1
2 **PROPHYLATIC EFFECTS OF SOME NIGERIAN HIGHER FUNGI ON**
3 **MALARIA PARASITE, *PLASMODIUM BERGHEI BERGHEI* IN BALB/C**
4 **STRAIN ALBINO MICE.**

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9 **ABSTRACT**

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The preventive abilities of some Nigerian higher fungi; *Pleurotus pulmonarius, Fomes lignosus, Lentinus subnudus, Termitomyces robustus, Pleurotus tuber-regium* collected from wood substrates, and the combined extracts (40mg/mL) were studied against malaria parasite, *Plasmodium berghei berghei* to exploit them as medicinal agents. The fruit bodies were extracted with ethanol using soxhlet apparatus. Six hundred and twelve 4- 5 weeks old BALB/c mice (average weight 21g) were assigned into 30 groups of twelve mice each. They were orally treated with 0.1mL extracts of 4, 0.4, 0.04, 0.004, 0.0004mg/mL before infecting with 0.2mL of 5×10^6 parasitized erythrocytes. Chloroquine,CQ (20mg/kg) served as controls. Liver and kidney were examined for histological changes. Parasitemic level, packed cell volume (PCV) and weights of animals were checked using standard methods. Descriptive statistics and ANOVA (at p= 0.05) were used for data analysis. *Lentinus subnudus* produced the highest decrease of parasitemia (30%), and recorded the least loss of PCV (27%), while *Pleurotus tuber-regium* produced the least weight loss of 26.5%. All the mushroom extracts produced same effect as CQ at concentrations 0.4 and 0.04mg/mL. The tissues of infected animals without extracts had abnormalities.There were no histological damage in tissues of uninfected animals. Pre-infection treatment produced mild abnormal conditions from severe periportal infiltration and marked sinusoidal congestion. The extracts could be formulated into drugs against malaria however, appropriate dosage must be known.

11
12 *Keywords:* Prophylactic, *Plasmodium berghei berghei,* Albino mice, mushrooms, malaria.

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15 **1. INTRODUCTION**
16 Malaria is the world's most devastating human parasitic infection (1) that causes death of their victims especially children
17 and pregnant women (2,3). It is one of the major health challenges that is endemic in West Africa, and African countries
18 including Nigeria (4,5).
19 Malaria is caused by species of *Plasmodium,* in which *P. malariae, P. vivax, P. ovale, P. falciparum* and the species
20 responsible for malaria in humans (6). Untreated malaria in tropics caused by *Plasmodium falciparum* can lead to lethal
21 effect. Malaria characterized by fever, anaemia, splenomegaly and some complications vary considerably, depending on
22 the species of *Plasmodium* causing the infection and the stage of development of the parasite (7,8).
23 *Plasmodium berghei berghei* is one of the four *Plasmodium* species described in African murine rodent, and infect
24 mammals other than humans (9,10*). P. berghei berghei* had sensitized the development of new drugs or vaccine through
25 their resistance and susceptibility potentials (11,12). Mushrooms are fleshy fungi, known to possess antimicrobial and
26 immunodulatory properties (13,14). Examples of *Pleurotus* species include *Pleurotus tuber-regium, P. pulmonarius;*
27 *Termitomyces and Lentinus* species. Reishi medicinal mushroom had reduced the amount of malaria parasites in infected
28 mice (15).
29 The treatment of malaria is becoming difficult and challenging due to the occurrence of drug resistant to parasites causing
30 infections. Also, many antimalarial drugs are ineffective (16). Many local antimalarial plant species had been reported to
31 be useful in treating malaria and fevers, but there are limited information on antiplasmodial potentials of mushrooms. This
32 study therefore investigated the antiplasmodial potentials of some higher fungi in Nigeria.

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34 **2. MATERIAL AND METHODS**
35 **Fungi samples and preparation of extracts**

36 Samples of *Pleurotus tuber-regium, P. pulmonarius, Termitomyces robustus and Lentinus subnudus* were collected from
37 Bodija market, Forestry Research Institute of Nigeria (FRIN), Iwo road market, University of Ibadan campus, all in Ibadan
38 (Longitude 3.9^{0}E, Latitude 7.4^{0}N) in Oyo state and Bowen University, Iwo (Longitude 4.1^0 E , Latitude 7.6^{0}N) in Osun
39 state, Nigeria respectively. The extracts were prepared by cleaning the samples using cotton swab, cutting into small
40 pieces before oven-drying the fresh fruit bodies at 40^{0}C, and extracting with ethanol using a soxhlet apparatus (17).
41 Combined extract was prepared by combining equal proportion of each of the fungi before extraction.
42 **2.2 Grouping of Experimental mice and Parasite inoculation (Passaging)**
43 Twenty-two (22) grammes of 4-5 weeks old of BALB/c strain albino mice (*Mus* musculus) were divided into 30 groups.
44 One millitre (1ml) parasitized blood was withdrawn from mouse infected with *Plasmodium berghei berghei* (NK-65 strain)
45 and injected into an uninfected mouse.
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47 **2.3 Prophylactic Experiment**
48 0.1ml of ethanol extract from the higher fungi were prepared into different concentrations of 4mg, ml, 0.4mg/ml,
49 00.4mg/ml, 0.004mg/ml and 0.0004mg/ml, and were orally administered to the mice separately for 4 consecutive days.
50 This was followed by the intraperitoneal injection of the animals with 0.2ml of 5x10^6 parasitized blood on the 8th day after
51 extract administration. The percentage parasitemia, PCV (Packed cell volume) and the weight loss of the albino mice
52 were monitored for 12 days.
53 The livers and kidneys of the dead animals were removed and kept in 10% formalin before carrying out histological
54 studies. Animals used for chloroquine (CQ) experiment were indicated as standard, and orally administered at the dosage
55 of 20mg/kg.
56 **2.4 Histological studies**
57 The excised livers and kidney kept in the formalin (10%) were studied for abnormalities using the procedures described by
58 Yoshida *et al.* (18)
59 **2.5 Statistical Analysis**
60 The effects of concentration of the extracts, extract types and their replicates were deduced from Analysis of variance
61 (ANOVA) using SAS version (2.0), while the means were separated by Duncan's Multiple Range Test (DMRT) at P <0.05.
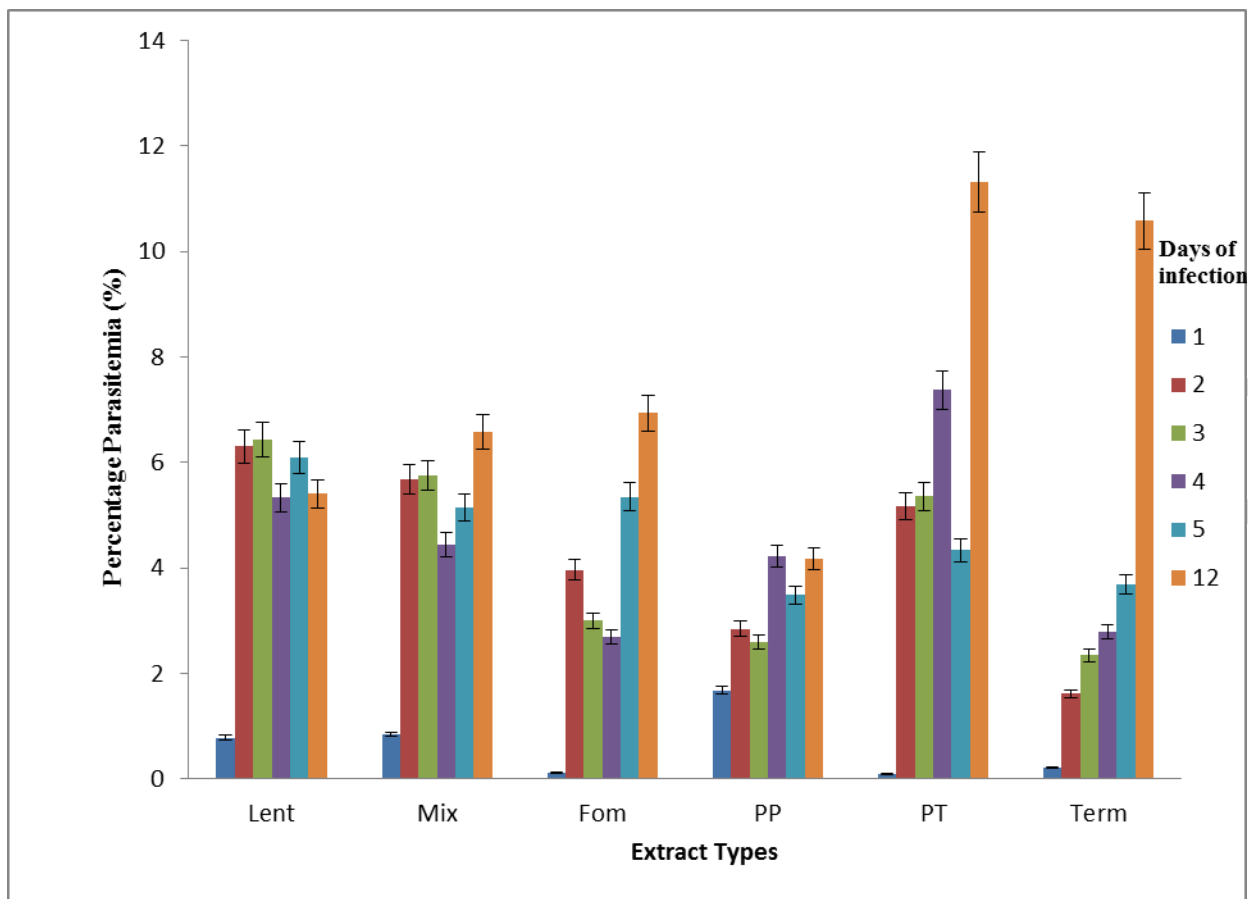62
63
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65 **3. RESULTS AND DISCUSSION**
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Figure 1: The effects of mushroom extracts types on parasitemia in mice infected with *Plasmodium berghei berghei*

