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Effects of fungus extract *Pestalotiopsis microspora* (Speg.) Bat. & Peres, 1966, on the antioxidant system of mice infected with *Plasmodium berghei* Vincke & Lips, 1948

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ABSTRACT

The experiment is focused on evaluating the effect of *Pestalotiopsis microspora* on the antioxidant system of mice infected with *Plasmodium berghei*. Twenty-five male albino mice of average weight of 20kg were distributed randomly into 5 groups. Infected untreated (group 1), Infected untreated (group 2), Infected treated with chloroquine (group 3) Infected treated with 25 mg/kg fungus extract (group 4), Infected treated with 50 mg/kg fungus extract (group 5). All the groups were infected with *Plasmodium berghei* via the intra-peritoneal route. After seven days' post infection, infected treated with chloroquine (group 3) Infected treated with 25 mg/kg fungus extract (group 4), Infected treated with 50 mg/kg fungus extract (group 5) were treated with chloroquine, 25 mg/kg and 50 mg/kg fungus extract respectively. The treatment lasted for 4 days. The results of this study revealed that, the effect of the fungus extract significantly increased the ALT, catalase, MDA, uric acid and XO in serum of infected treated group when compared with the decrease in the uninfected untreated group.

Keywords: *Pestalotiopsis microspora*, chloroquine, oxidative stress, Uric acid, *Plasmodium berghei*, male swiss albino mice, blood glucose

1. INTRODUCTION

Malaria is an endemic infectious disease in warm climate and particularly in under developed and developing countries of the world (Tesfaye and Alamneh, 2014). It is the deadliest parasitic infection in the world and a public health problem nationwide (Yoshida *et al.*, 2010; Innocent *et al.*, 2013), Malaria, a major tropical disease transmitted by *Anopheles* mosquitoes, remains a critical health problem. There were more than 247 million malaria cases and about 619 000 malaria deaths worldwide in 2022 (WHO 2022). Biocontrol of mosquito populations through entomopathogenic fungi is one of the recent research areas for vector control (Renuka *et al.*, 2023).

In Nigeria, malaria is a major primary healthcare challenge for the children and pregnant women, responsible for 30% childhood mortality and 11% maternal mortality (Katsayal and Obamiro, 2007; Okemwa and Omosa, 2017). Malaria disease continues to have severe socioeconomic impact on the population and it also affect all and sundry irrespective of social status, human race and other parameters in Nigeria. Malaria parasites multiply in hepatocytes (Exoerythrocytic stage) liver and erythrocytes (Erythrocytic stage) which occur in the red blood cell of their vertebrate hosts. The clearance and survival of parasite is a role played by the immune system, which has been hypothesized to depend on the redox metabolism of the host-parasite unit and rescue cell on pro-oxidant and antioxidant (Becker *et al.*, 2004; Sorci and Faivre, 2009; Jortzik and Becker, 2012; Muller, 2015). Previous studies show that malaria transmission entails extensive interactions between parasites and mosquitoes (Ramelow *et al.*, 2023; Zhang *et al.*, 2023).

During a malaria infection, both host and parasites are under oxidative stress. This can be caused in two ways: one, by the exogenous reactive oxidant species (ROS) and reactive nitrogen species (RNS) produced by the host immune system, and two, by the endogenous production of ROS generated during the digestion of host cell haemoglobin and concomitant biochemical reactions (Kanzok *et al.*, 2000; Rahlfs *et al.*, 2002; Muller *et al.*, 2003). Oxidative stress has been implicated in the development of chronic human diseases and degenerative ailments (Willcox *et al.*, 2014). Oxidative stress is elevated during malaria and has been associated with different complications (Vasquez *et al.*, 2021). Minimizing oxidative stress could therefore ameliorate many physical ailments and help prevent certain degenerative diseases caused by free radicals (Iyawe and Onigbinde 2009).

Oxidative stress is a cellular phenomenon or condition which occurs as a result of physiological imbalance levels between the antioxidants and oxidants (free radicals or reactive species) in favor of oxidants. *Pestalotiopsis microspora* is an endophytic fungus which is capable of breaking down and also digesting polyurethane (Jonathan *et al.*, 2011). These bioactive molecules according to Mustapha *et al.*, 2009; 2010 possess antimalarial, antimicrobial and also antioxidant activity.

