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Original article

Biosynthesized nanosilver as anti-oxidant, anti-apoptotic and anti-inflammatory agent against *Plasmodium chabaudi* infection in the mouse liver

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ABSTRACT

In recent years, the use of plant-mediated nanoparticle synthesis to combat infectious diseases has become increasingly significant. Malaria is one of the world's most infectious diseases caused by *Plasmodium* species. The antioxidant, anti-apoptotic, and anti-inflammatory properties of nanosilver biosynthesized from *Indigofera oblongifolia* leaf extracts (NS) against *Plasmodium chabaudi* infection of the mouse liver were investigated in this research. Male mice were infected with *P. chabaudi* infected erythrocytes then treated with NS for 7 days. The parasitemia was suppressed by approximately 24, 28, 47 and 75% on days 4, 5, 6 and 7 postinfection, respectively after treatment of mice with NS. Also, NS was able to regulate the leucocytes count and the *IL1β* and *TNF-α*-mRNA expression in mice. Ns could increase the antioxidant activity in liver of mice and was able to regulate the apoptotic genes, *Bcl2* and *Casp3*. We showed that NS has antioxidant, anti-apoptotic, and anti-inflammatory properties when it was used to treat the livers of mice infected with *P. chabaudi*.

1. Introduction

Malaria is a fatal disease induced by Plasmodium spp., a mosquito-borne protozoon that poses a risk to people all over the world (Mehlhorn 2014). *Plasmodium* resistance to antimalarial drugs has proven to be a major obstacle to the production of new therapeutic antimalarial drugs (Dkhil et al., 2021). Malaria has been treated with medicinal plants since ancient times. These plants have the ability to contribute to the development of new anti-malaria products Taherkhani et al., 2013).

Indigofera oblongifolia is a member of the Fabaceae family that is distributed across Asia and Africa. Splenomegaly and hepatomegaly can be treated with any part of the plant (Kirtikar and Basu 1984). The antioxidant and the antimalarial activity of I. oblongifolia had been reported (Lubbad et al., 2015).

Nanoparticle (NP) biosynthesis is a major research field due to its significant applications in medicine (Pantidos and Horsfall 2014). Because of its efficiency and environmental friendliness, synthesis of NPs from plant sources has become increasingly important (Lakshmanan et al., 2018). For example, Silver nanoparticles synthetized from plant sources like Artemisia species, *Azadirachta indica*, *Catharanthus roseus* were found to have antiplasmodial activity (Ponarulselvam et al., 2012; Avitabile et al., 2020; Dkhil et al., 2021).

Dkhil et al. (2020) used *I. oblongifolia* leaf extracts to synthesize silver NPs for the investigation of their hepatoprotective effects against *Plasmodium chabaudi* infection. Also, our group investigated the role of these NPs in the regulation of iron genes in the spleen of P. chabaudi-infected female mice (Murshed et al., 2020).

This study was conducted to assess the antioxidant, anti-apoptotic and anti-inflammatory activity of nanosilver (NS) synthesized by I. oblongifolia against P. chabaudi infection in the male

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mouse liver. More research is required to determine the mechanism of nanoparticle action on host organs.

2. Materials and methods

2.1. Biosynthesis and characterization of nanosilver (NS)

I. oblongifolia leaves have been collected from Jazan, Saudi Arabia "16°53′21″N 42°33′40″E". According to Lubbad et al. (2015), 70% of the methanol extract of *I. oblongifolia* was obtained. Following the method of Murugan et al. (2016), 5 mL of the extract was used for the biosynthesis of NS by mixing the extract with silver nitrate ($AgNO_3$, 8×10^{-3} M, ~0.06793 gm) in 45 mL of methanol. The reduced NS solution was measured with UV–visible spectroscopy (UV–vis). Then, the type and size of NS are characterized by JEOL JEM-2100 transmission electron microscopy (JEOL Ltd., Tokyo, Japan) (Jiang et al., 2008).

2.2. Infection and treatment of mice

Forty males of C57BL/6 mice aged from 9 to11 weeks old were used. Animals were housed and fed standard diet and water ad libitum. Mice were kept in a polycarbonate cage in an animal facility of Zoology Department that was accredited by the Assessment and Accreditation of Laboratory Animal Treatment and followed the National Institute of Health's Guide for the Care and Use of Laboratory Animals protocol.

