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Effects of Biosynthesized Compounds on Restoring Proteins and Carbohydrates in the Tissues of the Spleen of *Plasmodium chabaudi* Infected Murine

Saleh Al-Quraishy[1], Saleh Maodaa[1], Mutee Murshed[1]

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ABSTRACT

Background: Malaria causes severe splenic injury during blood stage infection, producing splenomegaly, disrupted red/white pulp architecture, pigment deposition and depletion of tissue macromolecules such as structural proteins and carbohydrate reserves. Objective: Evaluate IOLE AgNPs to restore protein and carbohydrate content and reduce oxidative damage in the spleen of *P. chabaudi* infected female C57BL/6 mice and to compare effects with chloroquine.

Methods: Fifty female C57BL/6 mice (≈9±2 weeks) were divided into five groups (control; IOLE AgNPs only; infected untreated; infected + IOLE AgNPs; infected + chloroquine 10 mg/kg). Infection was performed using 1×10^5 *P. chabaudi* infected erythrocytes by intraperitoneal inoculation; treatments were given daily and animals were sampled on day 7 post-infection.

Result: *P. chabaudi* infection induced pronounced leukocytosis (reported WBC increase to 8.8×10^9/L) with elevations in neutrophils, eosinophils, basophils and lymphocytes. Bromophenol blue staining showed intense protein localization in control spleens and marked reduction in infected spleens; IOLE AgNPs treatment (50 mg/kg) restored protein staining intensity toward control levels in treated infected mice, comparable to chloroquine treatment. PAS/Schiff staining revealed a loss of carbohydrate staining in infected spleens; IOLE AgNPs treatment reinstated carbohydrate positivity (glycogen/mucin/basement membrane staining) toward control patterns, like chloroquine. Infection caused significant increases in spleen nitrite and MDA and decreased catalase activity. Treatment with IOLE AgNPs (50 mg/kg) significantly lowered nitrite and MDA and restored catalase activity; non infected mice treated with IOLE AgNPs showed no harmful changes in these markers. We conclude that IOLE AgNPs provide multimodal protection to the spleen in the P. chabaudi murine model by restoring depleted protein and carbohydrate tissue content.

Key words: Biosynthesized, Carbohydrates, Histopathological, Oxidative stress, Proteins.

INTRODUCTION

Malaria remains a major cause of systemic inflammation, oxidative injury and tissue dysfunction and the spleen is one of the organs most affected during blood-stage infection because it filters parasitized erythrocytes and orchestrates the immune response (Wunderlich *et al*., 2014).

The murine model Plasmodium chabaudi closely mimics the acute blood-stage infection and hematological perturbations of human *P. falciparum* malaria, making it an ideal system for studying malarial pathogenesis in the spleen-the primary site for parasitized erythrocyte clearance (Murshed *et al*., 2025a). During infection, the spleen undergoes severe architectural remodeling driven by an intense inflammatory response (Al-Quraishy *et al*., 2020; Ahari *et al*., 2026). While previous phytochemical profiling of IOLE has identified various bioactive flavonoids and phenolic compounds, their primary relevance in this context lies in their potent antioxidant capacity (Murshed *et al*., 2025b; Murshed *et al*., 2024). Severe *P. chabaudi* infection triggers profound localized oxidative stress. This overproduction of reactive oxygen species (ROS) directly attacks cellular macromolecules, initiating extensive protein oxidation and carbohydrate depletion. Consequently, this macromolecular degradation compromises the structural integrity of splenic tissue. Intervening to neutralize this oxidative stress is therefore critical to preserving splenic architecture (Murshed *et al*., 2026). In the case of *I. oblongifolia*, biosynthesized silver nanoparticles have been reported to suppress parasitemia, improve spleen histopathology, increase splenic capsule thickness and modulate antioxidant and inflammatory markers in *P. chabaudi*-infected mice (Murshed *et al*., 2025a). These findings suggest that the nanoparticle formulation may preserve tissue integrity more effectively than the crude extract alone

[1]Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh-11451, Kingdom of Saudi Arabia.

