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## ORIGINAL RESEARCH

### Therapeutic evaluation of Lithium Chloride against Malaria: Antiparasitic, Antioxidant, and Hepatoprotective Properties

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#### Abstract

**Background:** Malaria remains a major cause of morbidity and mortality in tropical and subtropical regions, with drug resistance hindering eradication efforts. Lithium chloride (LiCl), an inhibitor of glycogen synthase kinase-3 $\beta$ , has shown therapeutic potential in several disease models and is being investigated as an antimalarial agent.

**Objective:** To evaluate the antimalarial, antioxidant, and hepatoprotective effects of LiCl in *Plasmodium berghei*-infected mice.

**Methods:** Fifty BALB/c mice were assigned to five groups: uninfected control, infected control, LiCl-treated (15 or 30 mg/kg), and chloroquine-treated (10 mg/kg). Treatments were given orally for three consecutive days beginning 72 hours after infection. Parasitemia, packed cell volume (PCV), mean survival time (MST), liver enzyme activities, antioxidant markers, and liver histology were assessed.

**Results:** LiCl significantly suppressed parasitemia from day 5 post-treatment, with 30 mg/kg producing effects comparable to chloroquine. Treated mice showed prolonged survival, with an MST of 30.3 days versus 31.6 days for chloroquine, and improved PCV values, indicating reduced malaria-associated anemia. LiCl also increased superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) levels while decreasing alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Histological findings demonstrated reduced hepatic necrosis and evidence of liver regeneration in LiCl-treated groups.

**Conclusion:** LiCl exhibits potent antimalarial, antioxidant, and hepatoprotective activities in *P. berghei*-infected mice, supporting its potential as a therapeutic candidate for malaria and justifying further mechanistic studies.

**Keywords:** Antimalarial, Antioxidant, Hepatoprotection, Lithium chloride, Malaria, *Plasmodium-berghei*.

## Introduction

Malaria remains one of the most significant mosquito-borne diseases affecting humans, caused by protozoan parasites of the genus *Plasmodium*. Transmission occurs primarily through the bite of infected female *Anopheles* mosquitoes. Despite decades of eradication campaigns, the disease continues to exert a heavy toll, especially in tropical and subtropical regions where transmission intensity is highest. Children under the age of five and pregnant women are disproportionately affected, experiencing the greatest risk of severe illness and death.<sup>[1]</sup> Among the five species that infect humans, *Plasmodium falciparum* is considered the most virulent, being responsible for the majority of lethal cases worldwide.<sup>[2]</sup>

Efforts to limit malaria spread have traditionally centered on vector management, including the use of insecticides, environmental modification, and biological control strategies.<sup>[3]</sup> More recently, advanced interventions such as the sterile insect technique (SIT) and the search for novel antimalarial compounds have been pursued to address growing resistance to both drugs and insecticides.<sup>[4; 5]</sup> While these approaches have contributed to declines in incidence, malaria still accounts for considerable morbidity and mortality globally.<sup>[6]</sup> The deployment of the RTS,S/AS01 (Mosquirix™) vaccine represents an important milestone in preventive measures. Nevertheless, its moderate efficacy and limited accessibility restrict its contribution to malaria elimination programs.<sup>[7]</sup> In addition, the emergence of multidrug-resistant *Plasmodium* strains remains a major

obstacle to sustainable control and eradication efforts.<sup>[8; 9]</sup>

The use of lithium chloride (LiCl) in this antimalarial study is justified by growing evidence that *Plasmodium falciparum* glycogen synthase kinase-3 (PfGSK3) is a viable and underexplored antimalarial target. Recent studies show that PfGSK3 plays an essential role in parasite invasion, development, and transmission, supporting kinase inhibition as a rational antimalarial strategy.<sup>[10]</sup> LiCl is a well-characterized inhibitor of GSK-3, acting through competition with magnesium at the enzyme's active site, and its effects on GSK-3-regulated signaling pathways have been extensively described in recent biochemical and cellular studies.<sup>[10]</sup> In addition, LiCl has demonstrated *in vivo* antiplasmodial activity in rodent malaria models, providing proof of concept for its suppressive effects on parasite growth. It has been studied for potential benefits in neuroprotection, oncology, and musculoskeletal disorders.<sup>[11]</sup> In malaria research, LiCl has demonstrated promise by significantly reducing parasitemia in *Plasmodium berghei*-infected murine models, with reductions of up to 86% under optimized conditions.<sup>[12]</sup> Beyond its antiparasitic action, LiCl has been reported to possess immunomodulatory and hepatoprotective activities, suggesting a multifaceted role in host parasite interactions.<sup>[13; 14]</sup>

**Objectives:** The present study evaluates the antimalarial potential of LiCl in *P. berghei*-infected mice, with particular attention to its effects on antioxidant parameters and liver functions.

