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Biosynthesized Nanosilver from Ginger Extract Exhibits Antioxidant and Hepatic Responses during *Eimeria papillata* Infection

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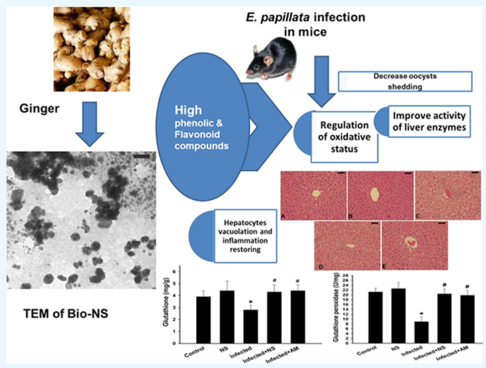
ABSTRACT: Although several anticoccidial medications have long been used to prevent coccidiosis, their adverse effects necessitate the use of alternative control methods. In this study, *Eimeria papillate* was used to infect the mouse jejunum, and the response of the liver to induced coccidiosis on treatment with nanosilver synthesized from *Zingiber officinale* (NS) and the reference anticoccidial drug amprolium was compared. Mice were infected with 1000 sporulated oocysts to induce coccidiosis. NS was able to inhibit the sporulation of *E. papillate* by approximately 73%, and also, the NS treatment improved the liver function in mice, as proven by lower levels of the liver enzymes AST, ALT, and ALP. Furthermore, treatment with NS improved the parasite-induced liver histological injury. Also, glutathione and glutathione peroxidase levels increased following treatment. Moreover, the concentrations of metal ions, Fe, Mg, and Cu, were studied, where only the Fe concentration was affected after treatment of the *E. papillate*-infected mice with Bio-NS. The presence of phenolic and flavonoid compounds in NS is thought to be responsible for its positive effects. Overall, the current study found that NS outperformed amprolium in *E. papillata*-induced mice.



1. INTRODUCTION

Eimeria spp. are intracytoplasmic coccidian parasites that cause coccidiosis in a wide range of animals, birds, and fish. It primarily damages the intestinal tract; however, it indirectly affects some accessory organs of the digestive tract such as the liver. The clinical course of the disease includes a range of symptoms, including diarrhea, weight loss, growth retardation, appetite loss, and mortality in severe instances.[1] The disease, therefore, causes massive economic loss, particularly in poultry farms around the globe.[2]

The predilection site of *E. papillata* is the jejunum of the mouse intestine, resulting in severe enteritis and also elevated oxidative conditions.[3] However, it has been found that the hepatic health status is also severely impacted; this is because of the deterioration of metabolism and elevation of some vital hepatic parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transferase (γ GT), and total bilirubin, in addition to hepatic tissue damage.[4]

Several anticoccidial medications of chemotherapeutic origin are still widely used in many control programs for coccidiosis, but the problem of drug resistance is still challenging, especially with no new anticoccidial drug available.[5,6] Besides, residues of medication in different body tissues resulted from the misuse and withdrawal activity of anticoccidial drugs. Therefore, much attention has been directed toward the use the herbal extracts in the treatment of many diseases. For instance, plant extracts have been found to be effective in treating coccidiosis with little chance of developing resistance and are environmentally friendly, such as pomegranate, garlic, mulberry, neem, ginger, and others.[7−10]

Ginger (*Z. officinale*) is considered one of the greatest vegetables in the family of Zingiberaceae.[11] Because of the high concentration of antioxidants and polyphenols in this rhizome, it is commonly used as food and seasoning,[12] in the manufacture of cosmetics, and recently in the treatment of some infectious diseases such as coronavirus disease. Besides, it is used to alleviate some general symptoms such as colic and cough and to cure nausea in cancer patients after chemotherapy intervention.[13,14] Furthermore, recently, ginger was found to have crucial anticoccidial activity in addition to its function in the regulation of goblet cells post-infection.[10] In addition to the important role of ginger in improving the feed intake and feed conversion rate in broilers,[15] it was proven to be an effective anticoccidial medication.[16]

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The emergence of nanotechnology was a breakthrough in multidisciplinary specialities of medicine and engineering.[17] Several metallic NPs such as copper, titanium, zinc, magnesium, gold, and silver have been considered, among which silver nanoparticles have been extensively experimented and found to be efficient;[18] they were proven to be antibacterial, antifungal, and antiviral agents,[19] in addition to their antiparasitic efficacy against some parasites such as *Giardia lamblia*, *Leishmania tropica*, *Entamoeba histolytica*, *Toxoplasma gondii*, *Cryptosporidium parvum*, *Ichthyophthirius multifiliis*, and *Cyclospora*.[20−25] Starting from this point, the possibility of utilizing nanosilver in the treatment of coccidiosis also arises. Therefore, our study aimed to assess the efficacy of silver nanoparticles biosynthesized from *Z. officinale* (NS) as antioxidants as well as to investigate its efficacy in restoring injured hepatic tissue during the clinical course of coccidiosis in mice experimentally infected with sporulated oocysts of *E. papillata*.