Glucose is vital for plasmodium as plasmodium has no capacity to store energy in the form of glycogen, so they rely on an exogenous supply of glucose. Malaria parasites are dependent on glucose as a nutrient source (Pierre-lutgen, 2013). According to Humeida *et al.*, 2011, infected erythrocytes exhibits a substantial increase in its permeability to low molecular weight sugar, while the metabolism of the parasite utilizes up to 75 times more glucose than uninfected erythrocytes. Some studies have compared oral, sublingual, or intravenous glucose administration (De buck *et al.*, 2019). According to the postulation, Srinivasan *et al.*, 2003, glucose stimulates the endothelial production of the pro-inflammatory interleukin-8 and can

probably stimulates the adhesion of plasmodium falciparum parasites. Uric acid is an organic molecule that can be either consumed through the diet or synthesized endogenously in the liver and adipose tissue from purines by the action of the enzyme xanthin oxidoreductase (Maiuolo *et al.*, 2016). Approximately, 65-75% of uric acid is excreted by the kidneys while the remaining 25-35% is eliminated through the intestines.

The overall balance of uric acid in the body is determined by the equilibrium between its excretion and synthesis (Maiuolo *et al.*, 2016). Although many studies have reported antioxidant effects of *Pestalotiopsis microspora*, there is paucity of information regarding the effect of the fungus on malaria-induced metabolic disorders. Therefore, this research work is aimed at evaluating the effect of filtrate extract *Pestalotiopsis microspora* on metabolic disorders induced by *P. berghei berghei* on male swiss albino mice and effects of fungus extract on the changes caused by malaria on antioxidants such as uric acid, blood glucose, xanthine oxidase and MDA.

2. MATERIALS AND METHODS

2. 1. Collection and preparation of fungus extracts *Pestalotiopsis microspora*

The stock cultures of *P. microspora* (Photo 1) was collected from Mycology Laboratory, Department of Plant Biology, University of Ilorin, Ilorin, Kwara State, Nigeria. The fungus was grown in 250 mL sterile conical flask containing 100 mL Potato Dextrose Broth and incubated at 25 ± 2 °C for 14 days (Garuba *et al.*, 2014). After incubation, the culture filtrates were filtered into pre-sterilized conical flasks using Whatman no. 1 filter paper. The filtrates were stored in a refrigerator at 4 ± 2 °C.

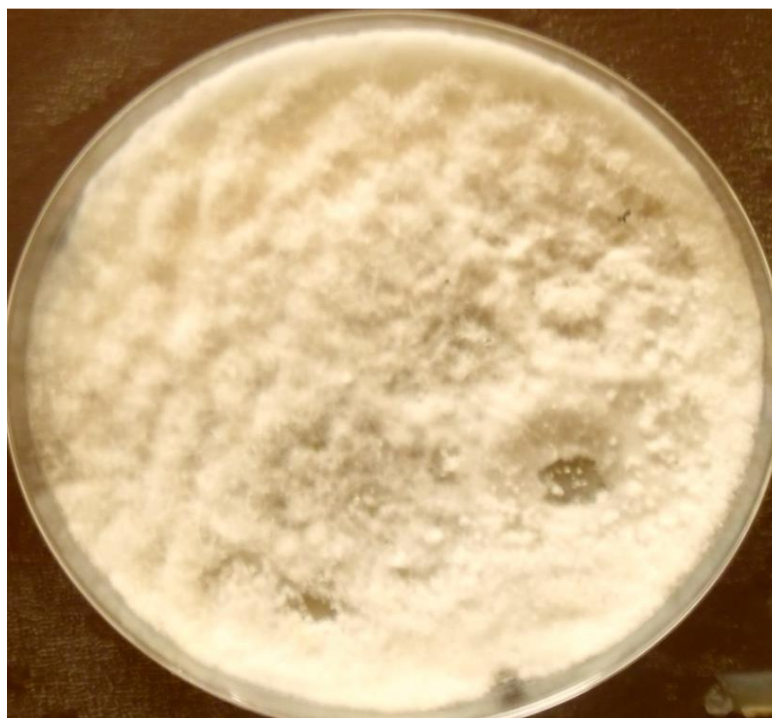


Photo 1. Pure culture of *Pestalotiopsis microspora* (Speg.) Bat. & Peres, 1966.