KEY:

FOM = *Fomes lignosus,* PT = *Pleurotus tuber-regium,* PP = *Pleurotus pulmonarius,* Term = *Termitomyces robustus,* Lent = *Lentinus subnudus,* Mix = Combined extract, I= Standard error
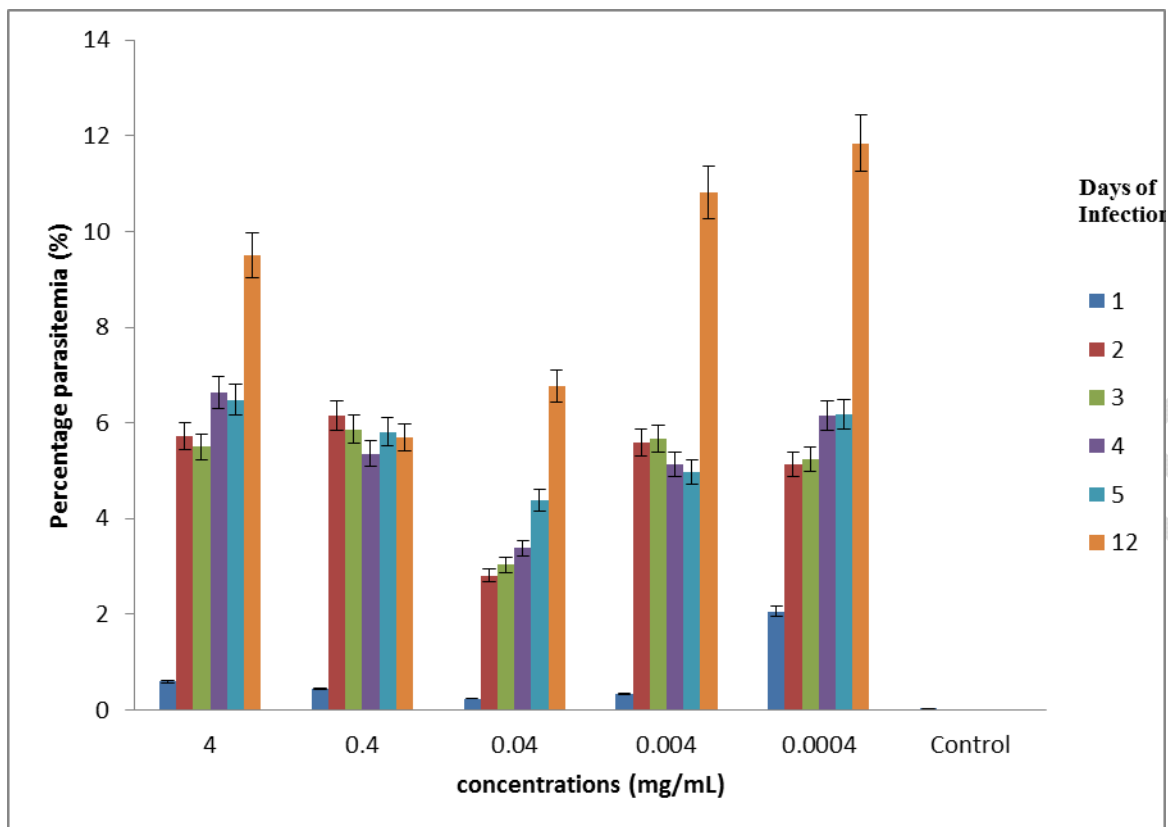
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Figure 2: The effects of different concentrations of mushroom extracts on parasitemia in mice infected with
Plasmodium berghei berghei.

