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Research Article

Assessment of antioxidant activity of *Conoclinium coelestinum* leaf extract in *Plasmodium berghei* infected mice

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Abstract

Conoclinium coelestinum, is a North American species of herbaceous perennial flowering plant belonging to the family Asteraceae. This study aimed to assess the antioxidant activity of *Conoclinium coelestinum* on *P. berghei* infected mice. Thirty (30) mice were divided into 6 (six) groups of five (5) mice each, with group A fed diet and water *ad libitum* to serve as control while groups B and C were infected with *P. berghei,* and *P. berghei* administered with artesunate respectively. Groups D, E and F were infected with *P. berghei* and administered with 250 500 and 1000 mg/kg of *C. coelestinum* leaf extract respectively, for 4 days. The mice were sacrificed and blood samples were collected for catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione peroxidase (GPx) and malondialdehyde (MDA) analyses using standard methods. The data were analysed using GraphPad Prism 8.0.1. The results of the study showed that infection with *P. berghei* caused a reduction in CAT, SOD, GSH and GPx with increased MDA concentration compared with the control. Administration of artesunate, a standard antimalarial drug increased CAT, SOD, GSH and GPx, along with reduced MDA concentration compared with *P. berghei* treatment. Administration of *C. coelestinum* extract caused a dose- dependent increase in CAT, SOD, GSH and GPx with a dose-dependent decrease in MDA concentrations compared to the *P. berghei* treated samples. The study showed that *P. berghei* caused a reduction in CAT, SOD, GSH and GPx with increased MDA, while administration of *C. coelestinum* caused a dose-dependent increased in CAT, SOD, GSH, GPx with dose dependent decreased in MDA which highlighting its antioxidant potential.

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Conoclinium coelestinum, anti-oxidant, catalase, superoxide dismutase, reduced glutathi-one, glutathione peroxidase, malondialdehyde.

1. Introduction

Conoclinium coelestinum (L) DC., the blue mistflower (Asteraceae), is native to the southeastern United States, from eastern Texas east to the Atlantic coast and from southern Missouri, southern Illinois, southern Ohio, south of the Gulf coast [1]. It is a perennial herb with long, slender rhizomes and bright blue flower heads [1]. Non-volatile compounds isolated from this plant include the pyrrolizidine alkaloid intermedine, flavonoids gardenin A, nobiletin, luteolin, and luteolin derivatives, amorphane sesquiterpenoid 5, 8-epoxy-4, 6-dihydroxy-3-cadinanone, with a benzofuran

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glucoside 7-hydroxytoxol 7-O-glucoside [2]. There are apparently no reports on the volatile components from this plant. However, the essential oil from the aerial parts of *C. coelestinum* has been collected and analyzed [3].

Malaria has been recognised as a serious health problem since its inception. This disease is caused by protozoan parasites of the genus *Plasmodium* [4]. The strong negative pressure of the disease has likely forced the evolution of human populations in malaria endemic regions and the selection of some unique genetic variants. For example, thalassemia and sickle-cell disease, both which are genetic disorders affecting red blood cells, are commonly found in malaria-endemic areas [4], people with these two disorders show resistance to malaria. Another well-known example is the Duffy-negative blood type that the majority of people living in Central and West Africa have [5]. This confers specific resistance to infection by one particular *Plasmodium* species, *P. vivax* [6]. The spread of this trait in the population is estimated to have begun around 42,000 years ago, and today, *P. vivax* malaria is rare in these areas whereas *P. falciparum* malaria is prevalent [7].

The emergence of malaria parasite resistance to antimalarial drugs has prompted the search for new, effective, and safe antimalarial agents. For this reason, certain studies of medicinal plants in discovering new antimalarial drugs-are important and remain a crucial step in the fight against malaria [8]. The lack of chemical diversity among the anti-malarial drugs in use, leads to cross-resistance between drugs of the same class of compounds, further aggravating drug resistance [9, 10]. To overcome these problems searching for new anti-malarial agents from medicinal plants is of great importance for malaria control measures [11], which have their own toxic effects on body antioxidants. This study investigated the antioxidant activity of *Conoclinium coelestinum* leaf extract in *Plasmodium berghei* infected mice using Catalase, Superoxide Dismutase, Reduced Glutathione, Glutathione Peroxidase and Malondialdehyde as indicators.