## **Methods**

### *Laboratory Materials and Reagents*

All experiments were carried out using standard laboratory glassware (flasks, test tubes, pipettes, and beakers) and equipment, including centrifuges, analytical balances, UV-visible spectrophotometers, compound microscopes, water baths, and pH meters. Oral cannulas and syringes were used for dosing and blood collection. Samples were stored at  $-20^{\circ}\text{C}$  and  $-80^{\circ}\text{C}$  until required. Commercial diagnostic kits for liver function assays (AST, ALT, and ALP) were obtained from Vital Diagnostic Spb Ltd. (Russia). Chemicals used for oxidative stress and antioxidant assays hydrogen peroxide, reduced glutathione (GSH), 5,5'-dithiobis-(2-nitrobenzoic acid, DTNB), and adrenaline were supplied by Sigma Chemical Co. (USA). Trichloroacetic acid, thiobarbituric acid, and ethanol were purchased from BDH Chemicals Ltd. (UK). Lithium chloride was obtained from BDH Chemicals Ltd. (England), while chloroquine phosphate was purchased from Laborate Pharmaceutical (India). All chemicals were of analytical grade.

### *Experimental Animals*

A total of fifty BALB/c mice weighing  $18 \pm 2$  g were obtained from the Institute for Advanced Medical Research and Training, University of Ibadan, Nigeria. The animals were kept in well-ventilated cages, maintained under standard laboratory conditions, and provided with a commercial rodent diet (Ladokun Feeds, Nigeria Ltd.) and clean water *ad libitum*. The *Plasmodium berghei* ANKA strain was sourced from the

Malaria Research and Reference Reagent Resource Center (MR4) and maintained in mice through serial passage. All procedures were conducted in strict accordance with the Institutional Animal Ethics Committee (IAEC) guidelines. The study also complied with the WMA Statement on the Use of Animals in Biomedical Research and the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes, as well as internationally accepted standards for experimental design in pharmaceutical and biomedical research. Ethical approval was obtained from the Health Research Ethics Committee, College of Medicine, University of Lagos, Nigeria (Ref: CMUL/HREC/10/24/1673).

### *Parasite Inoculation and Monitoring*

Infection was established by intraperitoneal injection of 0.2 mL of blood suspension containing approximately  $1 \times 10^7$  *P. berghei*-infected erythrocytes diluted in phosphate-buffered saline (PBS). To monitor parasitemia, thin blood smears were prepared daily from tail vein samples, fixed in methanol, stained with 10% Giemsa, and observed microscopically under oil immersion ( $\times 100$  objective). The curative (Rane's) test was used to assess antimalarial efficacy. In this model, untreated infected mice typically die within 12 days, while therapeutic activity is reflected by prolonged survival in treated groups.<sup>[15]</sup>

### *Experimental Design and Treatment Groups*

The mice were randomly allocated into five groups ( $n = 10$ ), namely:  
Group I (uninfected control): administered distilled water (10 mL/kg).

Group II (infected control): infected with *P. berghei*+ distilled water (10 mL/kg).

Group III: *P. berghei* + LiCl (15 mg/kg).

Group IV: *P. berghei* + LiCl (30 mg/kg).

Group V (standard control): *P. berghei*+ chloroquine (10 mg/kg).

Drug administration began 72 hours post-infection and continued for three consecutive days (days 3, 4, and 5-post-infection). The LiCl doses were selected based on previously reported LD<sub>50</sub> values.<sup>[16]</sup> and preliminary toxicity screening. Both LiCl and chloroquine were freshly prepared in distilled water and administered orally by gavage. Parasitemia and packed cell volume (PCV) were measured on days 3, 4, 5, 6, 7 and 10 post-infections,<sup>[17]</sup> before five (5) of the animals (n=5) were sacrificed. The blood and liver of sacrificed animals were obtained for biochemical assay, while survival time of the remaining five animals was monitored for 30 days.