KEY: FOM = _Fomes lignosus,_ PT = _Pleurotus tuber-regium,_ PP = _Pleurotus pulmonarius,_ Term = _Termitomyces robustus,_
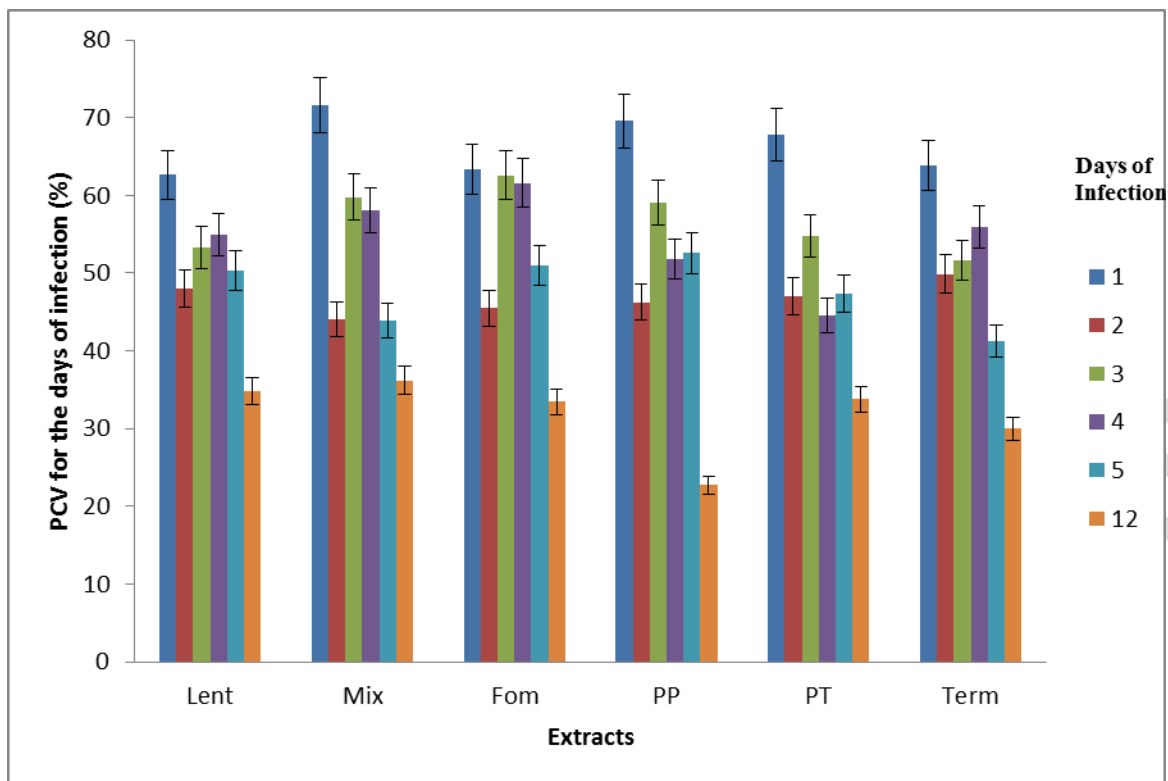Lent = _Lentinus subnudus,_ Mix = Combined extractI= Standard error

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Figure 3: The effect of mushroom extracts on packed cell volume (PCV) of mice infected with *Plasmodium berghei berghei*

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88 **KEY**: FOM = *Fomes lignosus,* PT = *Pleurotus tuber-regium,* PP = *Pleurotus pulmonarius,* Term = *Termitomyces robustus,*
89 Lent = *Lentinus subnudus,* Mix = Combined extract, I= Standard error
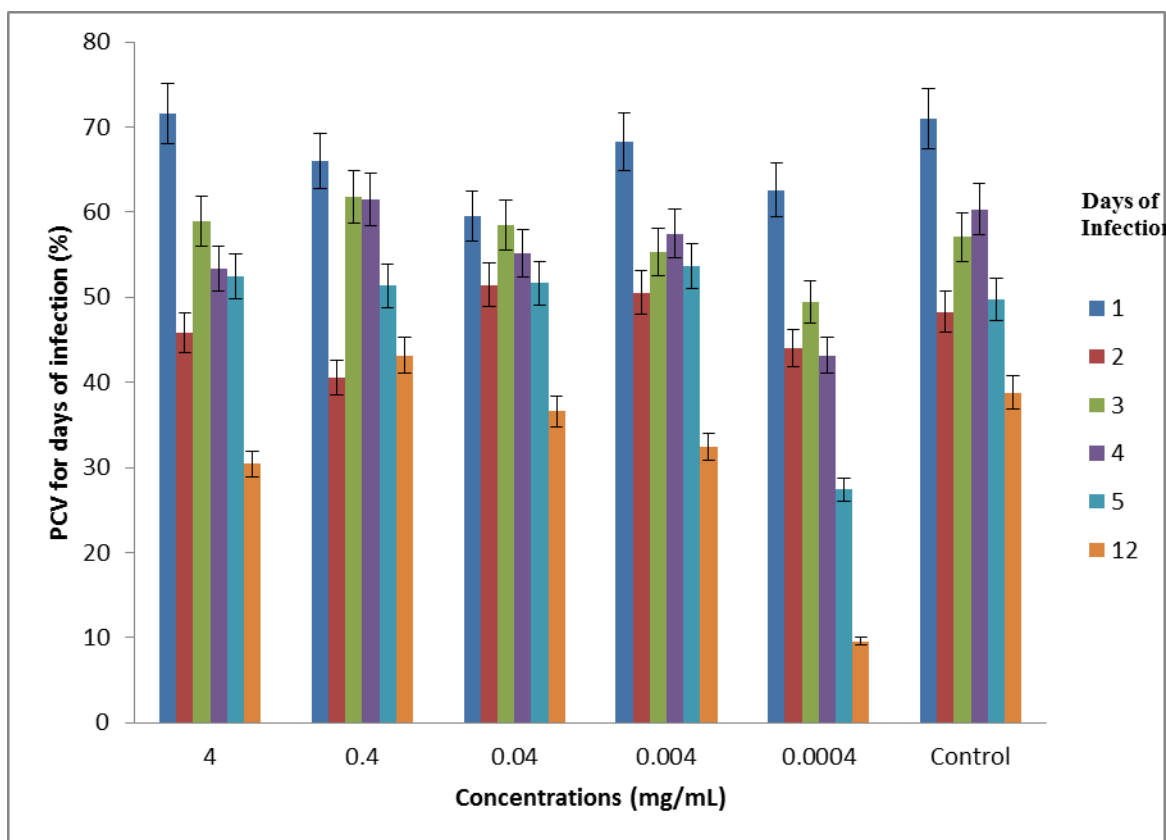
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Figure 4: The effects of different concentrations of mushroom extracts on packed cell volume (PCV) of the mice infected with *Plasmodium berghei berghei*

KEY: FOM = *Fomes lignosus,* PT = *Pleurotus tuber-regium,* PP = *Pleurotus pulmonarius,* Term = *Termitomyces robustus,* Lent = *Lentinus subnudus,* Mix = Combined extract, I= Standard error

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Figure 5: The prophylactic effects of Extracts on weights of mice infected with *Plasmodium berghei berghei*

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KEY: FOM = *Fomes lignosus,* PT = *Pleurotus tuber-regium,* PP = *Pleurotus pulmonarius,* Term = *Termitomyces robustus,*