2. 2. Reagents and materials

Distilled water, analar grade 0.9% normal saline, disposable plastic syringe, 70% ethanol, animal feed, Olympus research light microscope, microscope glass slide, weighing balance, latex powdered examination gloves, adjustable micropipette, dissecting set, immersion oil, Giemsa stain, stainless metallic feeding cannula, digital measuring scale, measuring cylinder, conical flask/beaker, magnetic stirrer and plain bottles were used in the present study.

2. 3. Experimental animals and parasite strains

30 male Swiss albino mice, 1-2 weeks of age and 12-18g of weight were obtained from animal house holding of LAUTECH. They were kept in the animal care facility of the zoology department, faculty of life sciences university of Ilorin for one week prior to the initiation of the experiment for acclimatization (Abdulkareem *et al.*, 2019) under standard environmental and laboratory conditions. The mice were kept at about 25 °C room temperature and were exposed to 12 hours' light and 12 hours' darkness and they had access to standard commercial grower mash diet and water *ad-libitum* every 12 hours. The animals were housed in plastic cages with perforated net-cover and soft wood shavings as beddings at Zoology's animal house. Handlings of animals were in conformity to the National Institutes of Health guidelines on the care and use of laboratory animals (ILAR, 2011). On weekly basis, the parasites were maintained by serial passage of blood from infected mice having a parasitemia level of 20-30% to non-infected mice.

2. 4. Parasite inoculation

Anka 1 N-strain of malaria parasite, *P. berghei* maintained in mice by serial passaging from mouse to mouse was used throughout the experiment. The donor mouse was obtained from the animal house of the pharmacology unit, of Obafemi Awolowo University (OAU) of Nigeria. The method of blood- induced plasmodium infection in mice was first described by Peters. Albino mice previously infected with *Plasmodium berghei* were used as donor animals. Blood was obtained from each donor mouse by cardiac puncture using a sterilized containing 10 IU of heparin. The blood was suitable diluted with sterile normal saline so that the final inoculum (0.2 ml) for each mouse contained the required number of 10^7 parasitized red blood cells as described by Peters and Knight and Williamson.

2. 4. 1. Drug preparation and administration

The standard drug (chloroquine) obtained at a pharmaceutical store at Tanke, Ilorin, was prepared in aqueous solution and administered orally at a dose level of 20 mg/kg body weight. 25g of the tablet was made into powdery form by grounding. The powdery form was then weighed (24g) and dissolved in 6ml distilled water. Preparation of *Pestaloitiopsis microspora* was done by dissolving 0.0026g in 12 ml distilled water, giving 100 mg/kg concentration of sodium acetate stock solution. Of this 100 mg/kg, preparation of 50 mg/kg and 25mg/kg were made through the process of serial dilution.

2. 5. Blood smear preparation

Thin blood smears were collected from the caudal tip of infected mice every two days and prepared on microscope slides before, during and after infection. The slides were air-dried

in the absence of dust and fixed in 70% methanol, stained for 30 minutes in 5% solution of Giemsa stain (WHO, 2000).

2. 6. Statistical analysis

Statistical analysis was performed with Graph-pad Prism software version 5 using one-way analysis of variance (ANOVA), significant difference was determined at 95% confidence levels $P \leq 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

As shown in Fig 1, there was a significant increase in uric acid in the serum of the uninfected control (group 1) and a significant decrease ($P < 0.05$) in the uric acid in the serum of infected untreated (group 2), showing a significant difference between all the groups. However, there was a significant decrease in uric acid in serum of the infected mice administered with 20 mg/kg chloroquine (group 3) and in the infected mice treated with 50 mg/kg fungus extract (group 5) when compared with the infected mice treated with 25 mg/kg fungus extract (group 4) where there was a significant increase.

