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Effect of Formulated Cookies from Pleurotus Pulmonarius Flour on Parasitemia Level and Hematological Indices in Plasmodium Berghei (Nk–65 Strain) Infected Mice

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ABSTRACT

A nutritious supplement that has both nutritional and therapeutic qualities is the mushroom. Malaria is a potentially fatal illness that strikes a wide range of individuals worldwide, with Nigeria having the highest incidence in Africa. It typically appears when the immune system is weakened. The antimalarial potential of cookies containing various formulations of Pleurotus Pulmonarius (10, 15, 20, and 25%) was examined in this study. It was examined how the cookies affected the mice's weight, temperature, parasitemia level, and hematological indicators. With the exception of the normal control group, 72 albino mice weighing between 24 and 26 g were divided into 9 (n = 8) groups and infected with a standard inoculum of the chloroquine-sensitive strain NK65 Plasmodium berghei. Comparing the parasitemia levels of the infected mice fed the formulated cookies to those of the positive and negative control showed a significant ($p<0.05$) decrease in parasitemia. Compared to animals treated with chloroquine (87.16%) and mice not treated (0.00%), the parasitemia level on the fifth day was 86.59% suppressed in the infected mice fed 25% cookies. Furthermore, compared to the untreated infected mice, the red blood cell, hemoglobin, and packed cell volume counts of the infected mice fed wheat-mushroom cookies showed a substantial ($p<0.05$) increase. According to this study, the bioactive chemicals in P. Pulmonarius have the ability to prevent malaria and improve the antioxidant state of the host when added to cookies.

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Introduction

Food ingredients called Pleurotus Pulmonarius are non-toxic and have been used to cure, prevent, and enhance health. Throughout history, traditional folkloric medicine has utilized mushrooms as a valuable food source, particularly in Africa. These mushroom species are consumed because they are readily available, produce great yields at low costs, are simple to cultivate, and require little labour [1,2].

The therapeutic (anti-inflammatory, antibacterial, antitumor, hypoglycemic, antiviral, anti-microbial, anti-hypercholesterolemic, anti-hypertensive, antineoplastic, anti-cancer, antifungal, and anti-oxidative) and nutritional qualities of mushrooms are well-known across the world. Because of their edibility, nutritional content, and delicacy, they are easily grown and consumed and have been utilized as food supplements in many civilizations. They are sources of biologically useful components and functional foods with significant therapeutic potential for the prevention and management of various diseases [3-7].

Nearly fifty percent of the world's population is at risk of malaria, a potentially fatal disease. In 2020, there were projected to be 241 million cases of malaria worldwide. Top of the list, Sub-Saharan Africa accounted for around 96% of worldwide malaria deaths and 95% of cases in 2020. The antiplasmodial effect of medicinal mushroom extracts is noteworthy, indicating the biological significance of mushrooms due to their multiple bioactive components. Thus, they may have potential use in the treatment and management of malaria. Because of their potential for therapeutic use, mushrooms are currently the subject of redoubled study efforts. Because of its therapeutic qualities and potential for healing, mushrooms are also an essential component of traditional medicine. Malaria is a potentially fatal disease that affects people all around the world [8-10].

Among baked foods worldwide, cookies make up one of the most popular snack categories. When provided to the customer in a convenient manner, it functions as a nutritious snack. These are typical, easily prepared, low-cost, ready-to-eat food items that include essential dietary and digestive guidelines. Pleurotus Pulmonarius is an important and valued medicinal fungus, as evidenced by its medicinal characteristics and therapeutic uses. The creation of novel, effective medications from plant sources in the form of snacks has proven beneficial and economical in light of the growing trend of drug resistance [11,12].

Developing products that suit the demands of individuals with health difficulties and diseases like malaria by serving as functional and nutraceuticals is one of the best strategies to increase the utilization of mushrooms. When creating mushroom cookies to treat illnesses, customers and nutritionists can use the generated data as a reference. Although mushroom extracts have been utilized in medicine, nothing is known about the scientific underpinnings and dietary value of antimalarial cookies. Thus, the purpose of this study is to investigate how cookies made with wheat and Pleurotus Pulmonarius mushroom flour affect the weight, temperature, parasitemia count, and hematological parameters (PCV, RBC level, and hemoglobin) of mice infected with Plasmodium berghei.