2. Materials and methods

2.1. Animals

Thirty mice (30) of two months old male Swiss albino mice (18-20 g.) used for this study were obtained from the University of Nigeria animal house, Nsukka Enugu, Nigeria. The animals were acclimatized and housed in wooden cages under standard conditions (ambient temperature, 28.0 + 2.0 ^{O}C, and humidity 46%, with a 12 h light/dark cycle) for a period of twelve days and were fed with growers mash. All mice were allowed to free access of food and water *ad libitum*, throughout the experiment. Good hygiene was maintained by constant cleaning and removal of feces and spilled feed from cages daily.

2.2. Reagent

Commercially prepared catalase, superoxide dismutase, gluthathione peroxidase and malondialdehyde reagents were purchased from Lagos.

2.3. Identification and preparation of plant materials

Fresh leaves of *Conoclinium coelestinum* were collected in May, 2024, at the Choba area, Port Harcourt, River State. The plant specimen was botanically identified by a botanist and a voucher specimen was deposited for future reference at the Herbarium of the Department of Pharmacognosy, Madonna University, Nigeria. The plant leaves were sorted to eliminate any dead matter and other unwanted particles. The voucher specimen was thinly spread on the flat clean tray to prevent spoilage by moisture condensation and allowed to dry at room temperature for seven days [12]. The dried plant materials were grounded into powder using an electric mill.

The crude extract was prepared by cold maceration technique [13]. The powdered material was extracted by refluxing 45 g of the specimen in 80% ethanol (2.5 L) for three consecutive days at room temperature. The extracts were then filtered using cotton and then filtrate was passed through whatman filter paper (No. 3.15 cm size with retention down to 0.1um in liquids). The ethanol extract was concentrated in a rotary evaporator (Buchi type TRE121, Switzerland) to a yield of 5.08 %. The extract was kept in a tightly closed bottle in a refrigerator until used for anti-malaria investigation.

2.4. Plasmodium berghei

A strain of *Plasmodium berghei* (artesunate sensitive) was obtained from the University of Nigeria, Nsukka, Nigeria. *P. berghei* was subsequently maintained in the

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laboratory by serial blood passages from mouse to mouse in every 5-7 days. Three animals were used at a time as infected donors and parasite reservoir.

2.5. Acute toxicity study

The crude extract of *Conoclinium coelestinum* leaf was evaluated for its toxicity in *P. berghei* using non-infected Swiss albino mice aged 2 months weighing 18-20 g by the modified Locke's [14] method of determining the toxicity level of extract in mice. The test was carried out in two phases. In the phase one of the study, twelve mice were randomized into three groups of four mice each and were given extract orally at the doses of 50, 100 and 200 mg/kg body weight, respectively. The mice were observed for changes in physical appearance, gross behavioral changes and death in the first four hours and subsequently daily for ten days. In view of the results obtained from phase 1 treatment, phase 11 treatment was carried out using another fresh set of twelve mice randomized into three groups of four mice each and were administrated with 500, 1000 and 2000 mg/kg (b.wt.) of the extract orally. These were observed for signs of toxicity and mortality for the first four hours and thereafter daily for ten days. The LD50 was then calculated as the square root of the product of the lowest lethal and highest non-lethal doses, i.e., the geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded in the second phase. The oral median lethal dose was calculated using the following formula:

LD50 =√maximum dose for all survival × minimum dose for all deaths [14].