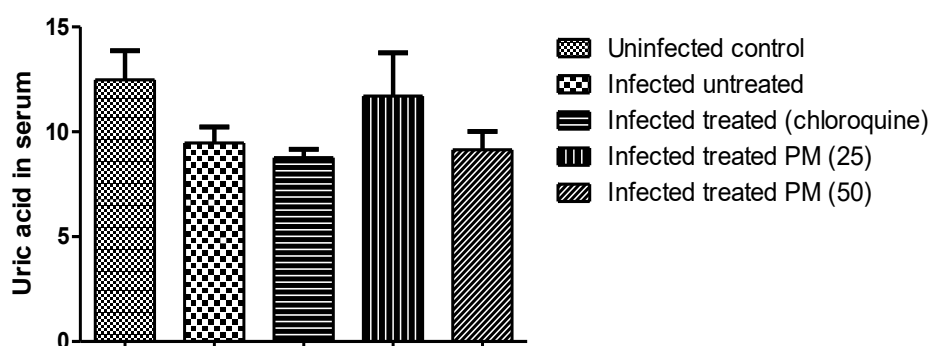


Figure 1. Effect of *Pestalotiopsis microspora* on uric acid in serum.

As shown in Figure 2, there was an increase in blood glucose level in the uninfected control (group 1) compared with the infected treated (group 2) where there was a slight decrease in blood glucose, showing no significant difference ($P > 0.05$) between the groups. However, there was a significant increase in blood glucose level of infected mice treated with 25 mg/kg fungus extract (group 4) when compared with infected mice treated with 20 mg/kg chloroquine (group 3) and in infected mice treated with 50 mg/kg fungus extract (group 5) where there was a slight decrease in blood glucose level.

Figure 4 shows that there was a slight decrease in MDA in the serum of the uninfected control (group 1) when compared with the significant increase ($P < 0.05$) in MDA in the serum of the infected untreated (group 2) showing no significant difference between the uninfected control (group 1) and infected untreated, there was a slight decrease in MDA in the serum of the uninfected control (group 1) when compared with the significant increase ($P < 0.05$) in MDA in the serum of the infected untreated (group 2) showing no significant difference between the

uninfected control (group 1) and infected untreated (group 2). However, there was a slight decrease in MDA in the serum of infected mice administered with 20 mg/kg chloroquine (group 3) and in the infected mice treated with 25 mg/kg fungus extract (group 4) when compared with the infected mice treated with 50 mg/kg fungus extract (group 5) where there was a significant increase.

This study investigated the effects of *Pestalotiopsis microspora* on xanthine oxidase, MDA, blood glucose and Uric acid in *P. berghei* infected mice, for the development of novel effective anti-malaria drugs. Findings of this study showed that *P. berghei* infection suppressed body weight and food intake. This study also demonstrated that the elevated serum level of MDA in infected untreated mice as compared to the uninfected group observed in this study, is in line with George *et al.* (2012), Adisa and Sulaimon (2017) and Iyawe and Onigbinde (2011).

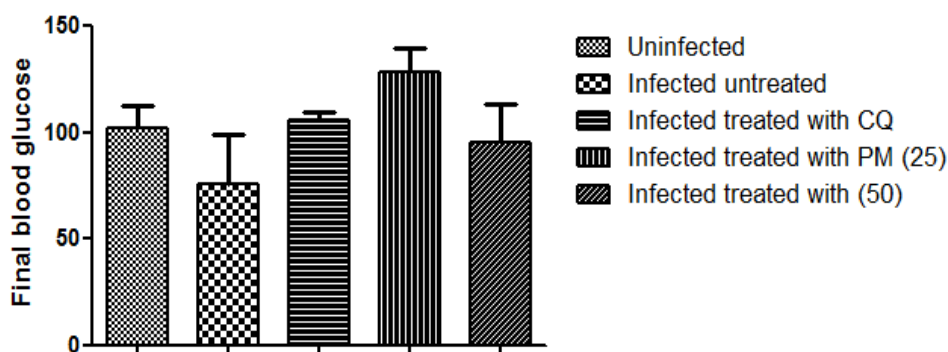


Figure 2. Effect of *Pestalotiopsis microspora* on blood glucose after treatment.