Materials and Methods

Sample collection

The LTC Mushroom Farm in Osogbo, Osun State provided the cultivated mushroom (Pleurotus Pulmonarius) fruiting bodies. In the Department of Pure and Applied Biology at Ladoke Akintola University of Technology, Ogbomosho, it was recognized and verified by a microbiologist. The Pleurotus Pulmonarius was characterized and registered with the National Centre for Biotechnology Information - GenBank Database under the accession number MK751847.

Chemicals

All Chemicals and reagents used were of analytical grade. Sulphosalicyclic, citrate buffer, sodium phosphate, trichloroacetic acid, glutathione, 5,5'–dithio-bis (2-nitrobenzoic acid),1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Sigma-Aldrich Chemical Co, USA and other reagents including chloroquine diphosphate. The standard antimalarial drug used in this study was chloroquine (Diphosphate salt –50- 63-5 EC No. 200-055-2) procured from Matador Dafon Pharmaceuticals Limited, Akure, Ondo State, Nigeria.

Diet Formulation

The major material used was Pleurotus Pulmonarius mushroom fruiting bodies, other materials for the production of the cookies are wheat flour, cocoa butter, milk, sugar, salt, fresh eggs and baking powder. All the chemicals and reagents used were of analytical grade.

Preparation of Pleurotus Pulmonarius Flour

The Pleurotus Pulmonarius mushroom fruiting bodies as presented in Figure.1 were collected from LTC Mushroom Farm at Ilupeju-Offatedo Osogbo, Osun State. It was cleaned with distilled water to remove specks of dirt and other field-damaged portions. The cleaned and sorted mushrooms were sliced with a knife into smaller sizes (3 ± 1 mm) thickness and placed on the tray with wax paper and dried at 40 oC for 24 hours using a heat drying oven, Model DHG-9053A. The dried mushroom was then ground in an electric grinder (model: heavy-duty motor 1100 Watts) and sieved through (150 μm) from the laboratory. The Pleurotus Pulmonarius mushroom powder was kept in airtight opaque zip-lock low-density polyethylene bags, labelled and kept inside a tight-fitting plastic container. The container was stored inside a freezer at -4 °C [5].

Figure 1: Freshly harvested cultivated Pleurotus Pulmonarius mushroom

Preparation of wheat Pleurotus Pulmonarius mushroom cookies

Four composite samples of wheat Pleurotus Pulmonarius were prepared by replacing 10%, 15%, 20%, and 25% of wheat flour with Pleurotus Pulmonarius flour adopting Biao method. All other ingredients were weighed accurately as in the formulation shown in Table 1, while control cookies were produced from 100% wheat flour. The cookies were produced using the formulated blend of refined wheat flour and mushroom flour was added alongside, milk, salt, and baking powder and mixed gently with the creamed mass of shortening and sugar powder mixture. The dough was prepared by adding the required amount of water to the mixture. The dough was rolled out and sheeted by a rolling pin and cut using a rectangular cutter. The formed batter was baked in an oven at 160 °C for 15-20 minutes, cooled, and stored in air-tight pouches for further analyses as shown in Figure 2 [13,14].

Table 1: Flour Blend Formulations and Other Ingredients

Samples	Wheat flour (g)	Mushroom (g)	Cocoa butter (g)	Egg Albumin (g)	Milk (g)	Salt (g)	Sugar (g)	Baking Powder (g)
A1	100	0	30	10	10	1	30	1
A2	90	10	30	10	10	1	30	1
A3	85	15	30	10	10	1	30	1
A4	80	20	30	10	10	1	30	1
A5	75	25	30	10	10	1	30	1

A1=10% MF and 90% WF; A2= 15% MF and 85% WF; A3= 20% MF and 80% WF;
A4=25% MF and 75% Wheat Flour (WF) and A5= 100% Mushroom Flour (MF)

Figure 2: Formulated Wheat-Mushroom Cookies

Animal and Parasite

A total of seventy-two (72) healthy male albino mice weighing between 18-25 g were purchased from the animal holding unit of the Institute of Advanced Medical Research and Training (IAMRAT), University College Hospital (UCH), Ibadan, Nigeria. The animals were housed in well-ventilated plastic cages under standard conditions (8 animals per cage). The cages were kept in a room where a 12-hour light and dark cycle was maintained coupled with free access to clean water and a standard animal pellets diet. They acclimatized for two weeks before the commencement of treatment.