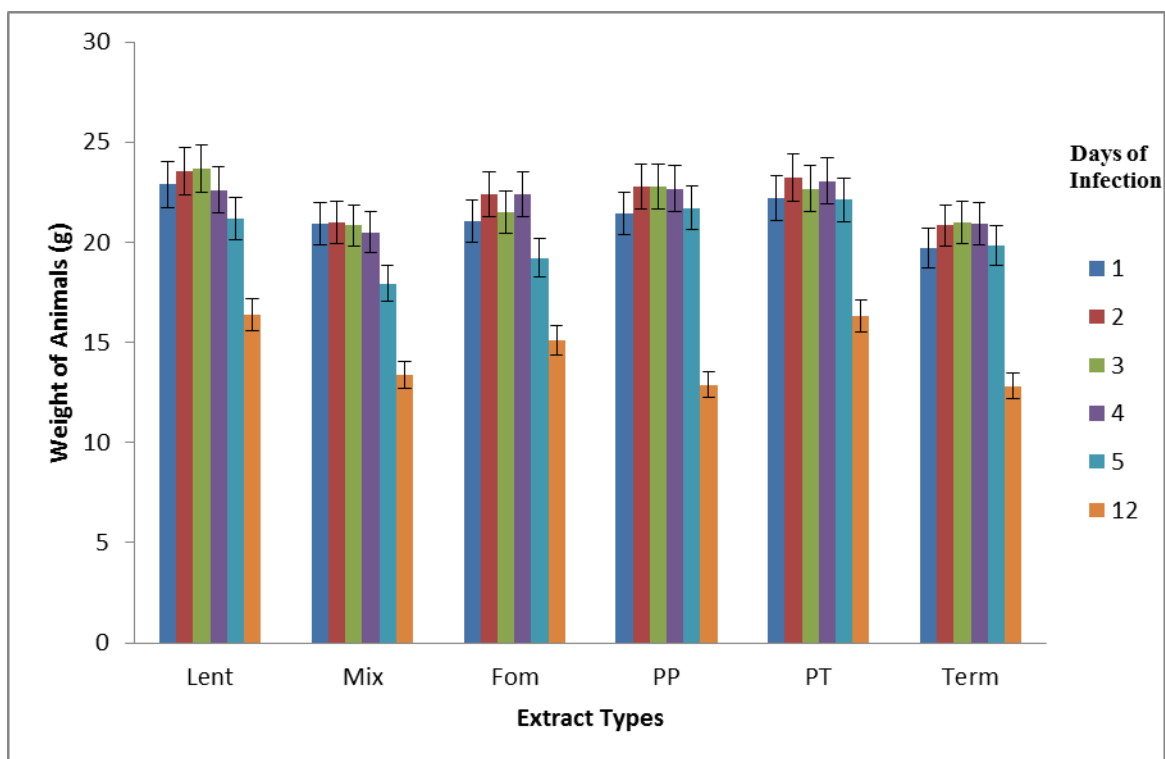Lent = *Lentinus subnudus,* Mix = Combined extract, I= Standard error

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Figure 6: The prophylactic effects of different concentrations of Extracts on weights of mice infected with
   Plasmodium berghei berghei

KEY: FOM = *Fomes lignosus,* PT = *Pleurotus tuber-regium,* PP = *Pleurotus pulmonarius,* Term = *Termitomyces robustus,*
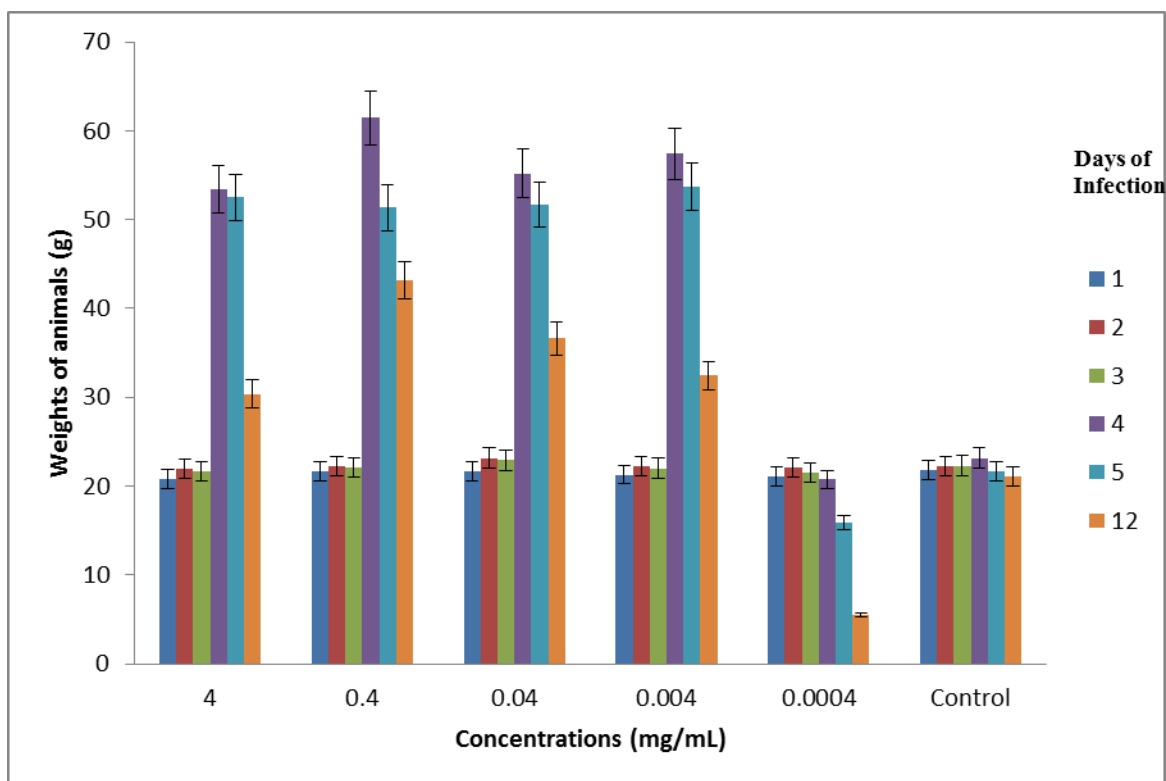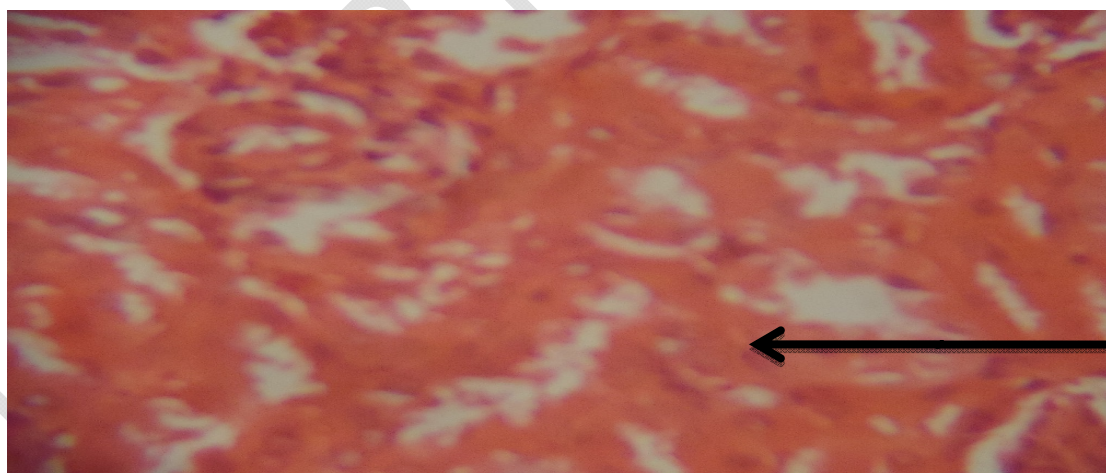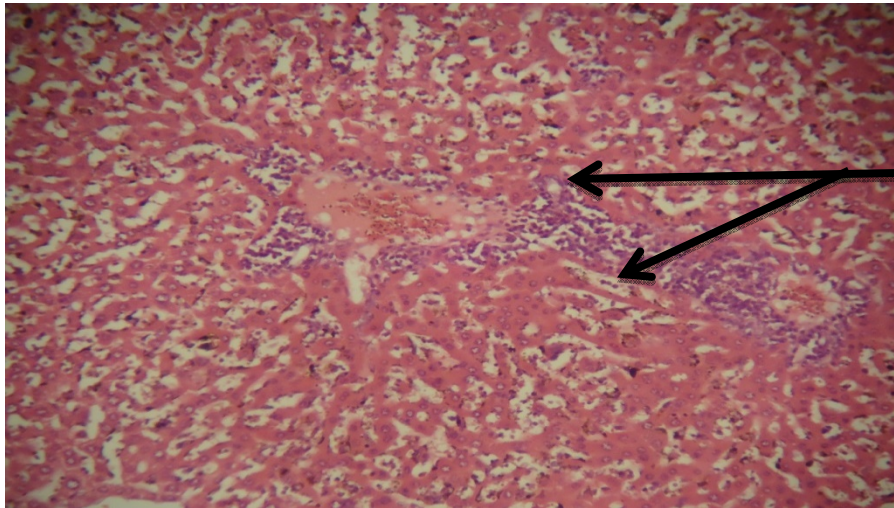Lent = *Lentinus subnudus,* Mix = Combined extract, I= Standard error