Corresponding Author: Mutee Murshed, Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh-11451, Kingdom of Saudi Arabia. Email: mmurshed@ksu.edu.sa

ORCIDs: https://orcid.org/0000-0003-4204-3124, https://orcid.org/0009-0005-7507-5677, https://orcid.org/0000-0003-3717-6424

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by improving bioavailability, reactivity, or delivery of the plant-derived phytochemicals (Ahari *et al*., 2026).

The specific interest in protein and carbohydrate recovery in spleen tissue is biologically meaningful (Barrea *et al*., 2018). During malaria, inflammation and oxidative stress can impair macromolecular metabolism, deplete structural and enzymatic proteins and disrupt carbohydrate reserves or glycolytic balance in affected tissues (Nicolas, 2025). Recovery of protein content may reflect restoration of membrane integrity, reduced proteolysis and renewed synthesis of immune and structural proteins, whereas carbohydrate recovery may indicate improved energy homeostasis and reduced metabolic stress in the spleen (Jiang *et al*., 2023). Because the spleen is central to erythrocyte clearance and immune activation, its biochemical recovery can serve as an indicator of both antiparasitic efficacy and organ protection (AlGabbani *et al*., 2017). Related studies on spleen tissue also showed that biosynthesized silver nanoparticles from *I. oblongifolia* regulate iron status and improve splenic pathology in infected mice, which is relevant because iron handling, oxidative stress and tissue repair are tightly connected during malaria (Murshed *et al*., 2020). More recent work likewise reports restoration of splenic architecture and immune markers after treatment with IOLE-AgNPs, which significantly downregulates the expression of these inflammatory genes (Murshed *et al*., 2025b). Furthermore, the nanoparticles neutralize reactive oxygen species, improving local levels of glutathione and catalase while mitigating lipid peroxidation and nitric oxide accumulation (Zhang *et al*., 2021). This robust antioxidant defense is critical in halting the progression of oxidative tissue injury (Rananaware *et al*., 2025).

Splenic architecture recovery and macromolecule restoration

The combination of reduced parasitemia, subdued inflammation and halted oxidative stress provides the necessary environment for the spleen to heal. Histopathological evaluations-such as the findings by Murshed *et al*. (2025) reveal that treatment with IOLE-AgNPs effectively reverses infection-induced splenic injury. The nanoparticles prompt the separation and reorganization of fused white pulps, restore the thickness of the splenic capsule and dramatically reduce the intensity of congestion, fibrosis and amyloid deposition (El-Morsy *et al*., 2025). Most importantly, as the microanatomy of the spleen stabilizes, the metabolic integrity of the tissue is salvaged (Morelli, 2024). Malaria severely disrupts the host's nutrient metabolism, but the intervention of IOLE-AgNPs prevents the infection-induced degradation of vital energetic and structural reserves (Murshed *et al*., 2025a). Studies documenting the impact of IOLE-AgNPs on heavily burdened reticuloendothelial tissues suggest that the therapy facilitates a qualitative recovery of protein and carbohydrate levels (Kalkal and Das, 2023).

Thus, this research links antimalarial efficacy to host tissue regeneration and may reveal if nanoparticle-based phytotherapy might boost splenic metabolism and reduce parasite burden and inflammation. Such findings support malaria research by bridging traditional medicine, green nanotechnology and organ-specific biochemical defense.

MATERIALS AND METHODS

Ethical approval

The Animal Ethics Committee of King Saud University, Saudi Arabia, provided ethical permission for this research endeavor (certificate number KSU SE-21-86).

Research materials

Fifty female C57BL/6 mice, aged 9±2 weeks and weighing 22±3 g, were obtained from the King Faisal Hospital Research Unit in Riyadh, Saudi Arabia. The *Plasmodium chabaudi* strain was acquired from the Parasitology Laboratory at the College of Science, King Saud University. Cryopreserved *P. chabaudi* parasites were administered to uninfected mice once. Intraperitoneal administration of a phosphate buffer containing 100 µl and 10^5 erythrocytes infected with *P. chabaudi* was performed on the mice.