Ethical Consideration

Animal handling and care during the study were according to ethical guidelines for handling laboratory animals. The experimental protocol for the animal study was approved by the Research and Ethics Committee (The Federal University of Technology Akure, Ondo State, Nigeria) with approval number (FUT/SOS/1411). All guidance for the care and use of laboratory animals, as contained in the National Institutes of Health Manual (National Research Council) were followed [15].

Parasite inoculation

The study utilized a mouse model of the P. berghei NK–65 strain of malaria parasite. The technique outlined by Dacie and Lewis was used to determine the parasitemia of malaria. Donor mouse blood from University College Hospital (UCH), Ibadan, Oyo State, Nigeria's Malaria Research Laboratories unit of the Institute of Advanced Medical Research and Training (IAMRAT) was infected with P. berghei (chloroquine-sensitive ANKA-65 strain) [16].

The mice were infected by obtaining parasitised blood from the cut-tip of the tail of infected mice (3–4 drops) and diluted in 0.9 ml of phosphate buffer (pH 7.4). They were inoculated via an intraperitoneal route with 0.2 ml P. berghei parasitised red blood cells maintained by passage and were considered to have cerebral malaria if they displayed neurological symptoms such as paralysis, deviation of the head, ataxia, convulsions or coma upon infection. They were given 0.2 ml of P. berghei-paralyzed red blood cells through an intraperitoneal injection, and if they showed neurological symptoms such paralysis, head deviation, ataxia, convulsions, or coma after infection, they were diagnosed with cerebral malaria. Next, using blood film (smears) created by taking a drop of blood from the mice's cut-tip tail on days 0 and 3, Giemsa stained the sample, and the proportion of parasitemia was computed as the number of parasitized red blood cells per 100 RBC [17].

Animal Groupings

The mice were divided into nine groups of 8 mice per grouping as follows:

Group 1 – Normal control (Uninfected and not treated mice fed with basal diet) (NC)

Group 2 – Infected and not treated (mice fed with basal diet) (PBIM)

Group 3 – Infected and treated with chloroquine drug (10 mg/kg) (PBIM+CQ)

Group 4 – Infected and treated with Commercial cookies (PBIM+CC)

Group 5 – Infected and treated with 100%WF cookies (PBIM+WFC)

Group 6 – Infected and treated with 90%WF10%MF cookies (PBIM+MFC10)

Group 7 – Infected and treated with 85%WF%15MF cookies (PBIM+MFC15)

Group 8 – Infected and treated with 80%WF20%MF cookies

(PBIM+MFC20)
Group 9 – Infected and treated with 75%WF25%MF cookies (PBIM+MFC25)

The mice were given the formulated feed (cookies) and drug, respectively in the above doses once daily for 5 consecutive days. The chloroquine drug was prepared by dissolving a tablet (250 mg) into 25 ml of distilled water, when fully dissolved, 0.30 ml of the drug solution was administered to the mice through oral gavage.

Parasite Evaluation
The animals received free access to food and water during the treatment periods, and for days 0–5, their weight, temperature, and slide smear readings were recorded. Cervical dislocation was used to sacrifice them at the conclusion of the therapy period. Temperature assessment, parasitemia count, and suppressive test were established. Complete blood counts were performed, blood films were generated, and the amount of parasites in the blood was assessed.