2. MATERIALS AND METHODS

2.1. Animals and Parasite Infection. This study used male mice (C57BL/6), aged 8−11 weeks. *E. papillata* oocysts were isolated from the feces of infected mice and processed using a method described in a previous study.[4] All experiments in this article were approved by the Committee of Research Ethics for Laboratory Animal Care Faculty of Science, Helwan University (approval no. HU-IACUC/Z/MA0901-22).

2.2. Preparation of the *Z. officinale* Extract. Rhizomes of *Z. officinale* were obtained from an Egyptian market, and plant specimens were authenticated by a taxonomist at the herbarium at Helwan University. Rhizomes were homogenized in 70% methanol in accordance with a process reported by Thagfan et al.[9] The *Z. officinale* extract was prepared, dried, and stored for use in synthesizing nanoparticles.

2.3. Biosynthesis and Characterization of Silver Nanoparticles. 5 mL of $AgNO_3$ (8×10^{-3} M, 0.06793 g) purchased from Sigma−Aldrich, Germany, was added to the *Z. officinale* extract (45 mL) before the mixture was placed in a dark chamber to produce silver nanoparticles (AgNPs). In order to ensure the formation of biosynthesized AgNPs (NS) in the solution, it was incubated until the color changed from brownish to black.

Transmission electron microscopy (TEM) was used to characterize the shape and size of NS using a Jeol JEM-2100 (JEOL Ltd., Tokyo, Japan) according to a method described by Jiang et al.[26]

2.4. In Vivo Infection and Experimental Design. Mice received 10^3 sporulated *E. papillata* oocysts orally, and the number of oocysts per gram of feces was calculated.[27] Thirty mice were divided into five groups (6 mice per group). Mice in group 1 (control group) received only distilled water (by oral gavage). Group 2 (NS) mice were administered 5 mg/kg NS daily via oral gavage inoculations.[28] Groups 3−5 were infected orally with 10^3 *E. papillata* oocysts.[27] After 1 h of infection, the fourth (Infected +NS) and fifth (Infected +Amprolium (AM)) groups were treated for 5 days with orally administered NS (5 mg/kg) and 25 mg/kg of the anticoccidial drug (AM; Sigma−Aldrich, Germany), respectively. On day 5, upon *E. papillata* infection, oocyst shedding was calculated using a McMaster chamber and expressed as the number of oocysts per gram of wet feces.

2.5. Determination of Total Phenolics and Total Flavonoids. The total phenolic content of NS was determined using a modified Folin−Ciocalteu technique. The samples were measured using a microplate reader (Thermo Fisher Scientific, Waltham, MA). The total phenolic content was calculated using a gallic acid standard curve.[29]

The total flavonoid concentration was determined using a colorimetric assay based on aluminum chloride. The absorbance was measured at 368 nm, with Quercetin, a reference flavonoid, to obtain a calibration curve to quantify the flavonoids in the samples.[30]

2.6. Antioxidant Activity by the DPPH Method. A methanolic solution (80 mL) of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma−Aldrich, Germany) was mixed with 20 mL of NS (1 mg/mL). This mixture was incubated for 30 min at 25 °C in the dark. At 517 nm, the absorbance was determined, and the radical-scavenging activity was estimated.[30]

2.7. Histopathology. Mice were sacrificed on day 5 post-infection and pieces of liver were isolated and fixed in neutral formalin, processed in ethanol, and then embedded in wax. Sections of liver were stained with hematoxylin and eosin (Biodiagnostic Co., Giza, Egypt).

2.8. Biochemical Study. Liver enzymes, AST, ALT, and ALP, were assayed in the homogenate of the liver using kits provided by Biodiagnostic Co. (Giza, Egypt). The liver homogenate was prepared as described in Dkhil et al.[4]

According to Ellman,[31] the glutathione (GSH) level was determined in the liver homogenate. Similarly, the glutathione peroxidase (GPx) activity was measured using the method of Paglia and Valentine.[32]

2.9. Metal Ions in the Liver. Metal ion concentrations (Fe, Mg, and Cu) were measured in liver samples from infected and noninfected mice using concentrated nitric acid and hydrogen peroxide, as described by UNEP/FAO/IOC/IAEA.[33] Metal ion concentrations were determined using the atomic emission spectrometer iCAP-6500 Duo with inductivity coupled plasma (Thermo Scientific, U.K.).