2.6. Research design

Thirty male Swiss albino mice randomly divided into six groups (A-F) of 5 rats each were used for this study. Group A which served as control was given 10 mL distilled water kg^{-1} body weight orally while the group B group was infected intraperitonally with 0.2 mL of infected blood containing about $1x10^7$ of *P. berghei* – parasitized erythrocyte per mL. The mice in group C was infected intraperitonally with 0.2 mL of infected blood containing about 1 x 10^7 of *P. berghei* – parasitized erythrocytes per mL and administered with 5 mg artesunate per kg body weight intraperitoneally. The experimental groups D, E and F were infected intraperitonally with 0.2 mL of infected blood containing about $1x10^7$ of *P. berghei* – parasitized erythrocyte per mL and administered with 250, 500 and 1000 mg/kg body weight of extract per day respectively. Treatments were initiated on day 0 and continued until day 4 and sacrificed.

2.7. Antimalaria study

The method of Peters [15] modified by Odeghe et al. [16] was adopted in the evaluation of the prophylactic potential of *Conoclinium coelestinum.* Blood smears were then made from each mouse 72 hours after treatment [17]. Increase or decrease in parasitaemia was then determined according to Girmaw and Engidawork [18].

$$\text{Parasitaemia (\%)} = \frac{\text{Number of infected red blood cells (RBC)}}{\text{Total Number of RBC}} \text{ X } 100$$

2.8. Inoculation

Each mouse (Groups B-F) except control (Group A) used in the experiment was infected intraperitonally with 0.2 mL of infected blood containing about $1x10^7$of *P. berghei*–parasitized erythrocyte per mL. This was prepared by determining both the percentage parasitemia and the erythrocyte count of the donor mouse and diluting the blood with isotonic 0.88% saline in proportions indicated by both determination [19]. The donor mice were monitored for signs of infection which include lethargy, anorexia, ruffled appearance, shivering and heat-seeking behavior. Blood was taken from the second day, to confirm level of parasitaemia in the donor mice, using the Iqbal et al., method [20]. Blood collected from the tail of the infected donor mouse was diluted with normal saline to produce a standard inoculum of 0.2 mL containing $1x10^7$ *P. berghei* infected erythrocytes [21]. Test mice were then inoculated with 0.2 mL of infected erythrocytes intraperitoneally (IP).

2.9. Antioxidant study

2.9.1. Determination of catalase activity

To measure catalase activity, the Goth method [22] was applied.

Procedure: A 50 µL reaction mixture was made on a 96-well microtiter plate using different I/L concentrations and 50 mM H_2O_2 in phosphate buffer (0.2 M, pH 7.4). It was then incubated for 5 min at 37 °C. An additional

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50 µL of catalase solution (50 µg mL-1 in phosphate buffer, 0.2 M, pH 7.4) was then added, and 30 min at 37 °C of incubation time was given after that. The enzyme activity was stopped using 100 µL of ammonium molybdate (64.8 mM). The absorbance at 405 nm was then measured using a microplate reader (SpectraMax M5, Molecular Devices, USA).

2.9.2. Superoxide dismutase (SOD)

Principle: The activities of the enzyme superoxide dismutase were estimated by measuring its ability to inhibit the auto-oxidation of pyrogallol in a superoxide anion-dependent manner as described by Marklund and Marklund, [23].

Procedure: A total volume of 2.6 mL was created by adding 0.01 mL of the tissue homogenate to 0.2 mL of EDTA (0.1M) containing 0.0015 percent NaCN, 0.1 mL of NBT (1.5 Mm) and 67 mM of phosphate buffer (pH=7.8). The absorbance of the solution was measured at 560nm against distill water after 0.05 mL of riboflavin was added. After consistently illuminating the test tubes for 15 min, the absorbance of the generated blue colour was once more measured. After comparing the absorbance of tubes with and without enzyme activity, the % inhibition was computed. One unit of enzyme activity, measured in U/mg of protein, was defined as the volume of the sample needed to eliminate 50% of the superoxide anion that was created.

2.9.3. Determination of reduced glutathione (GSH)

Principle: According to Ellman's [24] procedures, which involve the oxidation of GSH to GSSG and the enzyme-mediated degradation of cumen hydroperoxide, reduced glutathione (GSH) was measured.