Weight and Temperature
The weight of the mice was also checked (W0, W1, W3, W5). The readings were taken for a certain period of the experiment. The body weight of the mice was determined according to Krettli with modification by determining the change in body weight of each mouse in all groups before infection (W0) and after treatment (W5) with the aid of a sensitive electrical scale balance using the formula in (Equation 1). The body weight changes for the treated groups were compared with the control groups. The daily temperature of the mice was checked using a clinical thermometer (rector probe) (T0, T1, T3, T5). This was done by fixing the thermometer to their anus for 30 seconds after separating them into containers [18].

$$\text{Body weight gain}\left({}^{\text{g}}/_{\text{day}}\right) = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Days}} \quad (1)$$

Parasitaemia Count Determination
From 48 hours following the first day of treatment, the parasite counts and percentage suppressed were measured every day. The parasite count was determined using Kalra approach. The tails of the infected mice were used to make a thin blood smear, which was then dried and fixed with 100% methanol. After the slides were dried, each one was cleaned with water and dried again before being stained for fifteen minutes with 10% Giemsa in methanol. Then it was fixed with buffer water (pH 7.5). Each stained slide for each mouse was examined under a microscope (Olympus Corporation, Tokyo, Japan) using a 100-objective lens with immersion oil to estimate the number of red blood cells. The parasitaemia was determined by counting the number of parasitized erythrocytes in random fields of the microscope image for both infected and donor mice. Percentage parasitaemia and percentage suppression of both infected and donor mice were calculated as expressed in equations 2 and 3 [19].

$$\%\text{Parasitemia} = \frac{\text{Total number of parasitized red blood cells}}{\text{Total number of red blood cells}} \times 100 \quad (2)$$

$$\%\ \text{Parasitaemia suppression} = \frac{(P_c - P_t)}{\text{Parasitemia in negative control}} \times 100 \quad (3)$$

Pc = Parasitemia in negative control Pt = Parasitemia in study group

Determination of Haematological Parameters
After five days of treatment, the hemoglobin (Hb), red blood cell count (RBC), and packed cell volume (PCV) were assessed using the Cheesbrough standard method. An automated hematologic analyzer and a JENWAY colorimeter model 6030, serial No. 2087, United Kingdom were utilized. At 540 nm, the sample absorbance was measured [20].

Statistical Analysis
The data obtained were subjected to One-way ANOVA and Turkey's test for multiple comparisons. The significant difference was accepted at $p < 0.05$. Results are expressed as mean ± standard deviation. This analysis was carried out using IBM Statistical Package for Social Sciences (SPSS), IBM SPSS Statistics version 21.

Results
The result revealed the effect of the dietary supplementation of wheat-mushroom cookies on the body temperature of Plasmodium berghei-infected mice. A significant decrease in the body temperature of the untreated mice. The body temperature of the untreated-infected mice (<35.00 oC), the mice treated with commercial cookies (35.36 oC) and 100% wheat cookies (35.21oC) decreased significantly when compared with the other treated mice groups presented in Table 2. However, a normal body temperature was observed in the mice treated with a wheat-mushroom cookies-supplemented diet at different inclusion of 10%, 15%, 20% and 25% mushroom. There was no significant difference in the infected mice treated with chloroquine and wheat-mushroom cookies with different mushroom inclusion. A similar trend was reported by Ojueromi that treated Plasmodium berghei- infected with a supplemented diet from black seeds (Nigella sativa) [21].

Table 2: Effect of Wheat-Mushroom Cookies Formulated Diet on The Body Temperature (oc) of p.Berghei-Infected Mice

Groups	Temperature (°C)			
	Day 0	Day1	Day3	Day5
NC	37.20±0.89$^{a\#}$	37.34±0.60$^{a\#}$	37.19±0.57$^{a\#}$	36.99±0.68$^{ab\#}$
PBIM	36.65±0.51^{b}	35.43±0.35^{c}	35.00±0.53^{c}	<35.00*
PBIM+CQ	36.69±0.62^{b}	35.76±0.37^{b}	36.79±0.40^{ab}	37.18±0.59$^{a\#}$
PBIM+CC	36.91±0.65^{b}	36.64±0.59^{ab}	36.60±0.60^{ab}	35.36±0.24$^{c\#}$
PBIM+WFC	37.15±0.35^{ab}	36.84±0.85^{ab}	35.97±0.45^{bc}	35.21±1.04$^{c\#}$
PBIM+MFC10	36.13±0.19^{ab}	35.50±0.53^{b}	36.43±0.56^{b}	36.69±0.99$^{ab\#}$
PBIM+MFC15	36.66±0.50^{b}	36.49±0.36^{ab}	36.36±0.29^{b}	36.54±1.29$^{b\#}$
PBIM+MFC20	37.18±0.42^{ab}	36.35±0.61^{ab}	36.69±1.06^{ab}	36.94±0.77$^{ab\#}$
PBIM+MFC25	36.45±0.74^{b}	36.56±0.43^{ab}	36.74±0.43^{ab}	36.93±0.89$^{ab\#}$