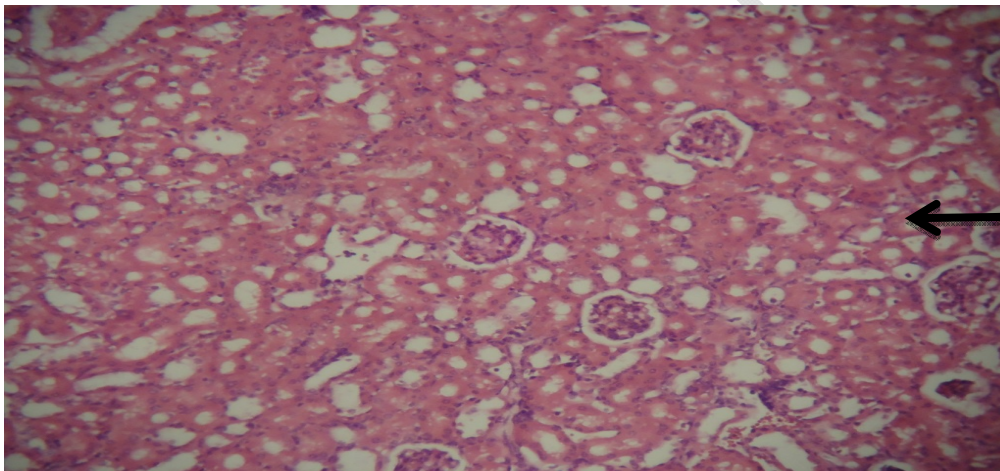**A**   No visible lesions   **X400**

Clusters of inflammatory cells at the portal area

X200

143   **B**   There is moderate periportal cellular infiltration by mononuclear cells.

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145   **Plate 1:   Photomicrograph of the kidney of mice infected with *P. berghei berghei* and administered with *L.**
146   ***subnudus* (0.4mg/mL) with no visible lesions (A) and the liver with moderate portal congestion and**
147   **periportal infiltration by mononuclear cells (B).**

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152   **Convoluted**
153   **Tubules (Normal)**
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156   **Renal**
157   **Corpuscles (Normal)**
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159   **A**   No visible lesions   **X400**
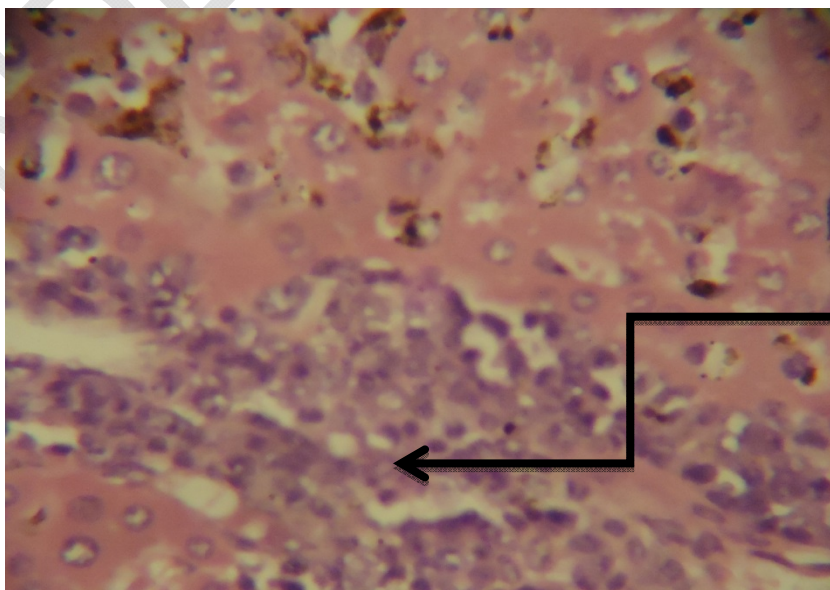
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168   **Clustered Inflammatory cells**
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B   There is severe periportal cellular infiltration by mononuclear cells   **X400**

Plate 2: Photomicrograph of the kidney of mice infected with *P. berghei berghei* with no visible lesions (A) and the liver with severe periportal infiltration after oral administration with *L. subnudus* (0.04mg/mL)