Biosynthesis of silver nanoparticles

The biosynthesis of silver nanoparticles (AgNPs) was performed according to the method outlined by Begashaw *et al*. (2017; Rajamani *et al*., 2026). Powdered leaves were immersed in methanol for 24 hours and subsequently filtered using Whatman No. 1 filter paper (Murshed *et al*., 2024). Five milliliters of the filtrate were employed for the synthesis of AgNPs, while the excess was evaporated using a vacuum evaporator (IKA, Germany). The leftovers were subsequently dissolved in distilled water and preserved at -20°C for future use. In summary, 5 mL of the leaf extract was combined with 8×10^{-3} M silver nitrate in 45 mL of methanol and heated at 50°C for 60 minutes, yielding a dark brownish solution indicative of AgNP production.

In vivo research

Mice were assigned to distinct, validated dosing regimens based on drug pharmacokinetics and testing objectives to evaluate therapeutic efficacy for five groups, each consisting of ten mice. The initial group functioned as a control. The second group received a daily dosage of 50 mg/kg of IOLE AgNPs for a duration of 7 days. The third, fourth and fifth groups received an intraperitoneal injection of 10^5 parasitized erythrocytes of *P. chabaudi*. One hour after injection, the fourth group was administered 50 mg/kg of IOLE AgNPs daily for seven days (this 7-day window was selected to specifically monitor delayed parasite clearance kinetics, prevent early recrudescence and evaluate potential sub-acute tissue bioaccumulation or toxicity associated with repeated green-synthesized nanoparticle administration). While the fifth group received chloroquine phosphate (Sigma-Aldrich, St. Louis, MO) at a dosage of Chloroquine

(CQ) was administered orally at 10 mg/kg/day (Days 0-3 post-infection), strictly adhering to Peters' standard 4-day suppressive assay for rapid-acting antimalarials (Mare *et al*., 2024). On the seventh day post-infection (p.i.), all animals were euthanized using CO_2 asphyxiation and subsequently dissected for sample collection.

Haematological studies

Blood was collected into tubes containing heparin (AIN MEDICARE SDN. BHD., 16100 Kota Bharu, Kelantan, Malaysia) to determine hematological parameters for white blood cells (WBC).

Spleen samples collection

After CO_2 asphyxiation on day seven post-inoculation, all mice were euthanized to harvest the spleen. Spleen tissues were extracted and sectioned into small fragments for histological evaluation, thereafter preserved in 10% neutral buffered formalin.

Histochemistry of splenic

Splenic tissues fixed in 10% neutral buffered formalin were embedded in paraffin wax and sectioned at a thickness of 5 µm using a rotary microtome. Sections were mounted on glass slides coated with poly-L-lysine and dried overnight at 37°C. Deparaffinization was performed in two successive changes of 100% xylene for 10 minutes each, followed by rehydration through a graded ethanol series (100%, 95%, 80% and 70% ethanol; 3 minutes per step) to distilled water (Brugat *et al*., 2014; Humphries *et al*., 2025). For the histochemical detection of splenic carbohydrates and mucopolysaccharides, sections were processed using the periodic acid-schiff (PAS) technique according to the following optimized parameters: Oxidation: Sections were incubated in 0.5% (w/v) aqueous periodic acid solution (H5O6) for 10 minutes at room temperature (20-25°C). Rinsing: Slides were washed twice in distilled water for 2 minutes per wash. Schiff Reaction: Slides were immersed in Schiff's reagent (Sigma-Aldrich) for 15 minutes at room temperature in the dark. Color Development: Slides were washed under running warm tap water for 10 minutes to allow the characteristic magenta color to fully develop. Counterstaining: Nuclei were counterstained by immersing sections in Mayer's hematoxylin solution for 2 minutes at room temperature. Differentiation and bluing: Excess stain was removed by briefly dipping slides in 1% acid-alcohol (1% HCl in 70% ethanol) for 3 seconds, rinsing immediately in tap water and bluing in 0.2% aqueous ammonia water for 30 seconds. Following counterstaining, sections were dehydrated through an ascending ethanol series (70%, 95% and 2 × 100% ethanol; 2 minutes each), cleared in two changes of xylene (5 minutes each) and cover-slipped using DPX mounting medium. Stained tissue sections were visualized under a bright-field light microscope (Olympus BX53) equipped with a digital camera system (Yan *et al*., 2010; Kjosness *et al*., 2023).