25%MFC: Plasmodium berghei infected mice +(25%) Mushroom flour cookies.
*p < 0.05 versus Plasmodium berghei + chloroquine (CQ).
#p < 0.05 versus Plasmodium berghei-infected group

The effect of the wheat-mushroom cookies formulated diet on the average final weight of P. berghei-infected mice treated (Table 3). A significant decrease was observed in the average weight of the untreated infected mice (24.16 g) compared with the normal group and all the treated infected mice groups. An increase in body weight was observed in the mice treated with chloroquine drug, commercial cookies and 100% wheat cookies as well as a mushroom-supplemented diet at varying mushroom inclusion of 10%, 15%, 20% and 25%. There was no significant difference in the final weight of infected mice treated with wheat-mushroom cookies from 15% and 25% mushroom inclusion. Also, there were no significant differences in the final weight of infected mice treated with commercial cookies,100% wheat cookies and wheat-mushroom cookies from 10% mushroom inclusion. There were no significant differences in the final weight of infected mice treated with chloroquine drug and wheat-mushroom cookies from 20% mushroom inclusion.

Table 3: Effect of Wheat-Mushroom Cookies Formulated Diet on Body Weight (G) in p. Berghei-Infected Mice

Groups	Weight (g)			
	Day 0	Day 1	Day 3	Day 5
NC	24.86±1.86	25.29±2.22	25.57±1.51	26.14±1.35
PBIM	24.66±2.33	24.43±2.04	24.25±3.65	24.16±0.36
PBIM+CQ	25.00±1.77	24.85±1.83	25.00±2.14	25.10±1.24
PBIM+CC	24.63±1.06	24.13±1.36	24.00±1.41	24.88±0.99
PBIM+WFC	24.50±4.07	23.75±4.03	24.10±3.60	24.69±0.11
PBIM+MFC10	24.62±3.46	24.00±3.21	24.45 ±2.77	24.85±0.61
PBIM+MFC15	25.62±2.92	25.63±2.77	25.63±3.11	25.74±0.79
PBIM+MFC20	25.15±2.31	24.75±2.90	25.10±2.20	25.30±1.44
PBIM+MFC25	25.63±3.74	25.13±2.90	25.49±3.50	25.66±1.24

Note: Mean values are of triplicates ± standard deviation.
Value within the columns with different superscript are significantly (P<0.05) different.
NC: Normal mice; PBIM: Plasmodium berghei infected mice; PBIM+CQ: Plasmodium berghei infected mice + Chloroquine; PBIM+CC: Plasmodium berghei infected mice + Commercial cookies; PBIM+ 100%WFC: Plasmodium berghei infected mice +Wheat flour cookies (100%); PBIM+ 10%MFC: Plasmodium berghei infected mice +(10%) Mushroom flour cookies; PBIM+ 15%MFC: Plasmodium berghei infected mice +(15%) Mushroom flour cookies; PBIM+ 20%MFC: Plasmodium berghei infected mice +(20%) Mushroom flour cookies; PBIM+ 25%MFC: Plasmodium berghei infected mice +(25%) Mushroom flour cookies.

Table 4 shows the percentage of parasitemia in P. berghei-infected mice treated with chloroquine, commercial cookies, 100% wheat cookies, and a diet enriched with mushrooms (at different inclusion percentages of 10%, 15%, 20%, and 25%). The parasitemia level of P. berghei-infected mice fed with commercial cookies (62%) and 100% wheat cookies (56%) exhibited a substantial increase on the fifth day compared to the untreated animals, who had an average parasitemia level of up to 85%.