2.10. Statistical Analysis. One-way ANOVA was used to analyze data for multiple variable comparisons. Duncan's test was used for the post hoc test to compare the class significance at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

Herbal remedies have been introduced in the treatment of variable types of diseases to overcome the drawbacks of resistance and residual activity of medications, while also characterized by a high safety margin. Among these herbal products, the ginger (*Z. officinale*) plant has been proven to have therapeutic benefits such as anti-inflammatory and analgesic effects[34] in addition to its antimicrobial efficacy and antioxidant behavior. It derives its medicinal effects from the large number of pharmacological compounds including antioxidants and phenolic compounds in its rhizomes. Transmission electron microscopy analysis revealed that the biosynthesized nanosilver is spherical in shape and with a size range of 20−40 nm (Figure 1).

In this study, the total phenolics and total flavonoids were measured in NS. It was found that ginger contains the appropriate concentration of phenolic (119.6 ± 7.5 μg/g) and flavonoid compounds (91.7 ± 16 μg/g) (Table 1). As a result, it might play a significant role as a reducing agent as well as a free radical scavenger.[35] Also, the antioxidant activity of ginger has been evaluated using the DPPH radical-scavenging assay. The resultant percentage of antioxidants (85.8 ± 3.5) in ginger might provide defense against the oxidative process (Table 1).

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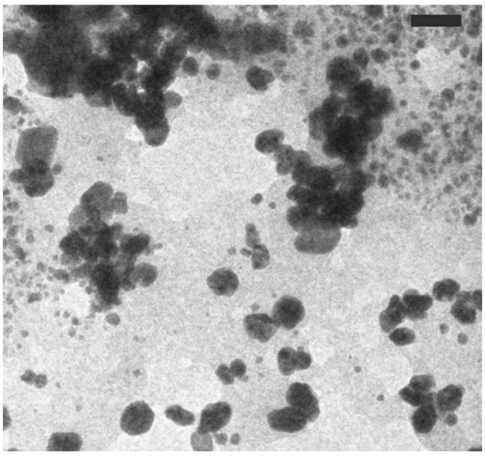

Figure 1. TEM of Bio-NS showing their shape and size. Scale bar = 50 nm.

Table 1. Total phenolic and flavonoid contents and radical-scavenging activity of nanosilver synthesized from ginger extract[a]

total phenolics (μg/g)	total flavonoids (μg/g)	antioxidant (%)
119.6 ± 7.5	91.7 ± 16	85.8 ± 3.5

[a]Values are mean and SD (n = 3).

Therefore, it is strongly recommended to consider ginger as a considerable antioxidant as well as a leading alternative treatment.

In the current study, the numbers of *E. papillata* oocystes excreted in the feces of the induced mice were estimated ($816.5 \times 10^3 \pm 112$). The treatment of *E. papillata*-induced mice with bio-AgNPs showed a substantial reduction ($218 \times 10^3 \pm 53$) in the overall oocyst number in feces compared to amprolium treatment ($404 \times 10^3 \pm 88$) (Table 2).

Table 2. Effects of biosynthesized nanosilver (NS) on oocyst shedding of mice infected with *E. papillata*

group	number of oocysts ($\times 10^3$)	suppression (%)
infected	816.5 ± 112	-
infected + NS	218 ± 53*	73.3± 9*
infected + AM	404 ± 88*	50.5 ± 7*

Results are presented as mean ± SD (n = 6). *Significant changes at $p < 0.05$ between the infected and treated groups.

This result proved the anticoccidial activity of NS. This finding might be attributed to the ability of NS to hinder the development and division of parasites in the intestinal epithelium. Furthermore, the coccidiostatic efficacy of ginger might be attributed to the presence of essential compounds derived from some phenolic compounds that harbor antioxidant activity.[34] It seems that NS perhaps has a certain disrupting effect on the cell membrane of parasites, which results in the incomplete development of *Eimeria* in the intestinal tract, particularly in the sporogony stage. In the same context, the oocyst output was reduced in response to the treatment of *E. papillata*-infected mice with chitosan.[3] Also, on the same lines, berberine showed a coccidiostatic effect on *Eimeria* infections in mice.[27] Moreover, consistent with our results, zinc oxide nanoparticles showed anticoccidial activity in mice experimentally infected with *E.papillata*.[36] On comparing NS and amprolium in terms of oocyst suppression, it was found that AgNPs (73.3% ± 9) were more effective than amprolium (50.5% ± 7) (Table 2).