#### *Calculation of Parameters*

Percentage Parasitaemia: Parasitaemia was estimated by counting infected erythrocytes among total erythrocytes in microscopic fields:

Parasitaemia (%) =

$$\frac{\text{Total Number of parasitized RBCs}}{\text{Total number of RBCs counted}} \times 100$$

RBC = Red blood cell

Mean Survival Time (MST): The survival time of each mouse was recorded over a 30-day observation period, and MST was calculated as:

MST (days) =

$$\frac{\text{Sum of survival days of all mice in a group}}{\text{Total number of mice in that group}}$$

Determination of Packed Cell Volume (PCV): Packed cell volume (PCV), which reflects the proportion of red blood cells to plasma, was measured using the microhematocrit method. Briefly, a small volume of blood was collected from the tail vein of each mouse into heparinized capillary tubes. The tubes were sealed at one end and centrifuged in a hematocrit centrifuge at 12,000 rpm for 10 minutes to allow separation of erythrocytes from plasma. The height of the packed red cell column was then compared with the total blood column using a standard hematocrit reader, and the PCV percentage was calculated as:

PCV (%) =

$$\frac{\text{Height of packed red cells}}{\text{Total Height of Blood column}} \times 100$$

#### *Preparation of Blood and Tissue Samples for Biochemical Analyses*

The mice were sacrificed on the 31<sup>st</sup> day - post infection under ether anesthesia. Blood samples obtained by cardiac puncture were placed in plain tubes and allowed to coagulate. After centrifuging the clotted blood samples at 3000 rpm for 15 minutes, the serum samples aspirated were used to evaluate the liver function enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Liver tissues were excised, rinsed thoroughly in cold normal saline (0.9%), blotted dry, weighed, and kept on ice. Each liver sample was homogenized in 0.1 M sodium phosphate buffer (pH 7.4) using a Teflon homogenizer. The homogenates were centrifuged at 10,000 × g for 20 minutes, after which the clear supernatant was collected for the estimation of antioxidant enzyme activities, including

catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH). The supernatant was stored at 4 °C until analysis. Additionally, a portion of the liver tissue was fixed in 10% neutral-buffered formalin for histopathological evaluation.

### ***In Vivo Biochemical Assays: Serum Liver Enzymes***

The activities of key liver function enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were determined in serum samples using commercially available diagnostic kits (Pars Azmun, Tehran, Iran). Enzyme levels were quantified spectrophotometrically according to the manufacturer's instructions

### ***Estimation of Antioxidant Enzyme Activities (GSH, SOD, and CAT)***

Reduced glutathione (GSH) levels in liver homogenates were determined following the method described by Kazak and Coskun.<sup>[18]</sup> Briefly, tissue homogenates were reacted with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and the absorbance of the resulting chromophore was measured at 412 nm. Superoxide dismutase (SOD) activity was assessed using the nitrobluetetrazolium (NBT) reduction assay as outlined by Adaramoye *et al.*,<sup>[17]</sup> with absorbance recorded at 560 nm. Catalase (CAT) activity was determined according to the procedure of Aebi,<sup>[19]</sup> which involves monitoring the rate of hydrogen peroxide decomposition at 240 nm.

### ***Histological Examination***

Liver tissues were fixed in 10% neutral-buffered formalin and processed using standard histological protocols. The samples were dehydrated through a graded series of ethanol, cleared, and subsequently embedded in paraffin wax. Paraffin blocks were sectioned at 4 µm thickness, and the sections were stained with hematoxylin and eosin (H&E) for microscopic evaluation, following the procedure described by Elshopakey and Elazab.<sup>[20]</sup>

### ***Statistical Analysis***

Data are presented as mean ± standard error of the mean (SEM). Statistical comparisons were performed using one-way analysis of variance (ANOVA), followed by Dunnett's or Tukey's post hoc test, as appropriate. Analyses were carried out with GraphPad Prism version 6.0 (GraphPad Software Inc., CA, USA), and differences were considered statistically significant at  $p < 0.05$ .

## **Results**

Effect of Lithium Chloride, Distilled Water, and Chloroquine on Parasitaemia in *P. berghei*-infected mice. Parasitaemia in the infected control group administered distilled water showed a steady increase across the study period, with a statistically significant ( $p < 0.05$ ) elevation detected on days 6, 7, and 10 when compared with day 3. In animals treated with lithium chloride (15 and 30 mg/kg), parasitaemia initially rose on days 3 and 4, but subsequently insignificant declined from day 5 through day 10.

Conversely, mice that received chloroquine (10 mg/kg) exhibited a slight elevation in parasitaemia on day 3, followed by a pronounced reduction from days 4 to 7, which was significant ( $p < 0.05$ ) on days 6

and 7. By day 10, parasitaemia was completely cleared in the chloroquine-treated group (Figure 1).