Nonetheless, the mice's parasite burden was significantly suppressed in all groups given wheat-mushroom cookies at different percentages of mushroom inclusion (10%, 15%, 20%, and 25%) at 57%, 68%, 81%, and 87%, respectively. The level of parasitemia suppression in P. berghei-infected mice treated with chloroquine (87.16%) and those fed with wheat-mushroom cookies at 25% mushroom inclusion (86.59%) did not differ significantly. More than half of the parasite was suppressed by the combination of chloroquine and wheat-mushroom cookies. This is consistent with the findings of Abubakar, who stated that an extract is deemed therapeutically beneficial when it exhibits 50% suppression or supression of parasitemia [22].

The rate of changes in haematological parameters depends on the level of the nutritional status, parasitemia effect, and immunity level against malaria. Table 5 revealed the results of haematological analysis on Haemoglobin, Packed cell volume (PCV) and Red blood cell (RBC) of the Plasmodium berghei¬-infected mice. Haemoglobin level of the normal control mice (not infected) was 13.12 Hbg/dl, untreated P. berghei-infected mice had 5.33 Hbg/dl while P. berghei-infected mice treated with chloroquine (12.00 Hbg/dl), commercial cookies (5.80 Hbg/dl), 100% wheat cookies (6.20 Hbg/dl) and mushroom-supplemented diet at varying percentage inclusion of 10%, 15%, 20% and 25% had 7.48, 8.58, 10.25, 11.50 Hbg/dl, respectively. An increase in the mushroom content of the cookies increased the haemoglobin level. There was no significant difference in the haemoglobin level of the P. berghei infected mice treated with chloroquine (12.00 Hbg/dl) and mushroom-supplemented diet of 25% inclusion (11.50 Hbg/dl). The findings were similar to the result of haemoglobin level of the Plasmodium berghei- infected mice treated with supplemented diet from black seeds (Nigella sativa) reported by Ojueromi [21,23].

Table 4: Effect Of Wheat-Mushroom Cookies on The Parasitemia Level in P. berghei-Infected Mice

Groups	Parasitemia Count (%)		Parasitemia Suppression (%)
	Pre-treatment Parasitaemia (Day 3)	Post-treatment Parasitaemia (Day 8)	
NC	0.00 ± 0.00^{d}	0.00 ± 0.00^{h}	$100.00\pm0.00^{a\#*}$
PBIM	21.77 ± 1.06^{a}	84.90 ± 1.60^{a}	
PBIM+CQ	19.6 ± 1.70^{bc}	8.33 ± 1.43^{g}	$87.16\pm1.43^{b\#}$
PBIM+CC	19.39 ± 1.27^{c}	61.50 ± 1.25^{b}	$5.24\pm1.25^{g\#*}$
PBIM+WFC	21.35 ± 2.30^{ab}	56.26 ± 1.10^{c}	$13.31\pm1.10f^{\#*}$
PBIM+MFC10	19.50 ± 1.00^{bc}	28.00 ± 1.10^{d}	$56.86\pm1.10^{e\#*}$
PBIM+MFC15	19.730 ± 1.10^{bc}	21.00 ± 0.21^{e}	$67.64\pm0.21^{d\#*}$
PBIM+MFC20	20.47 ± 2.01^{ab}	12.50 ± 1.10^{f}	$80.74\pm1.10^{c\#*}$
PBIM+MFC25	20.00 ± 1.26^{bc}	8.70 ± 1.15^{g}	$86.59\pm1.15^{b\#*}$