Procedure: At a speed of 3000 revolutions per minute, a mixture of tissue homogenate (0.2 mL), distill water (1.8 mL), and a precipitating agent (3 mL) was centrifuged. The supernatant was then mixed with 4.5 mL of Ellman reagent in a volume of 0.5 mL. The same procedure was used to create a control mixture devoid of tissue homogenate. Within 30 minutes of the colour shift, the absorbance of the mixture was measured at 412 nm on a microplate reader in comparison to the control mixture.

2.9.4. Determination of glutathione peroxidase (GPX)

GPX estimation was carried out using Spectrophotometric method of Charmagnol et al. [25] as modified by Sigma Aldrich diagnostic.

Principle: The Principle of this assay showed that Glutathione peroxidase catalyses the oxidation of Glutathione (GSH) by cumene hydroperoxides. The oxidized glutathione is converted to the reduced form in the presence of glutathione reductase and NADPH. In this reaction the NADPH is oxidized to NADP + simultaneously. The decrease in absorbance at 340 nm was then measured.

Procedure: 20 µL of sample was added into a clean cuvette and 20 µL of distilled water into another cuvette to serve as reagent Blank, then 1 mL of working reagent was added to each cuvette. 40 µL of cumene hydroperoxide solution was added to each cuvette. The mixture in each of the cuvette was mixed and initial absorbances were read after 1 min and timer was started simultaneously. It was read again after 1 and 2 min. The reagent blank value was subtracted from that of the sample

2.9.5. Determination of malondialdehyde (MDA)

Malondialdehyde is one of the final products of the decomposition of polyunsaturated fatty acids (PUFAs) under the effect of free radicals released during stress. MDA estimation was done by colorimetric method.

Principle: The principle of this assay is based on the reaction of a chromogenic reagent, 2-thiobarbituric acid, with MDA at 25°C. One molecule of MDA reacts with 2 molecules of 2-thiobarbituric acid via a Knoevenagel-type condensation to form a pink colored complex (chromophore) with absorbance reading at 532 nm [26].

Procedure: The malondialdehyde (MDA) estimation was done by estimating Free MDA and Total MDA. To estimate free MDA, 200 µL of standard, sample and 200 µL of indicator solution were added into glass tubes labelled standards, samples and blank. This was followed by addition of 200 µL of indicator solution to all the tubes, mixed well and allowed to react for 45 min at room temperature to develop a pink color that is stable for several hours at room temperature. The absorbance of the resulting solution was measured at 532 nm. Measurement of total MDA required heat at 65 °C. 200 µL of standard, sample and 200 µL of indicator solution were added into glass tubes labelled standards, samples and blank, followed by

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Table 1. Effects of *C. coelestinum* on antioxidants parameters in *P.berghei* infected mice.

Group	CAT (µmol/mL/min)	SOD (mL/mM)	GSH (mM)	GPx (uL)	MDA (µm)
Control	11.58 ± 0.14	0.55 ± 0.01	13.3 ± 0.01	5.14 ±0.01	14.31 ± 2.50
P.berghei (P.b)	7.50 ± 0.07a,	0.18 ± 0.06a	0.29 ± 0.01a	0.29 ± 0.01a	54.49 ± 3.22a
P.b+Artesunate	10.44 ± 0.06 b	0.40 ± 0.00 b	8.17 ± 0.14 a, b	3.61 ± 0.00 a, b	21.55 ± 0.07a b
P.b+ 250mg/kg extract of *C. coelestinum*	9.41 ± 0.02a, b	0.19 ± 0.00a	4.59 ± 0.01a, b	2.65 ± 0.00 a, b	19.28 ± 0.05a b
P.b+ 500mg/kg extract of *C. coelestinum*	9.57 ± 0.00 b	0.27 ± 0.00a, b	5.47 ± 0.08a, b	3.05 ± 0.00 a, b	19.90 ± 0.05a b
P.b+1000mg/kg extract of *C. coelestinum*	10.10±0.00 b	0.35 ± 0.00 a, b	6.88 ± 0.14a b	3.22 ± 0.01 a, b	20.75 ± 0.06a b
F	377.5	380.9	2303	18930	15338
P	0.0001	0.0001	0.0001	0.0001	0.0001

a= significant when compared with control, b= significant when compared with *P. bergei*

addition of 200 µL of indicator solution to all the tubes and mixed well. The content of sample tube was heated at 65°C in waterbath for 45 min. The absorbance of the resulting solution was measured at 532 nm.