Coccidian parasites is a broad term that covers a variety of parasites. The most harmful one is *Eimeria spp*., which has a destructive effect on the intestinal tract; however, it has a deteriorating effect on the hepatic tissue. Hepatic tissue samples from mice infected with *E. papillata* were histopathologically analyzed in the current investigation. (Figure 2).

Hepatocytes exhibited vacuolations, which may have been caused by the inflammatory response to *Eimeira* as well as by metabolic disruption brought on by the infection.[4] When mice were treated with NS, the injured hepatic tissue was surprisingly significantly restored. As silver nanoparticles are involved in quick tissue regeneration and cosmetic appearance, as well as in downregulating the expression of anti-inflammatory indicators, our results corroborate the research suggesting that silver nanoparticles are anti-inflammatory agents.[37,38]

As a consequence of infection, some biological inflammatory parameters, such as GSH and GPx, the activity of liver enzymes, and concentrations of some metal ions in the liver, were altered. Our study evaluated the levels of GSH and GPx (Figures 3 and 4). The result revealed a significant decrease in their concentration in the *E. papillata*-infected group. Remarkable amelioration was noted after treatment with biosynthesized AgNPs, achieving appropriate enhancement of GSH and GPx. It is known that GSH and GPx are involved in the oxidation process as master antioxidants; they reduce oxidative processes and play a vital role in hepatic tissue regeneration.[39] Therefore, as might be expected, NS has been found to stimulate GSH and, in turn, prevent liver damage. In addition, comparing the effects of NS and amprolium on the level of GSH, it was found that NS was nearly as effective as amprolium.

Any damage to liver tissues results in the release of hepatic biomarkers into the blood, such as AST, ALT, and ALP.[40,41] Therefore, it was necessary to estimate the activity of these enzymes.

The results presented in Table 3 show a drastic elevation of all of these markers in *E. papillata*-induced mice. After treatment with NS, however, their levels decrease drastically to almost normal values. This result also proves another regenerative therapeutic effect of NS. NS is fairly effective as a dietary supplement. In contrast, NS is more effective than amprolium in restoring the activity of liver enzymes. It seems that NS could be used as a prophylactic supplement in the diet of poultry.

An additional confirmatory analysis was performed to ensure the healthy status of the liver; the current study investigated the effect of NS on the liver of mice infected with *E. papillata*. The result presented no significant correlation between metal ion concentrations (Fe, Mg, and Cu) and the application of either NS supplementation or amprolium (Table 4). However, ferritin might be elevated in the case of infection. Furthermore, it is an indicator of acute-phase reactants. This might be because Fe plays a crucial role in the defense mechanism, whereby ferritin competes with pathogens on iron to prevent pathogen development, and also Fe reduces free radical generation.[42] Thus, it is strongly recommended to prolong the duration of treatment to more than 5 days. In our opinion, the relationship between metal ions, liver, and NS is not yet clear and should be further discussed.

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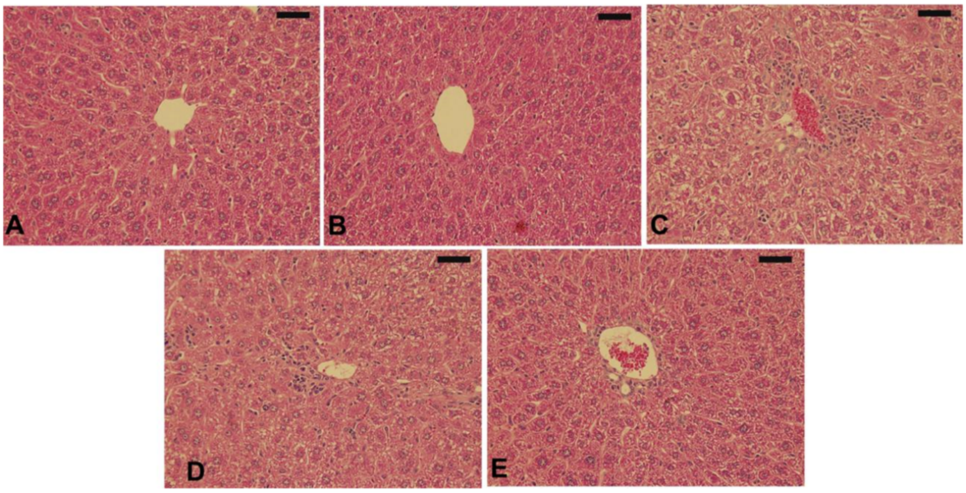

Figure 2. Effect of Bio-NS on coccidiosis-induced liver injury (n = 6). (A) Noninfected liver. (B) Bio-NS-treated liver. (C) Infected liver with inflammation and hepatocytic vacuolation. (D) Infected liver treated with Bio-NS. (E) Infected liver treated with amprolium. Sections stained with H&E. Scale bar = 50 μm.