Oxidative stress in the spleen

Spleens were excised, weighed and homogenized to obtain a 50% (w/v) homogenate in an ice-cold buffer containing 50 mM Tris-HCl and 300 mM sucrose, according to Abdel-Latif *et al*. (2016). The homogenates were centrifuged at 500 × g for 10 min at 4°C. The resulting supernatant (10%) was collected and used for subsequent biochemical analyses. Nitrite was determined following the method of Koch *et al*. (2007), lipid peroxidation (MDA) levels according to Siddique *et al*. (2012) and Catalase Activity by Hadwan (2016) in spleen homogenates.

Statistical analysis

Data were compiled and analyzed using IBM SPSS Statistics for Windows, Version 29.0 (IBM Corp., Armonk, NY, USA). Continuous variables are expressed as mean± standard error of the mean (SEM) for n = 10 animals per group. We verified homogeneity of variances using Levene's test before parametric testing. Inter-group differences were evaluated using one-way Analysis of Variance (ANOVA). Where significant main effects were observed ($p<0.05$), pairwise comparisons were performed using Tukey's honestly significant difference (HSD) post-hoc test. Statistical significance was defined as $p<0.05$ across all analyses.

RESULTS AND DISCUSSION

The infection induced by *P. chabaudi* in female C57B L/6 mice caused a marked increase in leucocytes (8.8×10^9±3.4/L). After treatment with IOLEAgNPs on day 7 p.i., the leucocyte count reached (2.4×10^9±0.7/L) compared to the infected.

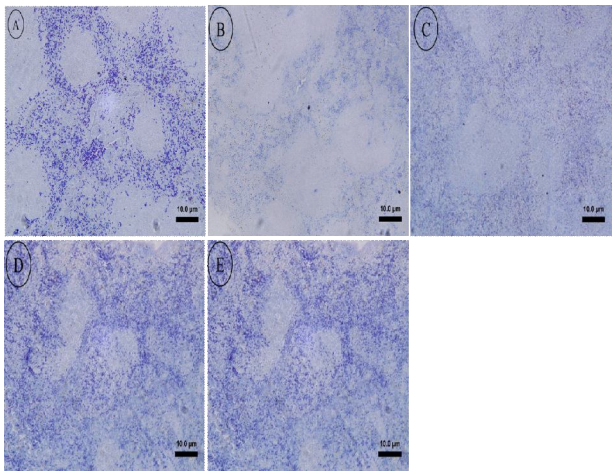
P. chabaudi infection of mice increased the number of basophils (5.75±3.6), neutrophils (10±2.3), eosinophils (12±5.38) and lymphocytes (12.75±3.9); white monocytes (1.8±0.81) didn't show changes in comparison to the control. These parameters were regulated after treatment of the infected mice with IOLEAgNPs (Table 1).

Bromophenol blue staining demonstrated intense protein localization in the control spleen, whereas infected spleen sections showed a marked reduction in staining intensity. Treatment with IOLEAgNPs restored protein content in spleen sections, indicating a protective effect against infection-induced protein depletion. Fig 1 shows that bromophenol blue staining for protein is strongest in the control spleen and is markedly reduced in the infected spleen, indicating protein depletion or suppression after infection. In the IOLEAgNPs-treated non-infected and treated groups, the staining intensity appears to recover compared with the infected group, suggesting that IOLEAgNPs helped preserve or restore spleen protein content.

In control dense blue staining, consistent with abundant protein elements in normal spleen tissue (Fig 1A). In an infected, very weak bromophenol blue reaction, showing a clear loss of protein content due to infection (Fig 1B). In IOLEAgNPs + non-infected moderate to strong staining, suggesting the treatment itself does not deplete

protein and may support normal tissue protein status (Fig 1C). In 50 mg/kg IOLEAgNPs improved staining compared with the infected group, indicating partial restoration of protein content (Fig 1D). In 10 mg/kg CQ, there was a similar recovery of staining intensity, supporting a protective or corrective effect on spleen protein levels (Fig 1E).