Renal Corpuscles

Severe Interstitial Infiltration

Inflammatory Cells in the Renal Interstitium

A
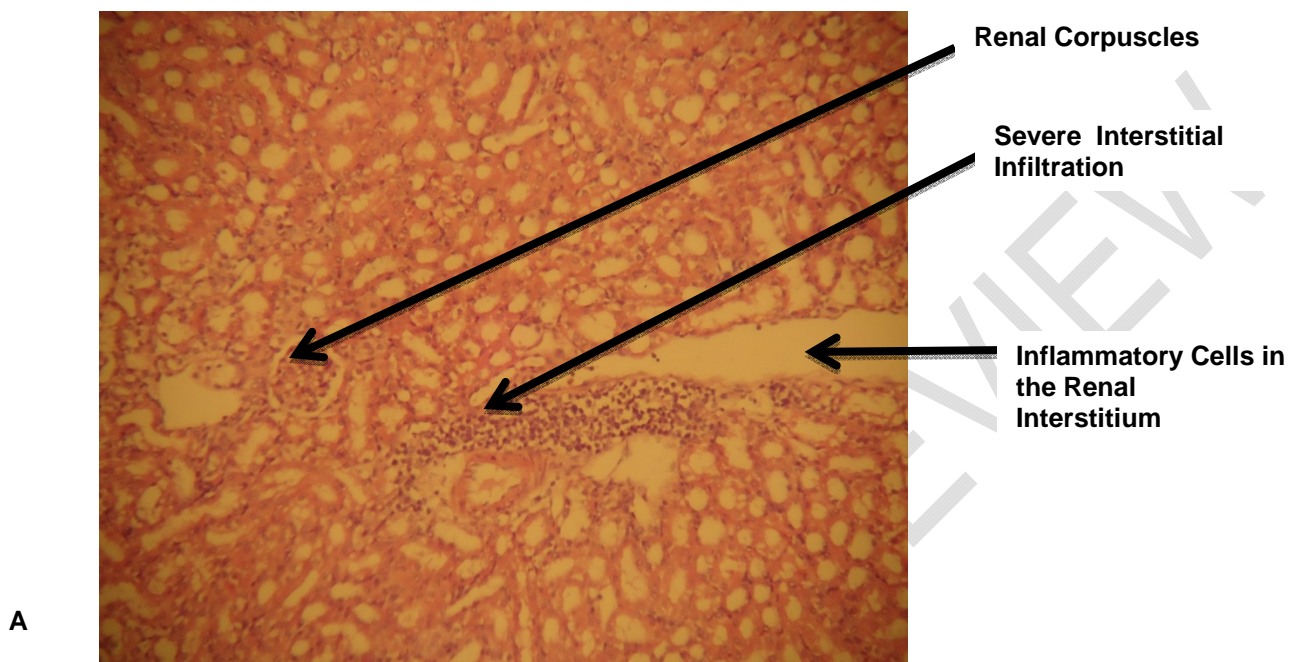
There is severe interstitial cellular infiltration   **X400**

Periportal Infiltration

Congested Portal Vein

B

X400
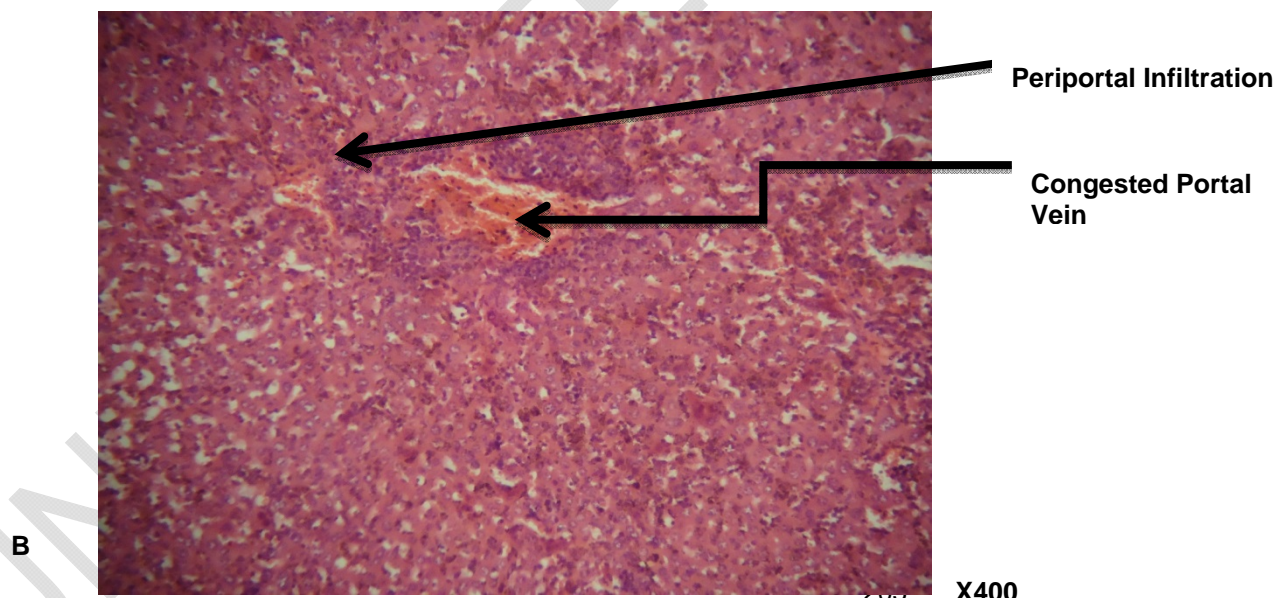
There is severe periportal infilration and marked sinusoidal congestion

Plate 3: Photomicrograph of the kidney (A) and liver (B) of mice infected with *P. berghei berghei* without extract (Control Experiment).
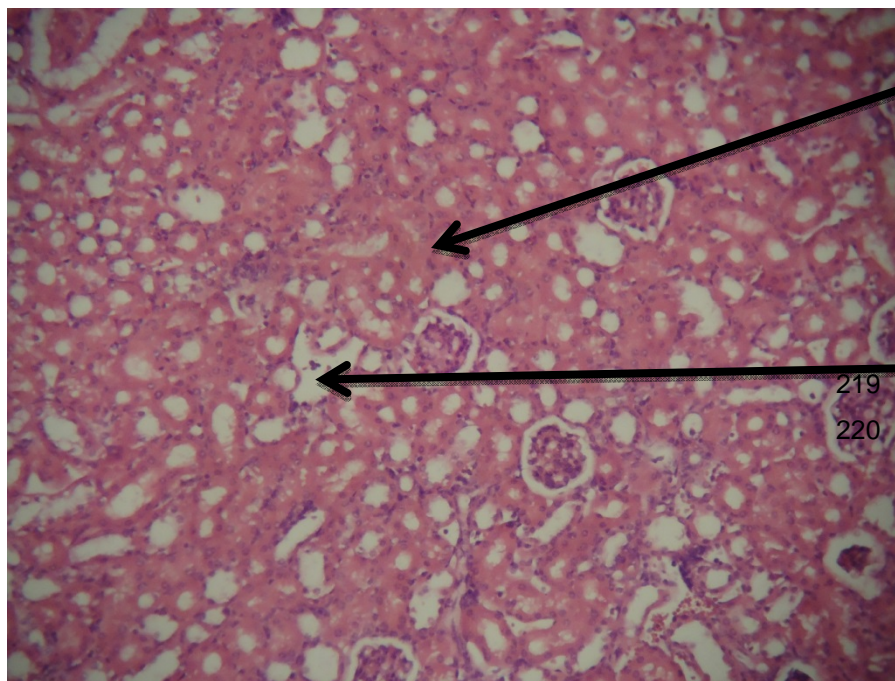
Renal Corpuscles (Normal)

Convoluted Tubules (Normal)