2.10. Statistical analysis

Results of the study were presented as a mean plus or minus standard error of mean (M ± SEM). Statistical significance was determined by one-way analysis of variance (ANOVA) while Bonferroni's test was used for multiple comparison tests to compare parameters within groups using computer software GraphPad Prism 8.0.1. Data from the groups were compared with their respective controls and differences at $P < 0.05$ were considered statistically significant.

3. Results

The result of the study showed that infection with *P. berghei* caused reduction in CAT, SOD, GSH and GPx with increase MDA concentration compared with the control. Administration of artesunate, a standard antimalarial drug increased CAT, SOD, GSH, GPx and reduce MDA concentration compared with the *P. berghei* treated. Administration of extract of *C. coelestinum* caused dose dependent increase in CAT, SOD, GSH and GPx with dose dependent decrease in MDA concentrations compared with *P. berghei* treatment as shown in Table 1 and Figs. 1-5.

4. Discussion

The emergence of malarial parasite resistance to antimalarial drugs has prompted the search for new, effective, and safe antimalarial agents. Since ancient times, plants and, in particular, edible flowers have found a place in people's diets. However, they are still considered as "modern" and have induced increasing interest. Fernandes et al. [8] had previously reported antimalarial activity of *Conoclinium coelestinum* and *Gymnema inodorum* leaf extract (GIE) in *Plasmodium berghei* infected mice.

In this study, the leaf extract of *C. coelestinum* was investigated for its antioxidant activity in *plasmodium berghei* infected mice using catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione peroxidase (GPx) and malondialdehyde (MDA) and as indicators in *Plasmodium berghei* infected mice. The results of the study showed that there was a significant decrease ($p < 0.05$) in CAT, SOD, GSH and GPx with increase in MDA, of *plasmodium berghei* infected mice, compared to their respective controls. This suggests that *P. berghei* caused a reduction in antioxidant activity. This is similar to the previous study of Adegoke et al. [27] who reported that *P berghei* caused oxidative stress in the brain by reducing brain antioxidants. Oxidative stress may play a central role in the onset of several diseases.

Malondialdehyde (MDA) is a marker of lipid peroxidation [28]. Free radicals are too short-lived to be detected directly in clinical systems, but oxygen free radicals react with lipids to produce lipid peroxidation products, which serve as indirect biomarkers of the in vivo oxidative stress status and related diseases. The extent of oxidative damage depends not only on ROS levels, but also on the mechanisms of cellular antioxidant defenses. Low levels of GSH, a molecule of critical importance in maintaining the stability of erythrocyte membranes, are related to cellular defense against xenobiotics and harmful compounds such as free radicals and hydroperoxides [29]. Glutathione acts as the first line