Blood stages of *P. chabaudi* prepared as previously described (Wunderlich et al., 1982). Mice of the first and second group received only distilled water and nanosilver (50 mg/kg) by oral gavage, respectively (Dkhil et al., 2020). The third and fourth groups were intraperitoneally infected with 10^5*P. chabaudi* infected erythrocytes (Timms et al., 2001). After 1 h, the forth group was treated with NS (50 mg/kg) for 7 days (Dkhil et al., 2020). Ot day 7p.i., all mice were sacrificed by CO_2 asphyxiation, dissected and then livers were obtained.

Giemsa stained blood films from the tail vein were prepared to calculate the parasitemia (Wunderlich et al., 1982).

2.3. Leucocytes count

Heparinized tubes were used to collect blood from the heart. A veterinary blood counter VET-530 CA Medonic; Medonic, Stockholm, Sweden) was used to count the number of leucocytes in the blood of mice.

2.4. Total antioxidant capacity

Liver homogenate was prepared according to Tsakiris et al. (2004). The total antioxidant capacity was measured by the colorimetric method and following Koracevic et al. (2001) using commercial kits (Biodiagnostic, Egypt).

2.5. Liver apoptosis by TUNEL assay

Pieces of livers were fixed in formalin (10%), processed and then, paraffin sections from the liver tissue were prepared (Drury and Wallington 1980). TUNEL assay for apoptosis was then performed according to the manufacturer's protocol of GeneScript (Piscataway, NJ, USA).

2.6. Gene expression

Total RNA was isolated from mouse liver using Trizol (Qiagen, Hilden, Germany). Also, following the manufacturer's instructions, RevertAidTM H Minus Reverse Transcriptase (Fermentas, Thermo Fisher Scientific Inc., Canada) was used to obtain cDNA. Quantitative real-time PCR was performed using the QuantiFast SYBR Green RT-PCR kit (Qiagen). Primers were obtained from Sigma-Aldrich (Table S1). The PCR reaction was carried out using the ViiATM 7 System (Thermo Fisher Scientific, CA, USA). (Livak and Schmittgen (2001) $^{\Delta\Delta}$Ct method was used to assess the differences in gene expression between groups. The reference gene was glyceraldehyde-3-phosphate dehydrogenase (Gapdh).

2.7. Statistical analysis

A one-way study of variance was used to determine significance, and Duncan's test was used to fulfil statistical comparisons between groups. For all statistical analysis in this study, $p \leq 0.05$ is considered significant.

3. Results

The synthesized NS particles are spherical in shape and range in size from 10 to 30 nm, as shown in Fig. 1A. The image also indicates

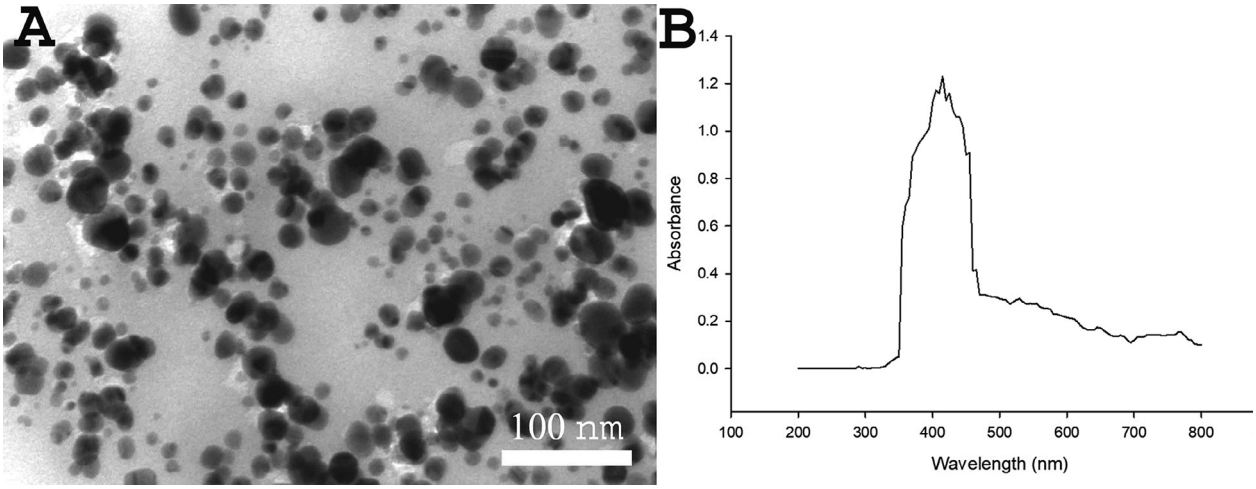

Fig. 1. Nanosilver characterization. (A) Transmission electron micrograph, (B) Absorption spectrum. Scale bar = 20 nm.