Note: Mean values are of triplicates± standard deviation. Value within the columns with different superscript are significantly ($P<0.05$) different.
NC: Normal mice; PBIM: Plasmodium berghei infected mice; PBIM+CQ: Plasmodium berghei infected mice + Chloroquine; PBIM+CC: Plasmodium berghei infected mice + Commercial cookies; PBIM + 100%WFC: Plasmodium berghei infected mice +Wheat flour cookies (100%);
PBIM+ 10%MFC: Plasmodium berghei infected mice + (10%) Mushroom flour cookies; PBIM + 15%MFC: Plasmodium berghei infected mice +(15%) Mushroom flour cookies; PBIM+ 20% MFC: Plasmodium berghei infected mice + (20%) Mushroom flour cookies; PBIM+ 25% MFC: Plasmodium berghei infected mice +(25%) Mushroom flour cookies.
*$p < 0.05$ versus Plasmodium berghei + chloroquine (CQ).
#$p < 0.05$ versus Plasmodium berghei-infected group

The packed cell volume level of the normal mice (not infected) was 37.50%, untreated P. berghei infected mice had 22.00% while P. berghei-infected mice treated with chloroquine (35.47%), commercial cookies (27.62%), 100% wheat cookies (25.62%) and mushroom-supplemented diet at varying percentage inclusion of 10%, 15%, 20% and 25% had 31.38, 32.43, 33.55 and 34.60%, respectively. There was no significant difference in the PCV level of the P. berghei-infected mice treated with standard drug (chloroquine) (35.47%) and mushroom-supplemented diet of 25% inclusion (34.60%). The value range of 22-42% for PCV results from Plasmodium berghei-infected mice treated with supplemented diet of black seeds (Nigella sativa). The wheat-mushroom-supplemented diet has a positive effect on the percentage PCV due to the changes obtained after treatment [21].

Also, the red blood cell level of the normal mice that were not infected was 8.45 $x10^6$/L, untreated P. berghei-infected mice had 3.14 x106/L while P. berghei-infected mice treated with chloroquine (7.86$x10^6$/L), commercial cookies (5.36$x10^6$/L), 100% wheat cookies (4.84$x10^6$/L) and mushroom-supplemented diet at varying percentage inclusion of 10%, 15%, 20% and 25% had 6.25, 6.74, 7.21 and 7.79 $x10^6$/L, respectively. However, the chloroquine-treated group and 25% mushroom-supplemented diet-treated group did not show significant ($p>0.05$) reduction in Haemoglobin, PCV and RBC.

Table 5: Effect of wheat-mushroom cookies on Haemoglobin, PCV and RBC level in P. berghei-infected mice

Treatment Groups	Haemoglobin (Hbg/dl)	Packed Cell Volume (PCV) (%)	Red Blood Cell (RBC) ($x10^6$/L)
NC	13.12 ± 0.71^{a}	37.50 ± 2.12^{a}	8.45 ± 0.05^{a}
PBIM	5.33 ± 0.10^{g}	22.00 ± 0.14^{h}	3.14 ± 0.20^{f}
PBIM+CQ	12.00 ± 1.15^{b}	35.47 ± 0.19^{b}	7.86 ± 0.57^{ab}
PBIM+CC	5.80 ± 0.28^{fg}	27.62 ± 0.71^{f}	5.36 ± 0.04^{d}
PBIM+WFC	6.20 ± 1.53^{f}	25.62 ± 1.61^{g}	4.84 ± 1.56^{de}
PBIM+MFC10	7.48 ± 0.41^{de}	31.38 ± 0.24^{e}	6.25 ± 0.25^{c}
PBIM+MFC15	8.58 ± 0.01^{d}	32.43 ± 0.65^{d}	6.74 ± 1.08^{bc}
PBIM+MFC20	10.25 ± 0.91^{c}	33.55 ± 0.15^{c}	7.21 ± 0.33^{b}
PBIM+MFC25	11.50 ± 0.42^{b}	34.60 ± 1.41^{bc}	7.79 ± 0.04^{ab}

Note: Mean values are of triplicates ± standard deviation. Value within the columns with different superscript are significantly ($P<0.05$) different.
NC – Normal control (Uninfected and not treated mice fed with basal diet)
PBIM – Infected and not treated (mice fed with basal diet)
PBIM+CQ – Infected and treated with chloroquine drug (10 mg/kg)
PBIM+CC – Infected and treated with Commercial cookies
PBIM+WFC – Infected and treated with 100%WF cookies
PBIM+MFC10 – Infected and treated with 90%WF10%MF cookies
PBIM+MFC15 – Infected and treated with 85%WF%15MF cookies
PBIM+MFC20 – Infected and treated with 80%WF20%MF cookies
PBIM+MFC25 – Infected and treated with 75%WF25%MF cookies