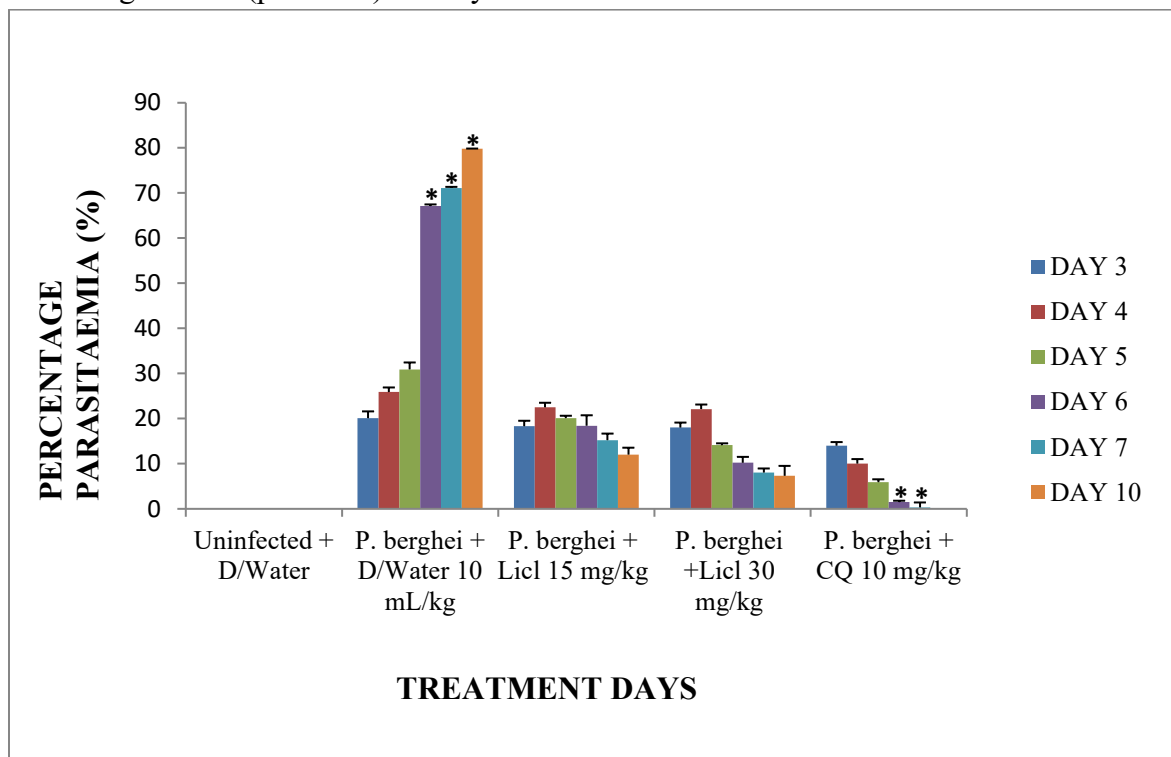


Figure 1: Effect of Lithium chloride, D/water and CQ on percentage parasitaemia in the *Plasmodium berghei*-infected mice

Value are given as mean  $\pm$ SD (n=10) \*  $p < 0.05$ , significant difference from day 3.

#### Effect of Lithium Chloride, Distilled Water, and Chloroquine on Mean Survival Time in *Plasmodium berghei*-Infected Mice

As presented in figure 1, the result revealed a significant difference in mean survival time among the groups ( $p < 0.05$ ). Compared with the uninfected control, *P. berghei*-infected mice treated with distilled water and those treated with LiCl at 15 mg/kg and 30 mg/kg

showed significantly reduced survival times ( $p < 0.05$ ), although LiCl treatment improved survival time relative to untreated infected mice. In contrast, chloroquine-treated mice showed no significant difference in survival time compared with the uninfected control ( $p > 0.05$ ), indicating near-complete restoration of survival (Figure2).