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X400
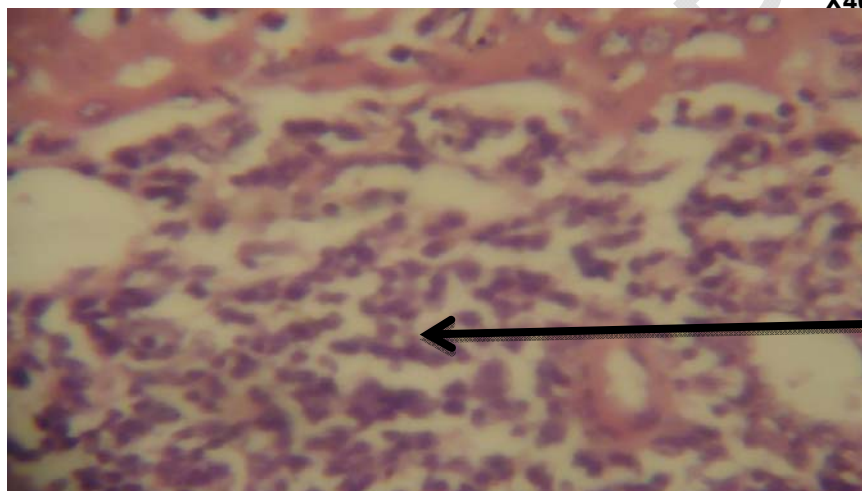
Clusters of Inflammatory Cells

Plate 4:   **Photomicrograph of the kidney (A) and liver (B) infected with** *P. berghei berghei* **and administered with chloroquine**

Normal renal corpuscles

Normal convoluted tubules

A   No visible lesions

Inflammatory cells (Neutrophils and fewer numbers of macrophages)
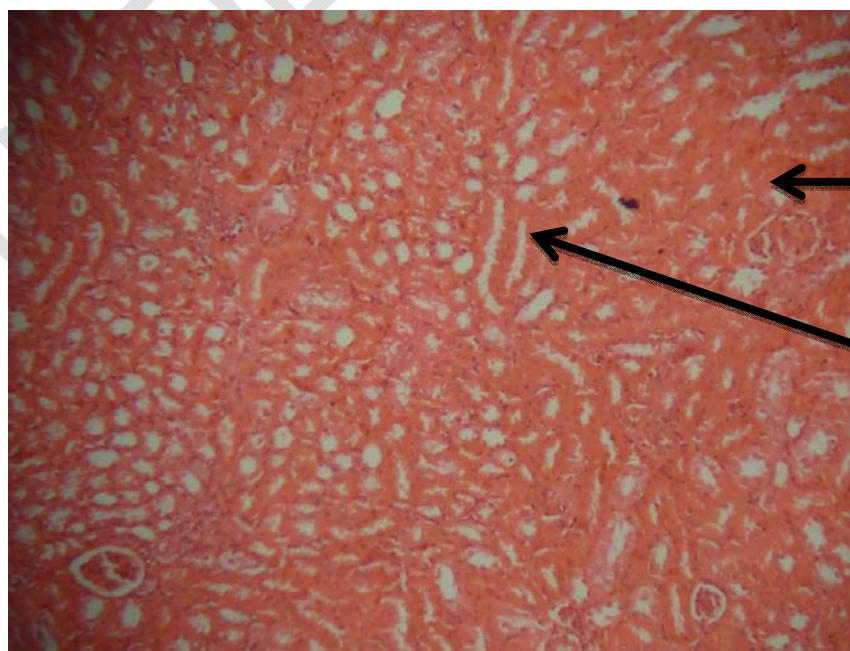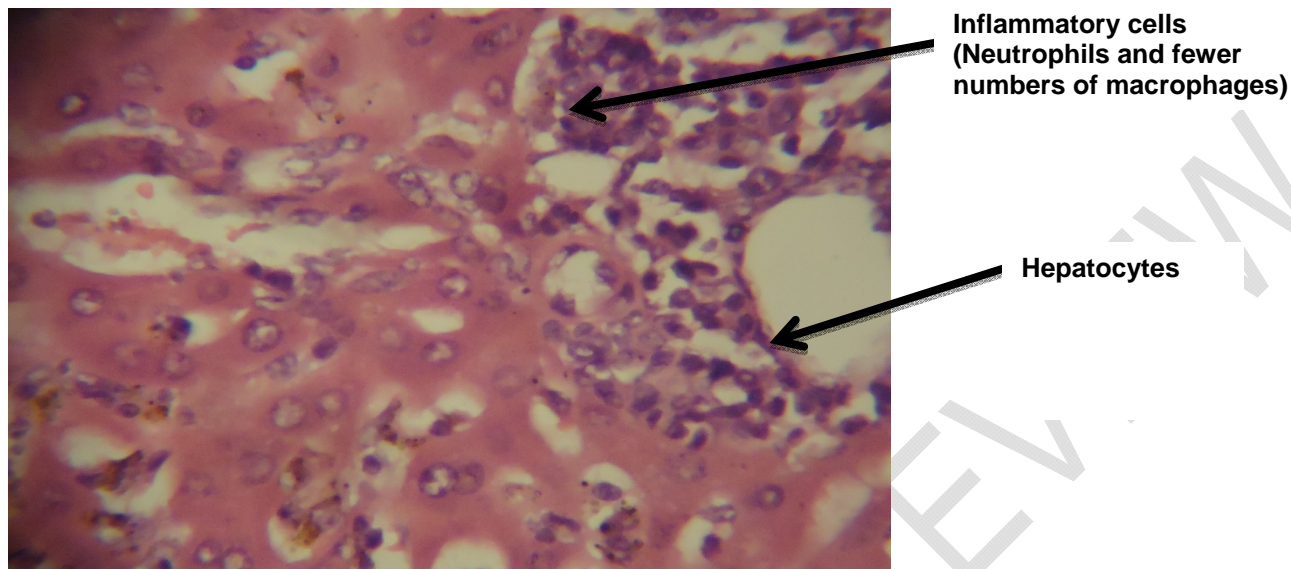
Hepatocytes

B   There is moderate portal congestion and periportal cellular infiltration

Plate 5: Photomicrograph of the liver of mice infected with *Plasmodium berghei* after oral administration of *Lentinus subnudus* (4mg/mL) (A) and the liver with no visible lesions (B)

The prophylactis of the higher fungi extracts had significant effect ($p<0.05$) on the parasitemia throughout the days of infection in the albino mice (Fig 1).

The percentage parasitemia infection was generally low with all the extracts on the first day of infection. The parasitemic effect of *Plasmodium berghei berghei* was reduced by *Pleurotus tuber-regium* (0.09%) followed by *Fomes lignosus* (0.11%), *Termitomyces robustus* (0.22%), *Lentinus subnudus* (0.78%), combined extract (0.84%), while the least parasitemic effect was in *Pleurotus pulmonarius* (1.68%).

The low parasitemic effect could be due to the efficacy of the fungi extracts in the blood of the animals after the infection (19). On the second day, there was an increase in the parasitemic level with highest percentage in *L. subnudus* (6.3%) and the least in *T. robustus* (1.61%). The increase in the infection level of the animals increased on the second day could be due to rapid multiplication and acclamatization of malaria parasites in the host to develop more resistance potentials to injected fungi extracts. There are profound changes in the parasites morphology which transform into new entities.

There was a decrease in parasitemia at the end of the experiment with *Lentinus subnudus.* This is in contrast to the increase in parasitemia after the administration of combined extracts; *F. lignosus, P. pulmonarius, P. tuber-regium and T. robustus*, which compared the second, fifth and twelveth days of infection. The decreased parasitemic infection could be due to prophylactic effect of *Lentinus subnudus* (20,21). *Lentinus* species had been reported to contain some pharmacological compunds such as lentinan which can be extracted from the cell wall of the fruiting body of mushrooms (22). Lentinan confers resistance against bacterial, parasitic and viral infections (23). Also, it was reported that the presence of lentinan in *L. subnudus* contributed to the prophylactic potential of the fungi in the treatment of malaria (24).