Discussions

Malaria is one of the most serious diseases in the world with acute life-threatening clinical symptoms and unique complications. In humans, this disease has caused a wide range of clinical symptoms and unique complications when the parasites invade the host cell (erythrocytes), multiply, and cause serious deformation. Body weight and temperature are parameters that can be used to assess the effect of the extra in treated mice. The changes in body weight and temperature of the mice before and after treatment were determined may be due to the depressant action on the appetite of the mice and the consequences of disturbed metabolic function and hypoglycemic effect of the parasite. Plasmodium parasites impair the ability of the main cells of the immune system to trigger an efficient inflammatory and immune response. The finding from this study showed a significant rise in the parasitemia level with increasing days after innoculation and a maximum parasitemia level was observed on the fifth and final day of the experiment in the P. berghei–infected mice. An elevated degree of parasitemia level with extreme mortality indicates the critical level of infection in this mice model. Also, the terminal complication of plasmodium parasites that occur in animals and humans is death. The anti-malarial drug like chloroquine possesses an integral mechanism to inhibit the formation of hemozoin from the heme released via the digestion of haemoglobin. Our study revealed a significant parasitemia suppression ($p<0.05$) in the groups treated with the dietary supplementation cookies of mushroom at varying percentage inclusion (10%, 15%, 20%, 25%). The treatment at 25% Pleurotus pulmonarius mushroom cookies of the combination was considered more effective for the treatment of malaria but all the cookies with mushroom inclusion suppressed more than 50% Plasmodium berghei in the infected mice. A good antimalaria agent should prevent weight reduction in plasmodium infected host; since weight is eminence of plasmodium infection [24,25].

This phenomenon is normal due to the effect of the wheat-mushroom supplemented diet on the cells in the blood especially the red blood cells since the plasmodium parasites are usually localized in these cells and any treatment procedure will involve lysing of the cells and consequently, this will affect the percentage packed cell volume. A significant reduction ($P<0.05$) was obtained in the haematological parameter (Haemoglobin, PCV and red blood cell) of the untreated mice infected with Plasmodium berghei. This is similar to the report by Ojueromi that treated P. berghei- infected with a supplemented diet from black seeds (Nigella sativa). Whereas, treatment with increase in mushroom inclusion in wheat-mushroom cookies (10%, 15%, 20% and 25%) caused a significant increase ($p<0.05$) in the Haemoglobin, PCV and red blood cell levels of P. berghei-infected mice as the mushroom inclusion increases. Joshua reported a similar trend when P. berghei- infected mice ameliorated with methanol extract of Erythrina senegalensis leaves (MEES) [21,26,27].

Conclusion

The formulated cookies from P. pulmonarius are useful in the treatment of malaria in the mouse model standard. This gives scientific evidence to claims that some cultivated mushrooms could be used in the management and treatment of malaria infection. The study also showed that the mushroom cookies exhibited hepatoprotective properties and high antioxidant levels which helps in restoring the antioxidant imbalance. These mushroom cookies possess antimalarial potential, owing to their nutritional, bioactive compounds and enormous therapeutic potential implications. Findings from this study revealed that the mushroom cookies possess potent antiplasmodial effects and may, therefore, serve as an alternative source for the development of a safe, effective and affordable antimalarial drug.

Author Contributions

Rachel O Adetola: Writing-original draft; investigation; methodology; Data curation, formal analysis; Writing-review & editing.

Bolanle A Akinwande: Conceptualization, project administration, supervision, validation, writing-review and editing, reading of final draft, reviewed manuscript.

Grace O Babarinde: Supervision, validation, writing-review and editing, reading of final draft, reviewed manuscript
Ganiyu Oboh: Methodology, supervision, validation.

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Conflict of Interests
The authors declare no conflict of interest

Data Availability Statement
Data will be made available on request

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