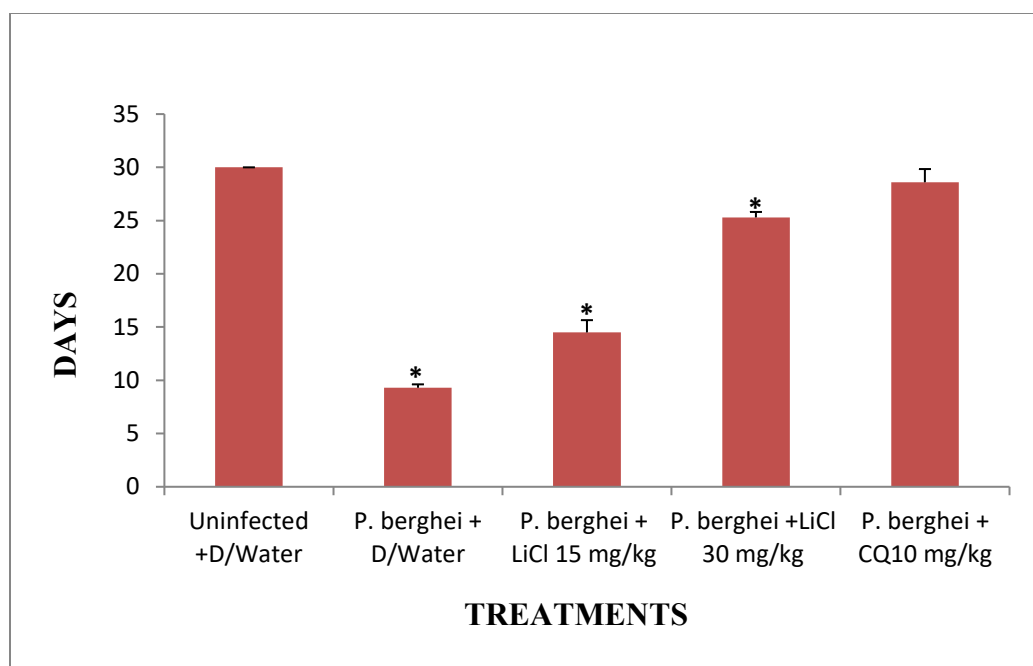


**Figure 2: Effect of Lithium Chloride, Distilled Water, and Chloroquine on Mean Survival Time in *Plasmodium berghei*-Infected Mice**

Value are given as mean  $\pm$ SD (n=5) \* p<0.05), significant difference relative to Uninfected + D/Water

*Effect of Lithium Chloride, Distilled Water, and Chloroquine on PCV in *P. berghei*-Infected Mice*

**Table I: Showing the effects of Lithium chloride, D/water and CQ on PCV (%) in the *P. berghei* infected mice**

| Days | Uninfected +D/Water | P. berghei + D/Water | P. berghei + LiCl 15 mg/kg | P. berghei + LiCl 30 mg/kg | P. berghei + CQ10 mg/kg |
|------|---------------------|----------------------|----------------------------|----------------------------|-------------------------|
| 3    | 51.4 $\pm$ 0.01     | 50 $\pm$ 0.16        | 47 $\pm$ 0.05              | 49.8 $\pm$ 0.3             | 50.8 $\pm$ 1.16         |
| 4    | 51.4 $\pm$ 0.21     | 41.5 $\pm$ 0.25      | 44.6 $\pm$ 0.09            | 46.2 $\pm$ 0.17            | 47.8 $\pm$ 0.26         |
| 5    | 50.6 $\pm$ 0.3      | 33.6 $\pm$ 0.16      | 41.4 $\pm$ 0.03            | 40.6 $\pm$ 1.20            | 44.2 $\pm$ 0.20         |
| 6    | 50.6 $\pm$ 1.3      | 29.6 $\pm$ 0.12*     | 38.6 $\pm$ 1.17            | 39.2 $\pm$ 0.31            | 45.1 $\pm$ 0.22         |
| 7    | 50.6 $\pm$ 0.2      | 26.2 $\pm$ 0.51*     | 39.3 $\pm$ 1.30            | 41.4 $\pm$ 1.53            | 45.9 $\pm$ 1.41         |
| 10   | 51.1 $\pm$ 1.1      | 22.8 $\pm$ 0.09*     | 40.8 $\pm$ 0.14            | 42.4 $\pm$ 0.13            | 46.3 $\pm$ 0.16         |

Value are given as Mean  $\pm$ SEM (n=10) \* p<0.05, significant difference from day 3

As shown in Table I, *P. berghei*-infected mice administered distilled water exhibited a significant (p < 0.05) reduction in packed cell volume (PCV) on days 6, 7, and 10 post-infections compared with baseline values {day 3}. In contrast, mice treated with lithium chloride at 15 and 30 mg/kg showed only a slight, decrease in PCV (p > 0.05), which is not statistically significant relative

to baseline. Similarly, treatment with chloroquine resulted in an initial decline in PCV; however, this change was not statistically significant (p > 0.05) when compared with baseline values.