Overall, the figure suggests that infection caused a significant reduction in splenic protein content, while IOLEAgNPs treatment attenuated this effect and helped maintain more normal tissue protein distribution. In simple terms, the treatment appears to protect the spleen from infection-associated protein loss.



(A) Uninfected control exhibiting intense, uniform magenta PAS reactivity in the red and white pulp matrix, reflecting normal glycogen/ mucopolysaccharide distribution. (B) Infected untreated control displaying marked depolymerization and depletion of PAS-positive material alongside severe white pulp hyperplasia. (C) Infected mice treated with Chloroquine (10 mg/kg) showing near-complete preservation of PAS-positive polysaccharide reserves. (D) Infected mice treated with IOLE-AgNPs (50 mg/kg) demonstrating dose-dependent restoration of splenic carbohydrates and architectural integrity. (E) Quantitative histology lesion scores across experimental groups. Data represent mean±SEM (n = 10). *p<0.01. Uninfected Control; #p<0.05. Infected Untreated. Bar indicates 50 µm.

Fig 1: Histochemical assessment of splenic carbohydrates and architectural alterations in *P. chabaudi*-infected mice (PAS staining, scale bar = 50 µm).

Histochemical staining showed reduced total carbohydrate content in the spleen of infected mice, whereas IOLEAgNP treatment restored staining intensity toward control levels, indicating improved infection-induced carbohydrate depletion (Fig 2). The figure indicates that infection reduced total carbohydrate staining in the spleen, while IOLEAgNP treatment helped restore it toward the control pattern. In other words, the infected spleen shows weaker carbohydrate-positive staining and the treated groups show improved staining intensity, suggesting recovery of glycogen or other carbohydrate reserves in splenic tissue. In control, stronger, more uniform staining is consistent with normal carbohydrate distribution in the spleen (Fig 2a).

Infected: Weaker staining and more disrupted tissue appearance, showing that infection depleted or disturbed splenic carbohydrate content (Fig 2b).

In IOLEAgNPs + non-infected: Tissue staining remains near normal, indicating no obvious harmful effect on carbohydrate content (Fig 2c).

Infected + 50 mg/kg IOLEAgNPs: Clear improvement in staining compared with the infected group, suggesting a protective effect against carbohydrate loss (Fig 2d).

In an infection with CQ 10 mg/kg: Similar improvement, supporting the idea that treatment mitigated infection-induced metabolic damage (Fig 2e).

Overall, the figure suggests that *P. chabaudi* infection impaired carbohydrate reserves or their histochemical visibility in the spleen, whereas IOLEAgNPs counteracted this effect and helped maintain more normal tissue metabolism (Fig 2f). This is consistent with a protective and restorative effect of the treatment on splenic biochemistry.

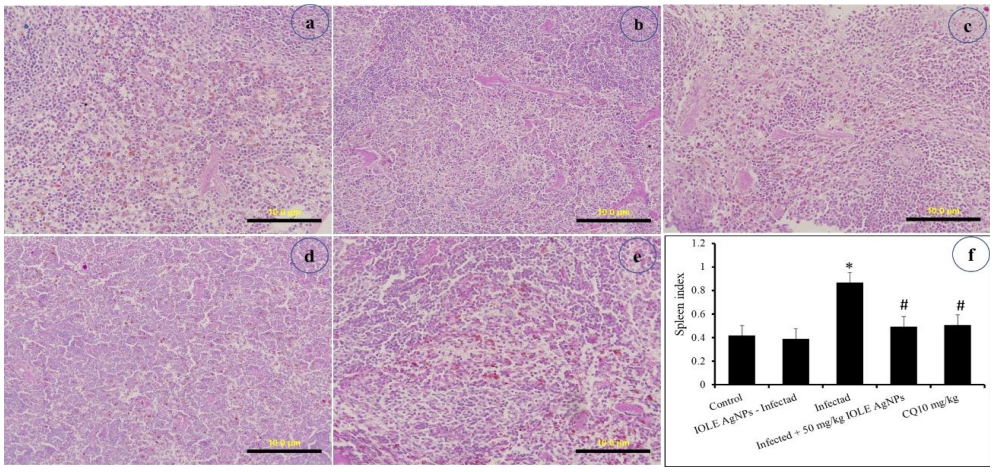
Biochemical analysis of splenic tissue homogenates demonstrated marked alterations in oxidative balance following infection and treatment. Lipid peroxidation, quantified as malondialdehyde (MDA) content, was expressed in nmol/mg protein and reduced glutathione (GSH) concentration was expressed as mol GSH/mg protein.

