

2.3 Determination of Biochemical Parameters

Aspartate amino transferase (AST) activity was assayed by the method of Reitman [13] spectrophotometrically using Randox kits. AST catalyses the reaction between L-aspartate and α-ketoglutarate to give oxaloacetate and L-glutamate.

Determination of ALT activity was done by the colorimetric method of Reitman [13]. The activity of ALP was assayed by the method of Walter [14] spectrophotometrically using Randox kits.
Total protein concentration was determined by the method of Wooten [15].

Albumin concentration was determined by the Bromo Cresol Green method of Spencer [16].
The concentration of total and direct bilirubin were determined using the method of Jandrassik [17] as outlined in Randox Kits.

2.4 Histopathological Studies of Liver Sections

This was carried out as described by Bancroft and Gamble, [18].Briefly, at the end of each experiment the liver from the various groups of mice were collected for histopathology after which they were fixed in 10% formol saline and dehydrated in ascending grades of ethanol. Thereafter, the tissues were cleared in chloroform overnight, infiltrated and embedded in molten paraffin wax. The blocks were later trimmed and sectioned at 5 – 6 microns. The sections were deparaffinized in xylene, taken to water and subsequently stained with Hematoxylin and Eosin (H and E) for light microscopy. Photomicrographs of the sections were taken using a motic camera fixed onto a light microscope.

2.5 Statistical Analysis

The data obtained for biochemical analysis was analyzed using one-way ANOVA using IBM statistical product and service solution (SPSS) version 23.0 to get the mean values and standard deviations. Further test for level of significance was done using LSD and Duncan tests. The level of significance was set at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Changes in enzymatic biomarkers of liver in *P. berghei*-infected mice treated with sodium bicarbonate

AST activity was significantly ($p<0.05$) higher in group 5 (13.33±0.707) and non-significant ($p<0.05$) change in groups 2(10.66±0.707), 3 (12.04±0.707) and 4 (11.70±0.707) when compared to the control (11.33±0.707). ALT activity increased non-significantly ($p>0.05$) in group 5 (9.00±0.707) and decreased non-significantly ($p<0.05$) in groups 2(8.00±0.707), 3 (8.00±0.707) and 4 (8.50±0.707) when compared to the control (8.33±0.707). ALP activity increased significantly ($p<0.05$) in group 5 (11.76±0.707) but the increased was not significant ($p<0.05$) in groups 2 (10.88±0.707), 3 and 4 (10.59±0.707) when compared to the control group (10.29±0.707).

3.1.2 Changes in non-enzymatic biomarkers of liver in *P. berghei*-infected mice treated with sodium bicarbonate

Total protein was found to increase significantly ($p<0.05$) in all the test groups; 2 (4.29±0.007), 3 (4.09±0.007), 4 (4.46±0.007) and 5 (4.65±0.007) when compared to the control (4.05±0.007). The concentration of albumin increased significantly ($p<0.05$) in all the test groups; 2 (3.58±0.007), 3 (3.76±0.007), 4 (3.61±0.007) and 5 (3.58±0.007) when compared to the control (3.57±0.007). Total bilirubin concentration was found to be significantly ($p<0.05$) lower in groups 3 (0.42±0.007), 4 (0.47±0.007) and 5 (0.48±0.007) but the concentration in group 2 (8.00±0.707) was not significant ($p>0.05$) when compared to the control. Direct bilirubin concentration was significantly ($p<0.05$) higher in groups 4 (0.20±.007) and 5 (0.22±.007) but non-significant ($p>0.05$) in groups 2 (0.14±.007) and 3 (0.17±.007) when compared to the control (0.15±.007).