Effect of Lithium Chloride, Distilled Water, and Chloroquine on Antioxidant parameters Levels in *P. berghei*-Infected Mice

**Table II: Effect of Lithium chloride on GSH, SOD and CAT antioxidant levels in *P. berghei* infected mice**

| Treatments                       | GSH ( $\mu\text{mol/mg protein}$ ) | SOD (U/mg protein) | CAT (U/mg protein) |
|----------------------------------|------------------------------------|--------------------|--------------------|
| Uninfected +D/Water              | 0.78 $\pm$ 0.03                    | 2.23 $\pm$ 0.12    | 0.69 $\pm$ 0.05    |
| <i>P. berghei</i> + D/Water      | 0.31 $\pm$ 0.05                    | 0.70 $\pm$ 0.01    | 0.46 $\pm$ 0.05    |
| <i>P. berghei</i> + LiCl15 mg/kg | 0.46 $\pm$ 0.03                    | 0.87 $\pm$ 0.13    | 0.55 $\pm$ 0.02    |
| <i>P. berghei</i> +LiCl30 mg/kg  | 0.53 $\pm$ 0.53                    | 1.12 $\pm$ 0.14    | 0.62 $\pm$ 0.05    |
| <i>P. berghei</i> + CQ10 mg/kg   | 0.65 $\pm$ 0.04*                   | 1.71 $\pm$ 0.02*   | 0.65 $\pm$ 0.02    |

Value are given as Mean  $\pm$ SEM (n=5) \* significant difference from normal (D/water only)  $p < 0.05$ .

GSH=Reduced glutathione, SOD=Superoxide dismutase, CAT=Catalase

As shown in Table II, treatment with lithium chloride (15 and 30 mg/kg) led to a statistically non-significant elevation in reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) levels compared with the infected group that received distilled water. The chloroquine-treated group showed a significant ( $p < 0.05$ ) increased antioxidant activity, evidenced by elevated reduced glutathione (GSH) and

catalase (CAT) levels, while, superoxide dismutase (SOD), activity demonstrated a non-significant activity, relative to the infected control.

Effect of Lithium Chloride, Distilled Water, and Chloroquine on Serum Liver Enzyme Activities in *P. berghei*-Infected Mice

**Table III: Effect of Lithium chloride on serum liver enzymes activities in the *P. berghei* infected mice**

| Groups                           | AST (IU/L)       | ALT (IU/L)       | ALP (IU/L)       |
|----------------------------------|------------------|------------------|------------------|
| Uninfected +D/Water              | 28.3 $\pm$ 2.3   | 21.4 $\pm$ 1.14  | 41.2 $\pm$ 0.02  |
| <i>P. berghei</i> + D/Water      | 38.7 $\pm$ 0.34# | 58.7 $\pm$ 1.23# | 66.2 $\pm$ 0.65# |
| <i>P. berghei</i> + LiCl15 mg/kg | 37.4 $\pm$ 2.21  | 51.1 $\pm$ 0.87* | 60.8 $\pm$ 4.10* |
| <i>P. berghei</i> +LiCl30 mg/kg  | 35.4 $\pm$ 0.54* | 39.7 $\pm$ 5.61* | 53.5 $\pm$ 3.31* |
| <i>P. berghei</i> + CQ10 mg/kg   | 30.1 $\pm$ 1.3*  | 28.9 $\pm$ 3.2*  | 45.4 $\pm$ 6.21* |

Value are given as Mean  $\pm$ SEM (n=5) #, \*  $p < 0.05$ , significant difference from Uninfected +D/Water and *P. berghei* + D/Water respectfully

Effects of *P. berghei* Infection on Liver Histology Following Treatment with Lithium Chloride and Chloroquine

As presented in Table III, the serum AST, ALT, and ALP levels were significantly ( $p < 0.05$ ) elevated in infected untreated mice (*P. berghei* + D/Water) compared with

uninfected control. Meanwhile, group infected with *P. berghei* and treated with lithium chloride (15 and 30 mg/kg) and chloroquine 10 mg/kg exhibited a significant

## Therapeutic Evaluation of Lithium Chloride Against Malaria

reduction in serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) compared with the infected group that received distilled water.

### Effects of *P. berghei* Infection on Liver Histology Following Treatment with Lithium Chloride and Chloroquine

Histological examination revealed marked hepatocellular necrosis and inflammatory

infiltration in the livers of *P. berghei*-infected mice treated with distilled water. In contrast, mice treated with LiCl at 15 mg/kg showed only occasional necrotic and inflammatory changes. Treatment with LiCl at 30 mg/kg demonstrated evidence of hepatic cord atrophy accompanied by signs of hepatocellular regeneration. Notably, livers from the chloroquine-treated group (10 mg/kg) appeared largely preserved, with no detectable histological lesions (Figure 3).