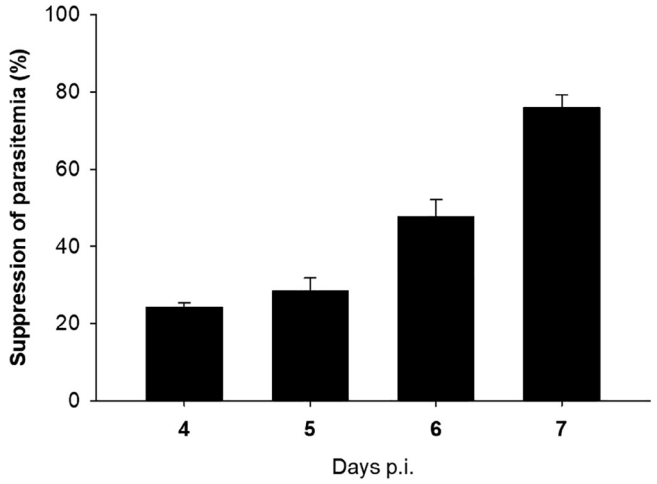

Fig. 2. Nanosilver induce suppression in parasitemia of mice at days 4,5,6 and 7 postinfection with *P. chabaudi*. Values are means ± SD.

that the prepared product contains no residues from the plant extract, implying that the nanostructure material is pure and morphologically stable. Also, the UV–Vis spectra of NS showed the peak position or plasmon resonance at 400 nm (Fig. 1B).

NS could suppress the parasitemia induced by *P. chabaudi* by approximately 24, 28, 47 and 75% on days 4, 5, 6 and 7 postinfection, respectively (Fig. 2).

The anti-inflammatory activity of NS was determined through the ability to regulate the leucocyte count and the *IL1β* and *TNF-α*-mRNA expression. In Fig. 3, the infection increased the leucocytes to 9.6 ± 1.2 × 10^3/mm^3 compared to 5.4 ± 0.9 × 10^3/mm^3 in the non-infected control group. Treatment of the infected mice with NS decreased the leucocytes to 7.5 ± 1.4 × 103/mm^3. Fig. 4 showed that NS could significantly lower the expression of *IL1β* and *TNF-α*-mRNA by about 50%.

The infection induced changes in liver apoptosis. This became clear through the examination of TUNEL assayed liver sections and also through the expression of *Bcl2* and *Casp3*-mRNA. Fig. 5 shows that apoptotic cells in the infected group's livers were

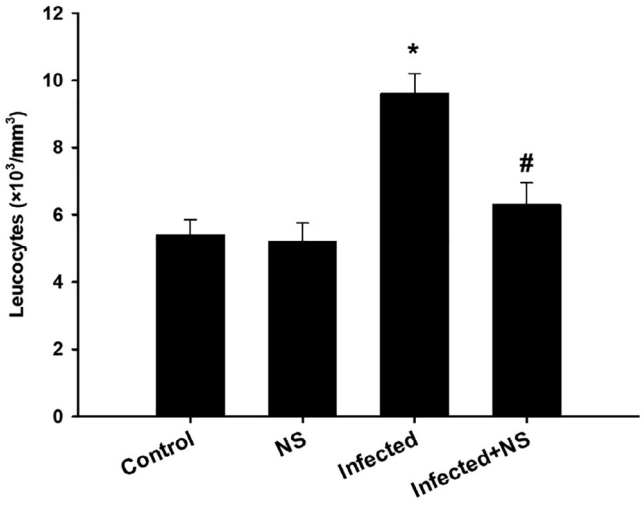

Fig. 3. Effect of nanosilver on the leucocytes count of mice infected with *P. chabaudi*. Values are mean ± SEM. *Significance against control group at P < 0.01. #Significance against infected group at P < 0.01.