There are variations in the efficacy of the different concentrations of the extracts on the parasitemia infection in the experimental mice (Fig 2). On the first and twelveth days of infection, the least concentration (0.0004mg/ml) had the highest parasitemic infection of 2.06 and 11.84 respectively. The infection levels of animals administrated with extracts of concentrations 0.4 and 0.0004mg/mL had the least percentage parasitemia value of 3.03, 3.38 and 4.3%, 5.67, 5.13 and 4.97%, between the third and fifth day. Comparing the second and twelveth days of infection, there was a decrease in parasitemia with extracts concentration of 0.04mg/mL. Others had increase in parasitemia with the least percentage of 5.69 at the end of the experiment.

302 The concentrations of 0.4mg/mL and 0.04mg/mL gave the best prophylactic effects against the malaria parasite in the
303 albino mice, thereby resulting to least parasitemia infection at the end of the experiment compared to concentration
304 0.0004mg/mL with the highest parasitemia. This is in agreement with the observation that increased dilution of a
305 compounded drug decrease the percentage of the larger particles in the drug (25). Therefore, the efficacy of the extract is
306 determined by the higher dilution of the extract. Also, minimum inhibitory concentrations are important in confirming
307 resistance of microorganisms to antimicrobial agents (26,27). There was virtually no infection in the control animals during
308 the prophylactic assay.
309 The effect of the prophylactic administration of the extract could be attributed to the low parasitemic infection in the mice
310 on the first day after the seventh day of fungi administration. The fungi extracts which are prophylactically active in the
311 order, *L. subnudus,* combined extract, *P. pulmonarius* similarly conforms to the observation made by **Iwalokun 2008**(28).
312 Significant effects of the extract on the PCV of animals were also recorded in the prophylactic experiment. There was a
313 general decrease in the packed cell volume (PCV) of the animals during the experimental period (Fig 3). Though the
314 combined extract had highest PCV values at the beginning and end of the experiment, the least PCV decrease of 27%
315 was observed in *L. subnudus.* The highest PCV loss of 46% was in *P. pulmonarius.*
316 The general decrease in PCV of animals could be due to the destruction or lysis of red blood cells targeted by the malaria
317 parasite (29,30). They undergo metamorphosis after invasion of the red blood cells leading to the subsequent lysis of the
318 cells and the release of merozoites that invade new cells (31). The mechanisms contributing to malarial anaemia are
319 increased destruction of red blood cells, lowering the level of iron in the body, decreased production of red blood cells,
320 development of badly functioning ones and destruction of uninfected red blood cells. The changes in red blood cells
321 functioning affects the level of iron within the body hence the body begins to show signs of iron deficiency anaemia (29).
322 The least PCV loss of *L. subnudus* could be attributed to its best prophylactic effect least parasitemia level against the
323 malaria parasite. PCV and parasitemia are interdependently related to each other with respect to invasion of malaria
324 parasites in red blood cells (32). The result from figure 4 shows that concentrations 0.04mg/ml and 0.004mg/ml had
325 similar effects on the PCV of the mice with the highest values of 51.44% and 50.56% respectively on the second day.
326 Both had the least loss of PCV while concentration 0.0004mg/ml had the highest PCV loss and the least PCV value of
327 49.44% at the end of the experiment.
328 The effects of the extracts on the weight of the albino mice are shown in Figure 5. There was a general loss of weight in
329 on the weight only on the first, third and fifth days of infections. *L. subnudus* extract on the first day had significant higher
330 weight (22.89g) than other fungi extracts. It maintained the highest weight of mice (23.67g) followed by *F. lignosus*
331 (21.50g), *P. pulmonarius* (22.78g), *T. robustus* (21.00g), while the least weight of 20.83g was observed in mice using
332 combined extract. The least weight loss of 26.5% was observed in *P. tuber-regium* during the experiment. The weight
333 decrease observed at the beginning and end of the experiment had been confirmed by **Homberger *et al.* (1999**)(33). The
334 highest weight loss was observed in *P. pulmonarius* extract. The effect of the different concentrations of the extracts on
335 the weight is shown in Figure 6. There was a general increase in weight from the first day till the fourth whereas, a loss in
336 weight was 0.0004mg/ml had the least weight while there was no significantly loss of weight in the control animals
337 throughout the experimental period.
338 The general decrease in weights of the experimental mice could be associated with heavy parasite infection. Though the
339 least weight of 26.50% was recorded with *P. tuber-regium,* there was a minimal weight loss with *L. subnudus* proving as
340 the best prophylactic against the malaria infection. Weight loss is one of the evidences of malaria attack because it often
341 causes loss of appetite leading to weight loss (34). Also, a number of nutritional factors are associated with malaria. In
342 pregnant women, maternal malaria contributes to up to 30% of low birth weight cases (35,36,37).
343 The pathological changes on the tissues of the mice infected with the malaria parasite after oral administration of extract
344 of *L. subnudus* 0.4mg/mL, 0.04mg/L; and control experiment (without extract) are shown in plates 1, 2 and 3 respectively.
345 In the histological studies, the abnormalities in the infected animals without extract (Plates 3) and the absence of visible
346 lesions in uninfected mice showed the damaging effects of the malaria parasite, *P. berghei berghei* on the organs of the
347 mice. The livers of the mice administered with *L. subnudus* (4mg/mL) before infection with malaria parasite had severe;
348 and mild portal congestion and periportal cellular infiltration (Plate 5). At concentration 4mg/mL, the extract is not as
349 effective as concentrations 0.4 and 0.04mg/mL.
350 The visible pathological changes on tissues of animals administered with chloroquine (20mg/kg) shown in plate 4 were
351 similar to those treated with extracts of concentrations 0.4 and 0.004 mg/mL. There were mild conditions with two different
352 concentrations and the chloroquine pre-infection experiment. This could be due to the preventive ability of the extract at
353 concentrations of 0.4 and 0.004mg/mL for prophylactic (pre-infective) administration. Plates 2, 3 and 4 revealed that
354 concentrations 0.4 and 0.04mg/mL had the same effect as the chloroquine.
355 *L. subnudus* can be considered a good prophylaxis in the prevention of malaria especially at concentrations 0.4 and
356 0.04mg/mL.

4. CONCLUSION
359 It is apparent from this study that the higher fungi had prophylactic antiplasmodial potentials and can be formulated into
360 antimalarial drugs. The extracts exerting prophylactic effect within 7 days at such low concentration confirms the
361 potentials of the higher extract against *Plasmodium* species. *Lentinus subnudus* and *Fomes lignosus* produced the

highest antiplasmodial effect against *Plasmodium berghei berghei* with a decrease of 30% and 36% respectively in parasitemia. These extracts could be potential sources of bioactive compounds and can be suggested as prophylaxis. However, further pharmacological studies are important.

COMPETING INTERESTS
Authors have no competing interest.

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