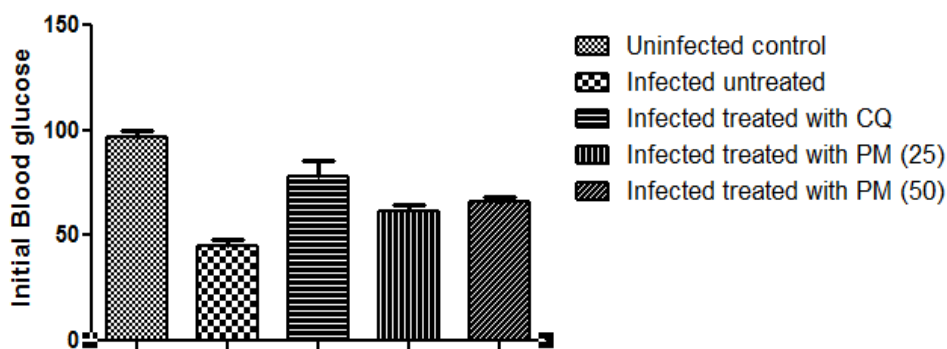


Figure 3. Effect of malaria on blood glucose during infection; showing a significant increase in the blood glucose of the infected control group when compared with the infected untreated group where there was a significant decrease.

This may imply that malaria parasite increases the risk of oxidative stress by increasing the concentration of the lipid peroxidation leading to high ROS generation in the host. Similarly, increased serum concentration of MDA in chloroquine treated group conformed to the work of Goerge *et al.* (2012) where chloroquine was reported to increase the production of ROS. In contrast, treatment with ethyl extract of *Pestalotiopsis microspora* reduced MDA, suggesting

that the extract is a better source of anti-malaria drug. Hyperuricaemia is an independent risk factor for metabolic diseases (Bagheri *et al.*, 2016). In this present study, *P. beghei* increased the level of uric acid suggesting a metabolic derangement in malaria infection. However, the administration of 50 mg/kg *Pestalotiopsis microspora* reduced the effect of hyperuricaemia, implying that it has ability to improve malaria-induced diseases.

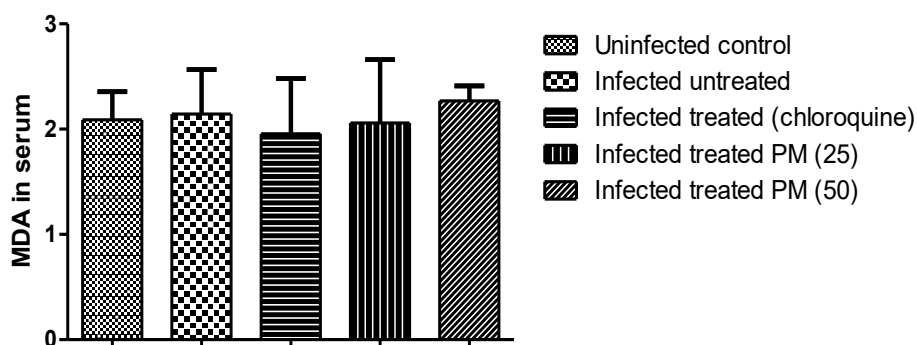


Figure 4. Effect of *Pestalotiopsis microspora* on MDA in serum of infected, uninfected and treated groups.

Table 1. The effect of *Pestalotiopsis microspora* on food intake during pre-infection, infection and treatment stages.

Group	Pre-infection	Infection	Treatment
Uninfected control	13.26 ±0.04564	12.65 ±0.04564	10.28 ±0.04564
Infected untreated	9.770 ±0.05773	7.760 ±0.05774	3.390 ±0.05774
Infected treated with chloroquine (20 mg/kg)	8.000 ±0.05774	6.953 ±0.08819	2.720 ±0.05774
Infected treated with fungus extract (25 mg/kg)	6.860 ±0.05773	7.820 ±0.05774	4.010 ±0.05774
Infected treated with fungus extract (50 mg/kg)	5.410 ±0.1000	4.680 ±0.1000	1.760 ±0.01000

Table 1 shows the effect of *Pestalotiopsis microspora* in all groups during the period of pre-infection where there was a significant increase in food intake by uninfected control (group 1) and a slight decrease in food intake by infected untreated group (group 2), during the period of infection where there was a significant increase in food intake by the uninfected control (group 1) when compared with infected untreated (group 2) where there was a decrease in food intake, showing a significant difference ($P < 0.05$) and during the period of treatment where

there was a significant increase in food intake by uninfected control (group 1) when compared with uninfected untreated (group 2) where there was a significant decrease, showing a significant difference ($P < 0.05$) between the two groups.