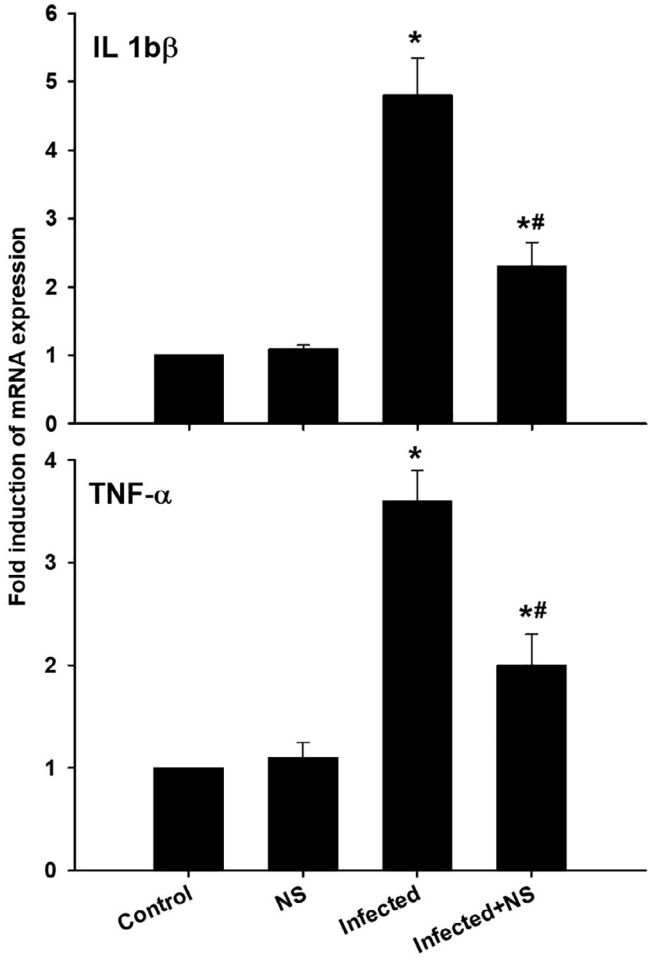

Fig. 4. Nanosilver regulates the expression of IL1β and TNF-α-mRNA in the liver of mice infected with *P. chabaudi*. The results were expressed as a fold change versus the control. (*) and (#) are significance at p < 0.01 against non-infected and infected groups, respectively.

brown in colour, with a higher number of apoptotic cells. The number of TUNEL-positive cells, on the other hand, decreased after NS treatment. NS treatment of the infected mice, was able to regulate the expression of *Bcl2* and *Casp3* (Fig. 6). The total antioxidant capacity has been determined in liver homogenate of all mice groups. Ns could increase the antioxidant activity in liver of mice (Fig. 7).

4. Discussion

Despite tremendous progress in the use of medicinal plants and nanotechnology as fight against malaria, about 3.2 billion people worldwide are still at risk of infection (WHO, 2019).

Although our group studied the effect of silver nanoparticles on the parasite and the host organs, spleen and liver (Al-Quraishy et al., 2020; Dkhil et al., 2020), but still more studies are needed to know several mechanisms of the parasite action and of the NS action. Here, we focused on the anti-inflammatory and the anti-apoptotic effect of NS against the induced infection in the liver of male mice.

Here, the rodent parasite, *P. chabaudi* was used where it has no risk to man and possesses similarities to the human parasite, *P. falciparum* (Carter et al., 1965). We recently used *P. chabaudi* as a model to investigate the antiplasmodial activity of silver nanopar-