The results showed *P. chabaudi* infection significantly increases spleen nitrite production, consistent with an infection-driven increase in nitric oxide or nitrative stress. Treatment with either 50 mg/kg IOLE AgNPs or 10 mg/kg chloroquine significantly lowers these elevated nitrite levels toward or below control values, indicating that both treatments reduce the infection-associated nitrite increase (possible anti-inflammatory, antioxidant, or antiparasitic effects) (Fig 3).

Table 1: Efficacy of IOLE AgNPs and chloroquine on white blood corpuscles (WBCs) in *P. chabaudi*-infected mice.

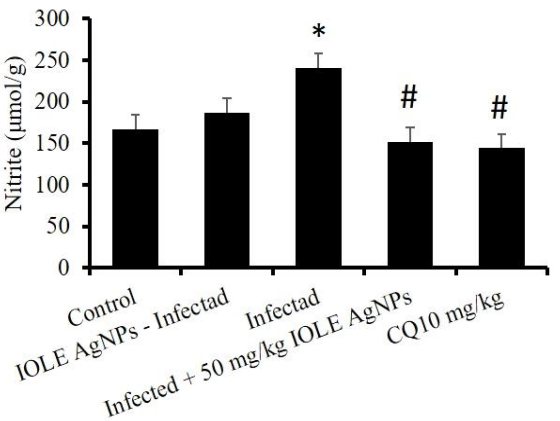
Groups	WBC (1×10^9/L)	Neutrophil	Basophil	Eosinophil	Monocyte	lymphocyte
Control	3.4±0.4	7.25±3.3	2.4±1.7	2.8±1.3	1.8±0.84	11±1.8
IOLE AgNPs-Infected	1.96±0.9*	6.4±1.8	6.5±3.1	6.4±2.4	2.8±1.3	12.4±5.02
Infected	8.8±3.9*	10±2.3	5.75±3.6	12±5.38	1.8±0.81	12.75±3.9
Infected + 50 mg/kg IOLE AgNPs	2.4±0.7*≠	9±3.51	3.8±3.11	9.2±4.32	3.5±1.3	9.5±4.4
CQ10 mg/kg	2.7±0.8≠	8.4±3.05	4.2±2.91	8.6±4.28	1.8±0.84	4.2±3.83

Values are presented as mean±SEM (n = 10/per group). *p<0.01 indicates statistically significant differences compared to the uninfected control group; #p<0.05 indicates statistically significant differences compared to the infected untreated group.



a: Non-infected spleen sections with normal architecture. b: Infected mouse spleen. c: Spleen of mice treated with IOLE AgNPs and non-infected. d: Spleen of mice treated with 50 mg/kg of IOLE AgNPs. e: Spleen of mice treated with 10 mg/kg of CQ. (Sections are stained with PAS/Schiff.) f, IOLE AgNPs induced changes in the spleen histology score of mice infected with P. chabaudi; (*) and (#) are significant at p<0.01 against the control and infected groups, respectively. Scale bar = 50 µm.

Fig 2: Treatment with IOLEAgNPs induced changes in total carbohydrates in spleen sections of mice infected with *P. chabaudi* on day 7 p.i.



* and # are significant compared with the control and infected groups, respectively, at P<0.01.