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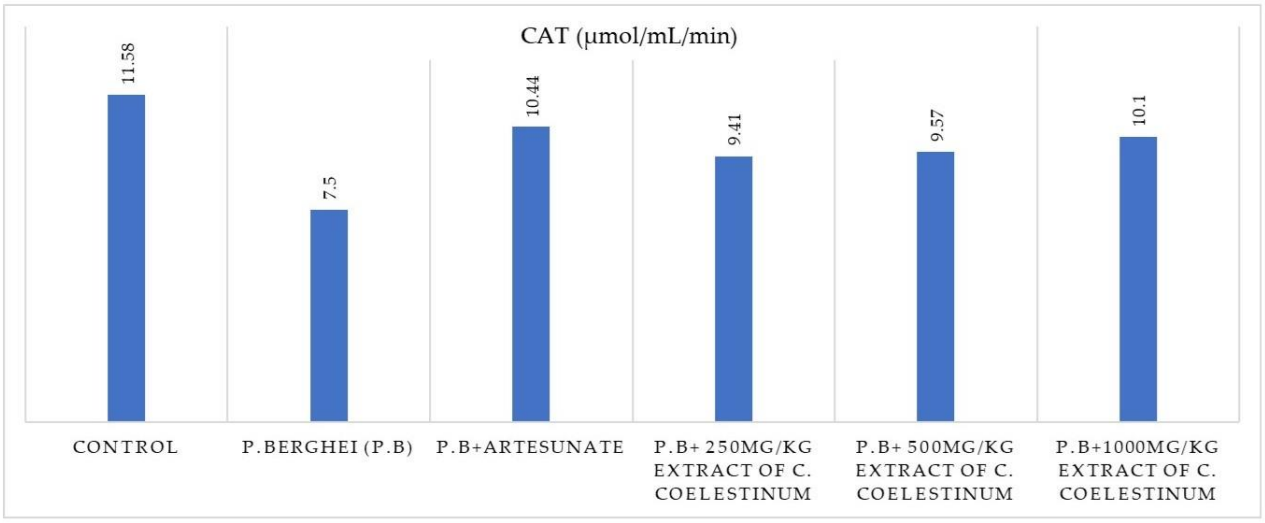

Figure 1. Effects of *C. coelestinum* on catalase in *P.berghei* infected mice.

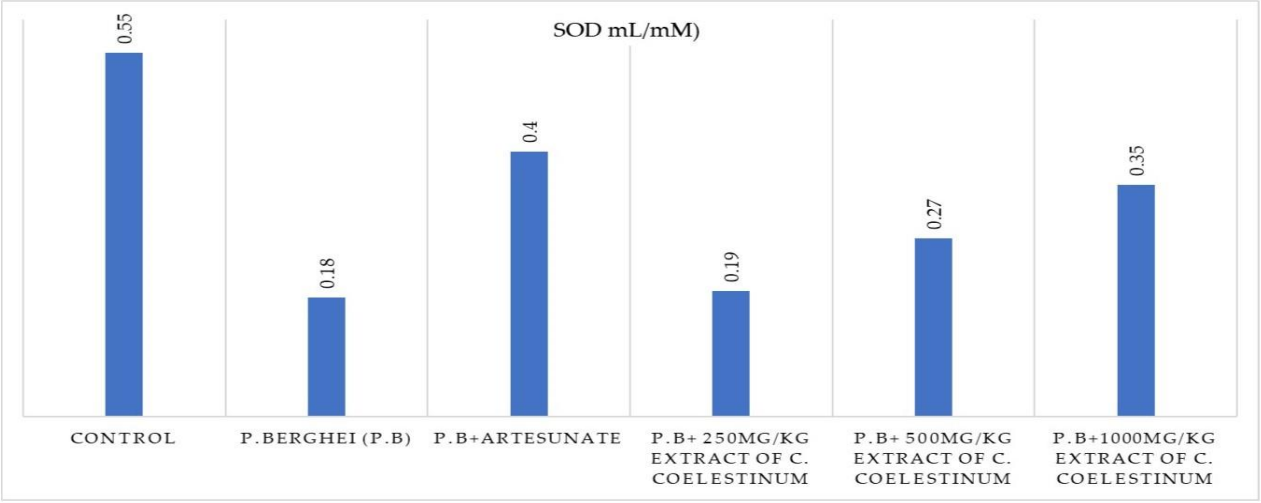

Figure 2. Effects of *C. coelestinum* on superoxide dismutase in *P.berghei* infected mice.