3.1.3 Photomicrograph images of the liver from *P. berghei*-infected mice treated with sodium bicarbonate

Results of the photomicrogaph images of liver sections from *P. berghei*-infected mice treated with sodium bicarbonate are presented in the slides below; Slide 1. Photomicrograph of sections of the liver from experimental rats of group 1 (normal control) shows normal hepatocytes and central vein in the liver section (black arrows). Slide 2. Photomicrograph of sections of the liver from experimental rats of groups 2 positive control (infected with *P. berghei* and not treated), showing the degeneration and necrosis in the (white arrow). Also note the veinous/sinusoidal congestion in the group 2 (white arrow). Slide 3. Photomicrograph of sections of the liver from experimental rats of group 3 infected with *P. beighei* and treated with 84 mg per kg body weight of sodium bicarbonate for once per day showing apparently normal

hepatocytes and normal central vein (black arrow). Slide 4. Photomicrograph of sections of the liver from experimental rats of group 4 infected with *P. beighei* and treated with 84 mg per kg body weight of sodium bicarbonate twice per day showing apparently normal hepatocytes and central vein (black arrow). Slide 5. Photomicrograph of sections of the liver from experimental mice of group 5 infected with *P. beighei* and treated with 84 mg per kg of sodium bicarbonate for thrice per day showing normal hepatocytes and central vein. All observations were carried out under hematoxylin and eosin (H and E) with x 400 objective.

Table 1. Changes in enzymatic biomarkers of liver in *P. berghei*-infected mice treated with sodium bicarbonate

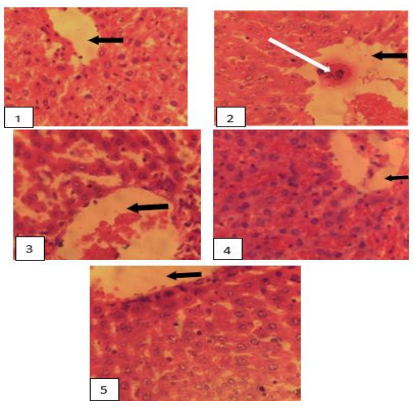
Group	AST Activity (IU/L)	ALT Activity (IU/L)	ALP Activity (IU/L)
Group 1	11.33±0.707 ^{a,b}	8.33±0.707^{a}	10.29±0.707^{a}
Group 2	10.66±0.707^{a}	8.00±0.707^{a}	10.88±0.707^{a,b}
Group 3	12.04±0.707^{b}	8.00±0.707^{a}	10.59±0.707^{a}
Group 4	11.70±0.707^{b}	8.50±0.707^{a}	11.00±0.707^{a,b}
Group 5	13.33±0.707^{c}	9.00±0.707 ^{a}	11.76±0.707^{b}

Results are expressed in Means ± SD (n = 4), Mean values with different letters as superscripts across the column are considered significant at p < 0.05

Table 2. Changes in non-enzymatic biomarkers of liver in *P. berghei*-infected mice treated with sodium bicarbonate

Group	Total protein (g/dl)	Albumin (g/dl)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
Group 1	4.05±0.007^{a}	3.57±0.007^{a}	8.00±0.707^{a}	0.15±.007^{a}
Group 2	4.29±0.007^{b}	3.58±0.007^{b}	8.00±0.707^{a}	0.14±.007^{a}
Group 3	4.09±0.007^{c}	3.76±0.007^{c}	0.42±0.007^{b}	0.17±.007^{a,b}
Group 4	4.46±0.007^{d}	3.61±0.007^{d}	0.47±0.007^{b}	0.20±.007^{b,c}
Group 5	4.65±0.007^{e}	3.58±0.007^{b}	0.48±0.007^{b}	0.22±.007^{c}

Results are expressed in Means ± SD (n = 4), Mean values with different letters as superscripts across the column are considered significant at p < 0.05



Slides 1-5. Photomicrograph images of the liver from *P. berghei*-infected mice treated with sodium bicarbonate

4. DISCUSSION

One of the greatest challenges of malaria management is the resistance of plasmodium species to available drugs [19]. There is a requirement to possess a replacement and effective agents. Therefore, we assessed the liver function parameters and histology of albino mice infected with *P. berghei* and treated with sodium bicarbonate because part of the life cycle of the parasite is usually spent in the liver such that an overwhelmed liver becomes inflamed, resulting to elevated serum biochemical parameters.