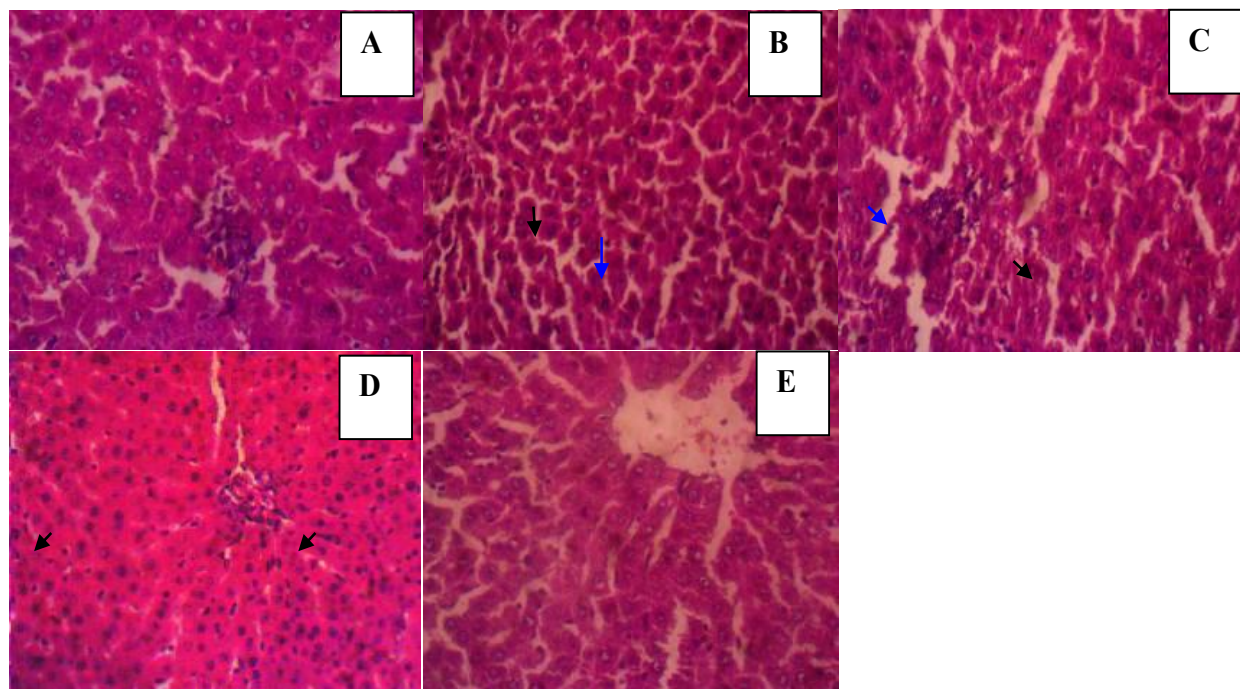


**Figure 3: Light microscopic examination of liver tissues (stained by hematoxylin and eosin stain {H&E}) showing the effects of *P. berghei* infected mice on the liver subjected to graded doses of lithium chloride and chloroquine, HE X 400.**

**Plates: -A= Uninfected +D/Water, B= *P. berghei* + D/Water, C= *P. berghei* + LiCl 15 mg/kg, D= *P. berghei* +LiCl 30 mg/kg, E= *P. berghei* + CQ 10 mg/kg.**

In the uninfected group administered with distilled water (Plate A), no histological alterations were observed. In contrast, *P. berghei*-infected mice treated with distilled water (Plate B) exhibited pronounced

hepatocellular necrosis and inflammatory infiltration (blue arrow). The group treated with LiCl at 15 mg/kg (Plate C) showed focal areas of hepatocellular necrosis (blue arrow) and inflammation (black arrow).

Treatment with LiCl at 30 mg/kg (Plate D) resulted in moderate hepatic cord atrophy alongside evidence of hepatocellular regeneration. Conversely, the chloroquine-treated group (10 mg/kg) (Plate E) displayed normal liver architecture without detectable lesions.