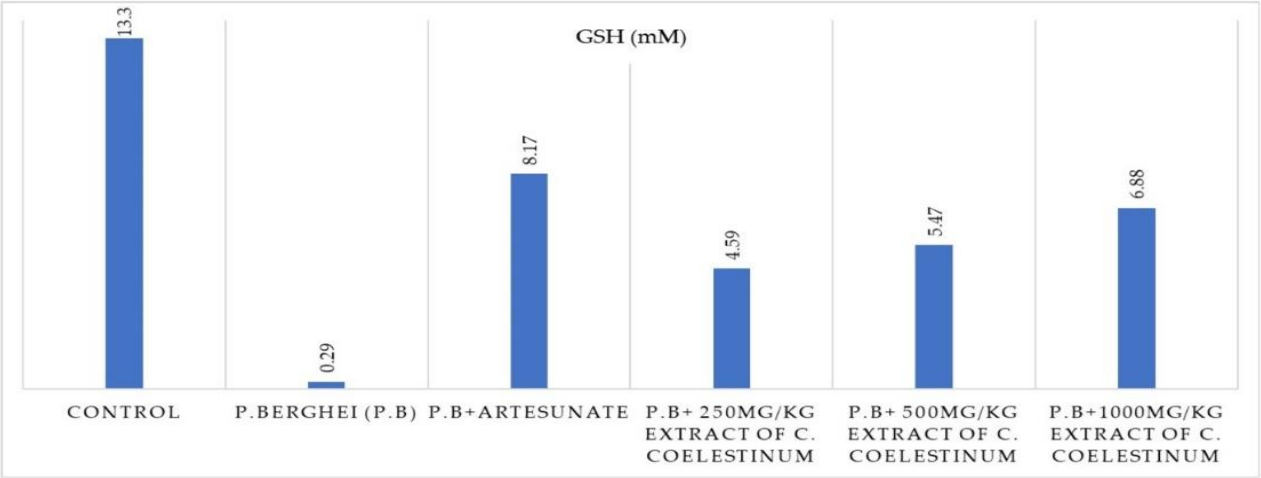

Figure 3. Effects of *C. coelestinum* on GSH in *P.berghei* infected mice.

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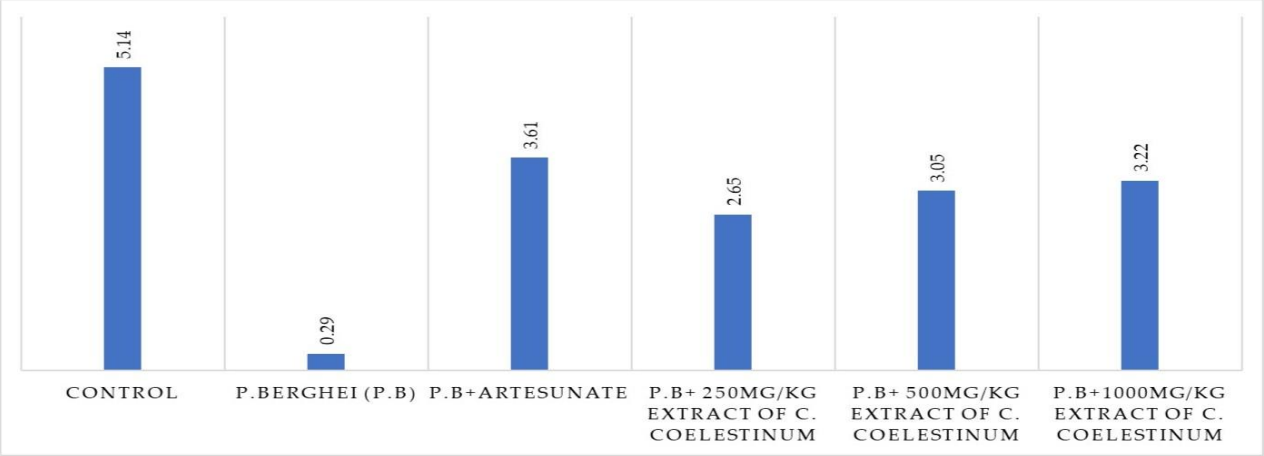

Figure 4. Effects of *C. coelestinum* on glutathione peroxidase in *P. berghei* infected mice.