In this study, administration of sodium bicarbonate to *P. berghei* infected mice significantly elevated AST and ALP activities in the group administered 84mg/kg body weight of sodium bicarbonate thrice per day when compared to the normal control, indicating that at that dose, sodium bicarbonate could not ameliorate the inflammatory effect on the liver induced by the parasite or sodium bicarbonate may have induced inflammation of the liver thereby leading to a leakage of the biomarker enzymes. Elevated amounts of these enzymes in the blood may be an indication of health challenge due to liver problems [20]. AST, ALT and ALT test are usually used for diagnostic purposes for liver diseases and to monitor liver disorder, also, to ascertain treatment efficacy and to ensure that medications are not causing liver damage.

Total protein is one of the biochemical indices for assessing the functional status of many organs such as the liver using serum samples. In this study, total protein was found to increase significantly in all the test groups when compared to the control. This may be due to slight leakages of proteins into the circulatory system or it may be due to increased protein synthesis in response to the invading parasite. Albumin is the most abundant circulating protein found in plasma. It represents half of the total protein content (3.5 g/dL to 5 g/dL) of plasma in healthy human patients. Albumin is synthesized by liver hepatocytes and rapidly excreted into the bloodstream at the rate of about 10 gm to 15 gm per day. Very little albumin is stored in the liver, and most of it rapidly excretes into the bloodstream. In humans, serum for example, albumin functions as a significant modulator of plasma oncotic pressure and a transporter of endogenous and exogenous (i.e. drugs) ligands. In clinical medicine, serum albumin can be measured via standard serum laboratory testing, and this measure has been advocated as a highly sensitive marker for an individual patient's nutritional status [21]. In this study which involved serum from mice infected with *P. berghei* and administered sodium bicarbonate, the concentration of albumin increased significantly ($p<0.05$) in all the test groups when compared to the control.

Unconjugated hyperbilirubinemia (albumin-bound) usually results from increased production, impaired hepatic uptake, and decreased conjugation of bilirubin [22];[23] and it is often used as a clinical biomarker for the diagnosis of liver disease. Increase in bilirubin concentration is an index of jaundice, a condition of liver injury, possibly due to increased production or decreased uptake by the liver, decreased conjugation and decreased secretion from the liver or blockage of bile duct In this study, Total bilirubin decreased significantly in the treatment groups while direct bilirubin increased significantly compared to the control. Total bilirubin is the bilirubin that is bond to glucuronic acid in the liver, it is also known as conjugated bilirubin while the bilirubin that is not bonded to glucuronic acid is referred to as indirect or unconjugated bilirubin. A combination of the both gives total bilirubin because it comprise of the conjugated and unconjugated bilirubin. The outcome of this study is an indication that much of the bilirubin in the system were been conjugated. It is worthy to note that conjugation of bilirubin makes it more soluble and less toxic hence it can be easily and safely excreted from the blood and leading to less possibility of developing jaundice also known as icterus.

The histological examination of the liver did not present any remarkable changes except groups 2 positive control (infected with *P. berghei* and not treated), which showed degeneration and necrosis of the hepatocyte and the central vein as manifested by veinous/sinusoidal congestion. The result indicates conspicuously that the infected *plasmodium berghei* parasite had histological damage on the liver of mice that were not treated.

5. CONCLUSION

The outcome of this study indicates that infection of albino mice with *P. berghei* may have caused inflammation of the liver as manifested by elevated activities and concentrations of the liver function biomarkers assessed as well as the

photomicrograph image of the group infected and not treated. Unexpectedly, the administration of sodium bicarbonate may not have ameliorated the situation since the activities and concentrations of the biomarkers did not reduce even in its presence, although this was not the case in the photomicrograph images of the treated groups as they appeared apparently normal in the treated groups.

CONSENT

It is not5 applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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