## Discussion

Malaria continues to pose a significant global health burden, largely due to the rise and spread of *Plasmodium* strains resistant to conventional antimalarial agents. The widespread resistance of *Plasmodium falciparum* to chloroquine has intensified the quest for new therapeutic candidates with novel mechanisms of action. One promising strategy focuses on parasite protein kinases such as glycogen synthase kinase-3 (GSK-3), cyclin-dependent kinases (CDKs), mitogen-activated protein kinases (MAPKs), and MAPK kinases which are critical for parasite growth and survival. Among these, GSK-3 is a multifunctional enzyme implicated in a variety of cellular processes and has been associated with the pathogenesis of diseases such as type II diabetes and Alzheimer's disease.<sup>[21]</sup> Lithium chloride (LiCl), a recognized GSK-3 $\beta$  inhibitor, has demonstrated antiparasitodal, hepatoprotective, and antioxidant properties. In the present study, LiCl produced notable antimalarial activity in *Plasmodium berghei*-infected mice. Suppression of parasitaemia was observed from day 5 in animals treated with LiCl (15 and 30 mg/kg), which was comparable to the chloroquine-treated group, where clearance began earlier (day 4). Remarkably, the mean survival time of mice

treated with 30 mg/kg LiCl (30.3 days) was nearly equivalent to that of chloroquine-treated animals (31.6 days), suggesting that LiCl could have therapeutic relevance in malaria control. These observations align with previous findings by Aiezzah *et al.*,<sup>[21]</sup> who reported the prophylactic and suppressive potential of LiCl against malaria. Anemia is one of the major complications of severe malaria, arising from the destruction of parasitized erythrocytes and impaired red cell production. Packed cell volume (PCV) is a key marker of anemia severity and blood oxygen-carrying capacity. In this study, infected mice given distilled water exhibited a marked reduction in PCV, consistent with severe anemia. Conversely, LiCl (15 and 30 mg/kg) mitigated the decline in PCV, indicating a protective role against malaria-induced anemia. This outcome supports earlier reports of PCV reduction in *Plasmodium*-infected rodents.<sup>[22; 23; 24]</sup>

Malaria pathology is closely associated with oxidative stress, which arises from the excessive generation of reactive oxygen species (ROS) during hemoglobin breakdown and the release of free heme in infected red blood cells. These ROS promote lipid peroxidation and cause damage to proteins and DNA, leading to tissue injury in the host.<sup>[25;26]</sup> In the present study, malaria-infected mice treated with distilled water showed marked reductions in key antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) reflecting a state of oxidative imbalance. In contrast, treatment with lithium chloride (LiCl) and chloroquine significantly restored antioxidant enzyme levels, with LiCl demonstrating a notable

ability to enhance GSH concentration. These results are in agreement with the findings of Ibrahim et al. [27], who reported impaired antioxidant defense mechanisms in malaria as a result of excessive ROS production. Therefore, the antioxidant activity of LiCl may play an important protective role by limiting oxidative damage and preserving cellular integrity.

Pathological changes in the liver and spleen are characteristic of malaria, driven by parasite sequestration, heightened macrophage activity, and inflammatory responses. [28; 29] In this study, untreated infected mice displayed elevated serum liver enzymes (ALT, AST, ALP) alongside histological evidence of hepatocellular necrosis and inflammation, consistent with hepatic injury. LiCl treatment, especially at 30 mg/kg, reversed these biochemical abnormalities and improved hepatic histoarchitecture, with observations including reduced necrosis, mild hepatic cord atrophy, and signs of hepatocellular regeneration. These hepatoprotective effects parallel the findings of Plirat *et al.*, [30] who demonstrated similar protective outcomes of medicinal plant extracts against *P. berghei*-induced liver damage. Despite the numerous pharmacological activities previously reported for lithium chloride, the present study on antimalarial study has several limitations. This study was conducted in a *Plasmodium berghei* mouse model, which may not fully reflect human malaria. Long-term safety, pharmacokinetics, and cumulative toxicity of lithium chloride were not evaluated. The molecular mechanisms underlying its antimalarial effects were not directly investigated. Biochemical and

histological analyses involved relatively small sample sizes. In addition, efficacy against drug-resistant strains and combination therapy were not assessed. These factors limit direct clinical extrapolation and warrant further investigation.

## **Conclusion**

Lithium chloride (LiCl) showed antimalarial activity in *Plasmodium berghei*-infected mice, with effects comparable to chloroquine, and was associated with improved antioxidant status and liver function. These findings indicate that LiCl may have potential relevance in malaria management and warrant further studies to clarify its mechanisms of action and therapeutic significance.

**Ethical approval:** All procedures were conducted in strict accordance with the Institutional Animal Ethics Committee (IAEC) guidelines. The study also complied with the WMA Statement on the Use of Animals in Biomedical Research and the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes, as well as internationally accepted standards for experimental design in pharmaceutical and biomedical research. Ethical approval was obtained from the Health Research Ethics Committee, College of Medicine, University of Lagos, Nigeria (Ref: CMUL/HREC/10/24/1673).

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