Summary of results obtained from the analysis of food intake during the experiment showing the effect of *Pestalotiopsis microspora* on the antioxidant system of mice infected with *P. berghei* are presented below:

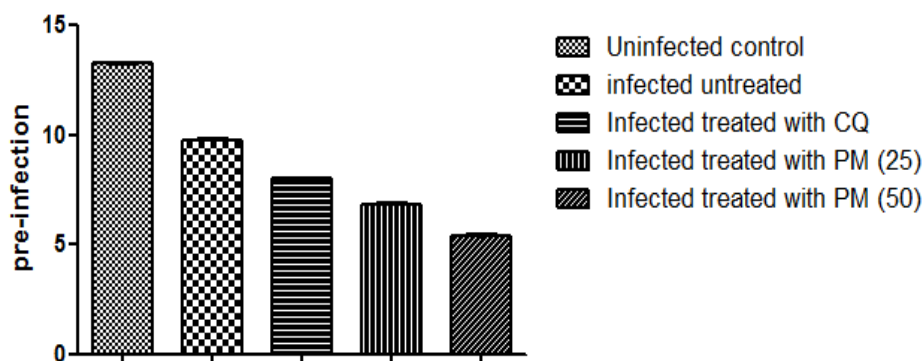


Figure 5. Effect of *Pestalotiopsis microspora* on food intake pre-infection stage.

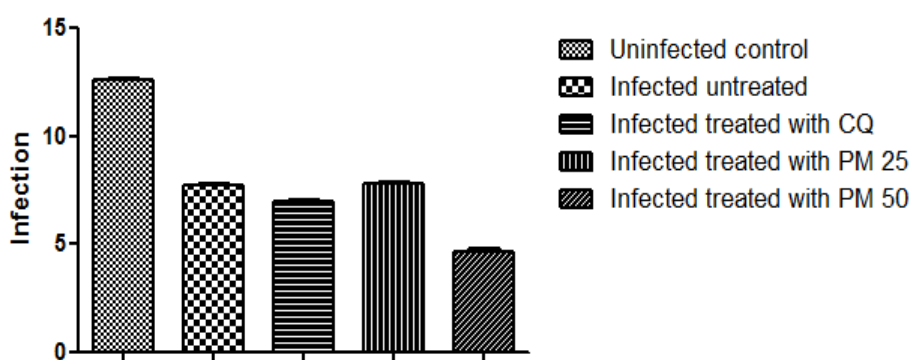


Figure 6. Effect of *Pestalotiopsis microspora* on food intake infection stage.

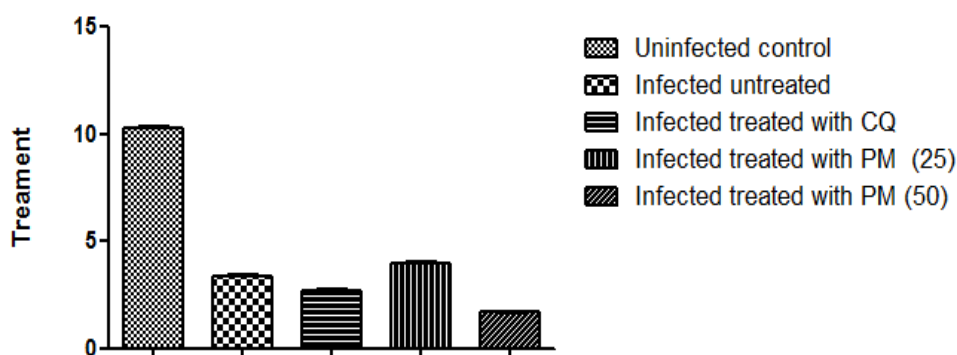


Figure 7. Effect of *Pestalotiopsis microspora* on food intake post-infection stage.

When compared side by side, the three figures representing the pre-infection, infection and treatment stages revealed that the fungus extract produced varying effects on food intake in mice, showing a slight increase in infected treated with 25 mg/kg fungus extract and a significant decrease in infected treated with 50 mg/kg. Throughout all stages, the uninfected control group consistently exhibited higher food intake compared to the infected groups.

4. CONCLUSION

The study showed that *Plasmodium berghei* infection induces stress especially during high infection in subjects. However, during malaria treatment, the administration of the fungus extract reduced the oxidative stress induced by the malaria parasite, showing that the fungus extract is a good therapeutical project reducing metabolic syndrome in malaria infection.

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