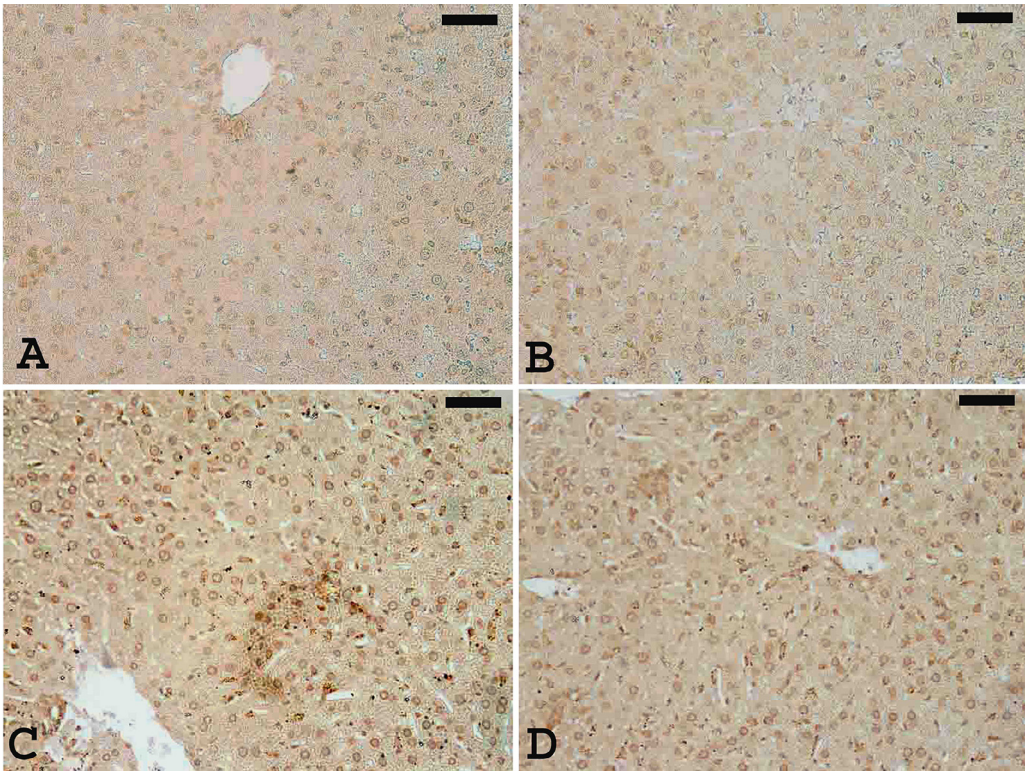

Fig. 5. Nanosilver induced changes in liver apoptotic cells of mice infected with *P. chabaudi*. (A) Non-infected liver section, (B) NS-treated liver sections, (C), Infected liver sections with more TUNEL-positive apoptotic cells. (D), Infected-NS treated liver sections. Scale bar = 50 μm.

ticles (Al-Quraishy et al., 2020; Dkhil et al., 2020). Drug resistance is an emerging issue, despite the fact that many widely available antimalarial drugs can be used to treat malaria. The removal of parasites from the patient's blood is disrupted or incomplete due to parasite resistance (Korenromp et al., 2003; Petersen et al., 2011). The development of new antimalarial compounds from different sources, especially traditional medicinal plants, is an excellent method in combating parasite resistance.

The biosynthesized NS from *I. oblongifolia* is rapid, cheap and ecofriendly and also could suppress the induced parasitemia on day 7 postinfection with *P. chabaudi*. This is due to the presence of active compounds like octadecenoic acid and 2,6-di-t-butyl-p-benzoquinone in the *I. oblongifolia* leaves (Chen et al., 2019; Dkhil et al., 2020).

The regulation of the leucocytes counts and the expression of *IL1β* and *TNF-α*- mRNA by NS is an indication to the anti-inflammatory activity. Gowda and Wu (2018) reported that early and strong cytokine-mediated effector mechanisms that destroy or eliminate parasite-infected cells is associated with both acquired and innate immune responses.

NS has a broad-spectrum behavior and can interact with the parasite (Lara et al., 2011). Moreover, NS can stay in the bloodstream for a long time, allowing for further contact with parasitized erythrocytes (Rai et al., 2017). This induce a protective role to the liver infected with *P. chabaudi* (Dkhil et al., 2020).

The immune response required to remove parasites resulted in severe cellular damage and the activation of phagocytes, which were required to kill the parasites and release proinflammatory cytokines (Al-Quraishy et al., 2020). Research findings have shown that cytokines are linked to the appearance of symptoms of disease, the degree of parasitaemia, and the nature and complications of the disease (Malaguarnera and Musumeci, 2002). On day 7 after infection with *P. chabaudi*, we found a significant increase in IL1β and TNF-α. Here, we discovered that NS derived from *I. oblongifolia* had anti-inflammatory and hepatoprotective properties.

During *P. chabaudi* infection, the formation of reactive oxygen species in tissues and cells suggested cellular damage in liver cells (Halliwell and Gutteridge, 2007). The antioxidant capacity of NS is detected in the liver of mice group treated with NS after infection (Fig. 7). The imbalance between oxidants and antioxidants causes cell stress in the liver, resulting in the formation of hydroxyl radicals, which cause oxidative damage and apoptosis (Guha et al., 2006). Furthermore, since the parasite utilizes hemoglobin as food, it releases heme, which causes hepatic oxidative imbalance, allowing the parasite to easily enter the host (Kumar and Bandyopadhyay, 2005).

Membranes of hepatocytes were affected due to apoptosis forming apoptotic bodies that could be easily phagocytozed by the liver macrophages, Kupffer cells (Savil and Fadok, 2000). These Kupffer cells may phagocytize the plasmodium infected erythrocytes (Kim et al., 2003). Treatment of the infected mice with NS, increased the number of Kupffer cells and reduced the parasitemia by phagocytosis.