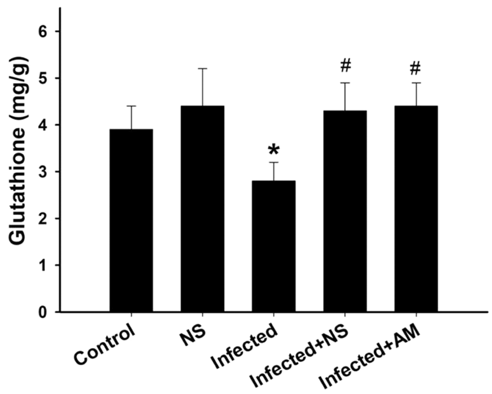

Figure 3. Effect of NS on coccidiosis-induced changes in the glutathione level. Values are mean ± SD (n = 6). Significance against noninfected (*) and infected (#) groups at $P < 0.05$.

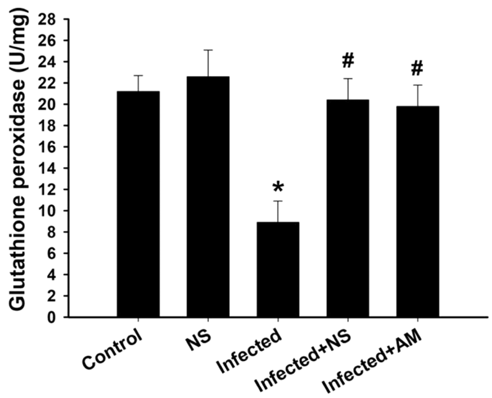

Figure 4. Effect of NS on coccidiosis-induced changes in glutathione peroxidase activity. Values are mean ± SD (n = 6). Significance against noninfected (*) and infected (#) groups at $P < 0.05$.

4. CONCLUSIONS

In general, the current study showed that biosynthesized silver nanoparticles produced encouraging outcomes in comparison to amprolium in *E. papillata*-induced mice and that it might also be added to the diet as a supplement and prophylactic in poultry farms. Therefore, biosynthesized nanosilver might be a potential alternative.

Table 3. Effects of biosynthesized nanosilver (NS) on liver function enzymes, AST, ALT, and ALP, in the liver of mice infected with *E. papillata*

group	AST	ALT	ALP
control	44.3 ± 6	13 ± 1	39.2 ± 8
NS	47 ± 4	17 ± 2*	51.7 ± 4
infected	67.5 ± 4*	33.5 ± 1*	94.7 ± 3*
infected + NS	48 ± 6$^{\#}$	18 ± 2$^{*,\#}$	47.8 ± 15$^{\#}$
infected + AM	47.7 ± 5$^{\#}$	22 ± 3$^{*,\#}$	65.12 ± 14$^{*,\#}$

Results are presented as mean ± SD. Significance against control (*) and infected (#) groups at **P** < 0.05. AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; NS, nanosilver; AM, Amprolium.

Table 4. Effects of Bio-NS on Fe, Mg, and Cu concentrations in the liver of mice infected with *E. papillata*

group	Fe	Mg	Cu
control	161.2 ± 11	385 ± 82	9.2 ± 2.5
NS	170.7 ± 9	717 ± 51	11.8 ± 3.5
infected	233.4 ± 22*	494 ± 47	11.8 ± 2.4
infected + NS	272.3 ± 61*	520 ± 104	17.6 ± 5.2
infected + AM	127.5 ± 41$^{\#}$	449 ± 86	10.1 ± 3.2

Results are presented as mean ± SD. Significance against control (*) and infected (#) groups at **P** < 0.05.

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Author Contributions

F.A.T., M.A.D., and R.A. contributed to the study design. M.A.D., E.M.A., S.E., and S.A. contributed to data acquisition. F.A.T., M.A.D., S.E., and R.A. performed the statistical analysis. All authors revised, improved, read, and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

Notes

The authors declare no competing financial interest.

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