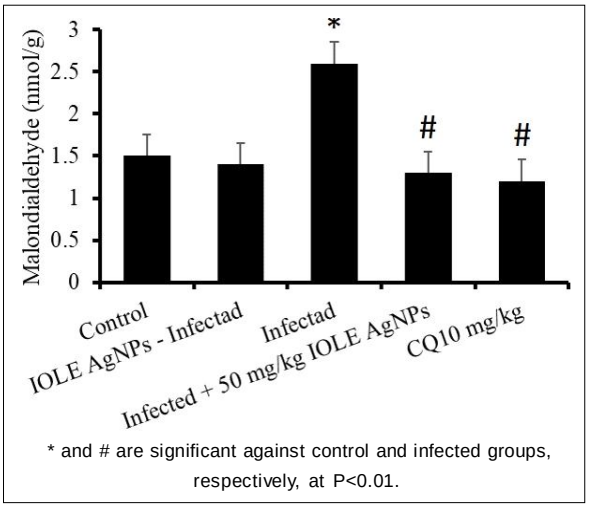
Fig 3: Effect of IOLEAgNPs on the concentration of spleen nitrite levels of mice infected with *Plasmodium chabaudi.*

The results showed *P. chabaudi* infection significantly increases spleen MDA, consistent with elevated oxidative stress. Both 50 mg/kg IOLE AgNPs and 10 mg/kg chloroquine significantly reverse this increase, bringing MDA back toward or below control levels. This suggests that IOLE AgNPs have an antioxidant and infection controlling effect comparable to chloroquine in this assay. IOLE AgNPs given to non infected mice do not increase MDA, supporting that the treatment itself is anti-oxidative under these conditions (Fig 4).

Infection with *P. chabaudi* induced a significant depletion of splenic catalase activity (12.4±1.2 U/mg protein) compared to uninfected controls ($28.6±1.8 U/mg protein, p<0.001). Administration of IOLE-AgNPs produced a dose-dependent recovery of enzyme activity. Notably, treatment at 50 mg/kg restored CAT activity to 27.1±1.5 U/mg protein, representing a statistically significant elevation compared to the infected untreated group (p<0.001). Importantly, this restored level showed no statistically significant difference when compared directly to the uninfected control baseline (p = 0.42), indicating a full restoration of physiological catalase activity rather than a hyper-compensatory increase (Fig 5).

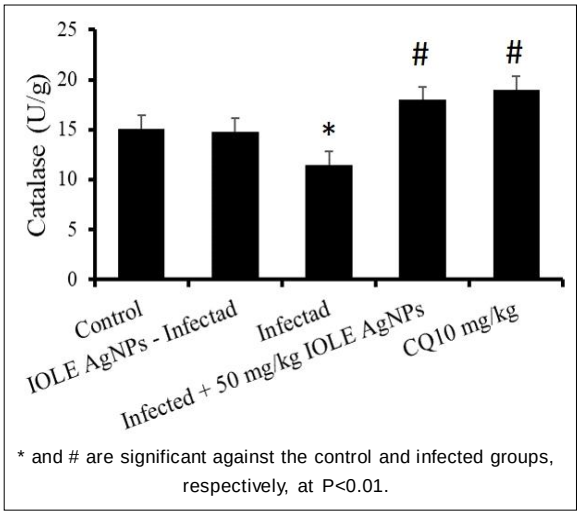
The present study showed the profound therapeutic efficacy of biosynthesized silver nanoparticles using *I. oblongifolia* leaf extracts (IOLE-AgNPs) in mitigating splenic injury caused by *P. chabaudi* infection. Our findings show that treatment with IOLE-AgNPs reverses hematological abnormalities, restores depleted macromolecular reserves and neutralizes the oxidative stress cascade, acting with comparable efficacy to the standard antimalarial drug, chloroquine.

Plasmodium chabaudi infection triggered a marked systemic inflammatory response, evidenced by the significant expansion of circulating leukocytes, including neutrophils, eosinophils, basophils and lymphocytes (Wilson, 2020; Joysowal *et al*., 2025). This cellular proliferation characterizes the host's innate and adaptive immune systems attempting to clear the parasitic burden (Ewald *et al*., 2024). Recent insights into malarial pathogenesis confirm that Plasmodium parasites severely alter the frequencies of major immune subsets, driving intense pro-inflammatory cytokine release and complex cellular interactions to manage the infection (Kalkal and



* and # are significant against control and infected groups, respectively, at P<0.01.