Guha et al. (2006) reported that malaria infection led to oxidative stress, apoptosis and hepatic disfunction. The induced apoptosis started by the downregulation of *Bcl2* through the mitochondrial pathway. Also, Alkahtani (2010) studied the apoptotic gene *Bcl2* and *Casp3* expression during *P. chabaudi* infection in the liver of mice. The results of this study agreed with our findings with the upregulation of *Casp3*. NS treatment could regulate the change in apoptotic gene expression due to their anti-oxidant properties. The anti-apoptotic activity of biosynthesized NS had been reported in MCF-7 cells by Baharara et al. (2015).

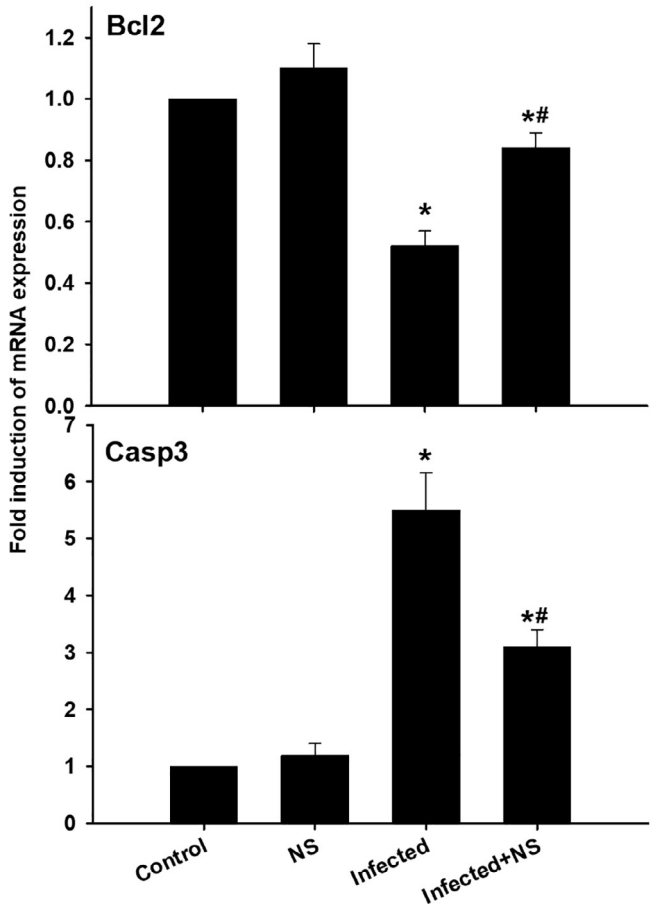

Fig. 6. Nanosilver regulates the expression of *Bcl2* and *Casp3*-mRNA in the liver of mice infected with *P. chabaudi*. The results were expressed as a fold change versus the control. (*) and (#) are significance at $p < 0.01$ against non-infected and infected groups, respectively.

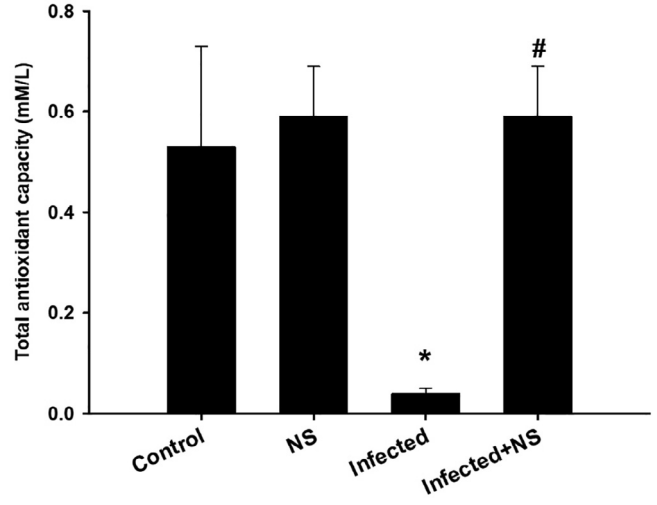

Fig. 7. Effect of nanosilver on the liver total anti-oxidant capacity. Values are means ± SD.* and # are significant at $p < 0.01$ against non-infected and infected groups, respectively.

5. Conclusions

In the livers of mice infected with blood-stage malaria, we found that NS has antioxidant, anti-apoptotic, and anti-inflammatory properties. To use NS in medical applications, further research on immune-regulatory mechanisms in the liver and other organs is needed.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.06.089.

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