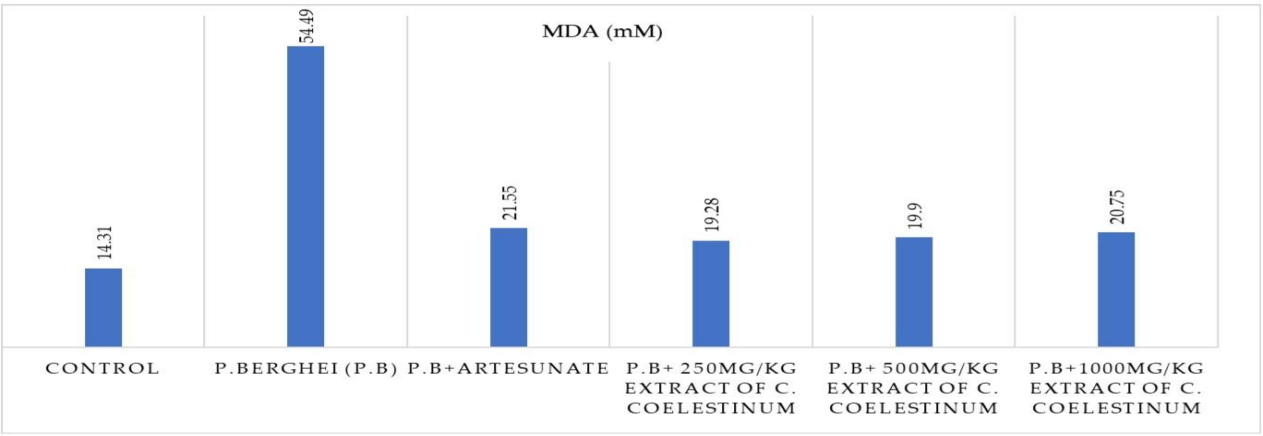

Figure 5. Effects of *C. coelestinum* on malondialdehyde in *P. berghei* infected mice.

of defense against free radicals produced by antitumor molecules. Decreased GSH levels can be explained by a decrease in GSH synthesis and/or increased consumption to remove peroxides and xenobiotic [30].

There was a dose-dependent increase in CAT, SOD, GSH and GPx with decrease in the MDA of infected mice treated with *C. coelestinum* compared with *P. berghei* infected mice. This suggests that *C. coelestinum* reversed the decrease in CAT, SOD, GSH and GPx with increased in MDA, caused by *P. berghei.* This results further support the antioxidant capacity of *C. coelestinum,* as reported by Odeghe et al. [31]. *C. coelestinum* contain alkaloids, saponins, glycosides, proteins, flavonoids, steroids, tanins and triterpenoids which have been documented to inhibit oxidative stress associate with neurodegenerative disorder and inflammation [32].

Antioxidants are substances that can remove free radicals from cells and prevent free radical induced oxidative damage. They are present in many plant derived foods such as spinach, arugula etc. A previous study by Odeghe et al. [31] reported that *Conoclinium coelestinum* is rich in antioxidant due to its rich bioactive compound profile. The *Conoclinium coelestinum* extract in this study reversed the oxidative damage caused by *P. berghei* by increasing CAT, SOD, GSH and GPx and reducing MDA concentrations. A previous study by Odeghe et al. [31] showed that *C. coelestinum* exhibited potent antioxidant activity due to its rich bioactive compound profiles. Odeghe et al. [31] reported that *C. coelestinum* inhibit lipid peroxidation, and scavenge H_2O_2, Nitric oxide and superoxide which shows its ability to neutralise reactive oxygen species and protect against oxidative damage.

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5. Conclusions

This study showed that infection with *P. berghei* caused a reduction in catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and glutathione peroxidase (GPx), with increased malondialdehyde (MDA) levels suggesting that it caused oxidative stress. Administration of *C. coelestinum* caused dose-dependent increases in CAT, SOD, GSH and GPx with decrease in MDA concentration highlighting its antioxidant activity. This may be due to the presence of a rich profile of bioactive compounds in *C. coelestinum*.

Disclaimer (artificial intelligence)

Author(s) hereby state that no generative AI tools such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators were utilized in the preparation or editing of this manuscript.

Authors' contributions

Concept and design of the study and correspondence, A.O.A.; biochemical analysis, I.M.G.O.; biochemical analysis, E.M.A.; statistical analysis, C.P.I.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest.

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