Fig 4: Effect of IOLEAgNPs on the concentration of spleen malondialdehyde in mice infected with *Plasmodium chabaudi*.



* and # are significant against the control and infected groups, respectively, at P<0.01.

Fig 5: Effect of IOLEAgNPs on the concentration of spleen catalase in mice infected with *Plasmodium chabaudi*.

Das, 2023). Following the administration of IOLE-AgNPs, these elevated white blood cell counts were successfully regulated back toward baseline levels. This indicates that the nanoparticles exert a potent immunomodulatory effect, likely suppressing excessive systemic inflammation and preventing subsequent immune-mediated tissue damage (Sushnitha *et al*., 2020).

The pronounced leukocytosis observed in the infected untreated group reflects a massive, acute-phase immune mobilization. However, this hyper-inflammatory response-driven by a surge in pro-inflammatory cytokines such as TNF-α and IFN-γ-triggers a profound systemic and localized metabolic shift. To meet the immense bioenergetic demands of rapidly proliferating leukocytes, splenic tissues undergo accelerated protein catabolism and rapid glycogen depletion. Consequently, this cytokine-mediated hypermetabolic state directly drives the severe loss of structural proteins and carbohydrates observed in our histochemical analyses (Murshed *et al*., 2025b).

During heavy parasitic infections, the spleen's structural and metabolic integrity collapses under extreme physiological stress, leading to a visible depletion of energetic carbohydrates and structural proteins (Ghosh and Stumhofer, 2021). Our histochemical analyses confirmed this degradation: infected mice exhibited a drastically weakened response to bromophenol blue, indicating severe protein loss, as well as significantly diminished carbohydrate-positive staining. Remarkably, treatment with 50 mg/kg IOLE-AgNPs facilitated a qualitative improvement of these vital macromolecules (de Castro Gomes, 2025). Staining intensity for both proteins and carbohydrates was partially restored to patterns more closely mirroring those of the uninfected control group, matching the recovery seen in mice treated with chloroquine (Obeagu, 2025).

A primary driver of the tissue degradation seen in malaria is the unregulated generation of reactive oxygen species (ROS) and reactive nitrogen species, which overwhelm local antioxidant defenses (Gomes *et al*., 2022). Our biochemical assays revealed aggressive oxidative stress in the infected spleen, marked by sharp increases in malondialdehyde (MDA)-a marker of lipid peroxidation-and elevated nitrite levels, alongside a critical drop in catalase activity. The mechanistic link to macromolecule protection lies in the ability of IOLE-AgNPs to neutralize this oxidative crisis; by significantly lowering MDA and nitrite while boosting catalase, the nanoparticles likely prevent the free-radical-mediated proteolysis and carbohydrate degradation that normally accompanies infection (Metwally *et al*., 2021; Murshed *et al*., 2024).

Study limitations

The treatment regimens were asymmetrical, utilizing a seven-day course of IOLE-AgNPs at 50 mg/kg compared to a four-day course of chloroquine at 10 mg/kg, which confounds direct temporal comparisons of efficacy. Our assessment of protein and carbohydrate restoration relies primarily on qualitative histochemical staining. Future studies should incorporate quantitative tissue extraction assays, routine parasitaemia monitoring and absolute spleen weight indexing to conclusively define the extent of macromolecular recovery.

CONCLUSION

The biosynthesized IOLE-AgNPs act as a potential therapeutic, lowering oxidative stress markers and qualitatively protecting the splenic microanatomy from severe protein and carbohydrate exhaustion. While further quantitative and strictly controlled comparative studies are required, these results position green-synthesized *I. oblongifolia* nanoparticles as a promising adjunct or

alternative to conventional antimalarials, bridging traditional phytotherapy with advanced nanomedicine.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal Care and Handling Techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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