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# Interactions of Extracts of Selected Macrofungi and Malaria Parasite, *Plasmodium berghei berghei* in BALB/c Strain Albino Mice

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author SGJ designed the study. Author OOO wrote the protocol and wrote the first draft of the manuscript. Author OJO performed the statistical analysis and interpreted the analyses of the study. All authors managed the literature searches, read and approved the final manuscript.*

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## ABSTRACT

Malaria is a global menace that claimed many lives. The potential of mushroom at appropriate dosage, concentrations and suitable condition especially as antiplasmodial agents against malaria is important. Therefore, this study investigated the interactive effects of some fungi extracts (*Pleurotus tuber-regium, Pleurotus pulmonarius, Fomes lignosus, Lentinus subnudus, Termitomyces robustus*) and their combinations with malaria parasite, *Plasmodium berghei berghei* in BALB/c strain albino mice. Intraperitoneal injection of experimental animals with 0.2 mL of $5x10^6$ parasitized blood was done before or after oral administration of the extracts of 0.1 mL fungi extracts at five concentrations. There were 3 replicates. The percentage parasitemia, packed cell volume (PCV), the weight loss of the albino mice were monitored. The *extract*; and *concentration levels* recorded highly significant ($p< 0.01$) effects on the parasitemic level (137.96; 329.26), PCV (4539.48; 2357.93) and weights (53.46; 510.56) of experimental animals in prophylactic and

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therapeutic experiments. Also, highly significant interactions (of 521.30) was obtained from *extracts* x *concentrations. Lentinus subnudus* and *Fomes lignosus* as well as *P. tuber-regium* had the best prophylactic and therapeutic potentials of 30%; 36% and 36% respectively. *Lentinus subnudus* could be considered a good prophylaxis in prevention of malaria as it exceeds therapeutic effect. Concentrations 0.4 mg/mL and 0.04 mg/mL were found to be the most effective; producing similar effect as chloroquine (20 mg/kg body weight) used as control. Therefore, the optimum activity of the fungi extracts was interactive against the malaria parasite, *Plasmodium berghei berghei* in the albino mice.

*Keywords: Fungi extracts; Plasmodium species; antiplasmodial potentials; albino mice; interactive effects.*

## 1. INTRODUCTION

Mushrooms are higher fungi growing on decaying wastes [1]. They are highly rich in nutrients and medicinal compounds, such as lentinan, glycans etc. [2]. These in addition to other bioactive compounds enhanced human's health [3]. According to World Health Organization [4], malaria outbreak is a global problem associated with resistant *Plasmodium* strains. There is the need to search for drugs especially of natural origin that are effective against strains of *Plasmodium* responsible for the spread of malaria parasite. Therefore, this work aimed at studying the interactions of fungi extracts, and their concentrations that enhance therapeutic potentials of selected higher fungi against malaria parasite, *Plasmodium berghei berghei* in albino mice.

## 2. MATERIALS AND METHODS

### 2.1 Sources of Fungi Extracts, Experimental Animals and Malaria Parasite

Fungi samples (*Pleurotus tuber-regium, P. pulmonarius, Termitomyces robustus, Fomes lignosus* and *Lentinus subnudus*) were collected from different locations. Extraction of the five fungi was done separately with ethanol using soxhlet apparatus [5]. The extracts (40 mg/mL) were serially diluted to 4, 0.4, 0.004 and 0.0004 mg/mL before administering orally to the mice. The malaria parasite, *Plasmodium berghei berghei*; and BALB/C strain albino mice *(Mus musculus*) of 4-5 weeks old of an average weight of 22 grammes were used. Passaging was carried out as the albino mice were intraperitoneally injected with 0.2 mL of 5 x $10^6$ *Plasmodium berghei berghei* infected blood sample. They were monitored for about 12 days for parasitemia. Also, the packed cell volume (PCV) and weights of animals were determined.

### 2.2 Statistical Analysis

Data collected were analysed using SAS version 2.0 to compute Analysis of Variance (ANOVA) while Means were separated by Duncan's Multiple Range Tests (DMRT) at $p < 0.05$.

## 3. RESULTS AND DISCUSSION

The prophylactic effects of extract types, replicates, concentration and their interactions on parasitemia in albino mice for the days of infection (Table 1). The fungi species produced a highly significant ($p< 0.01$) prophylactic and therapeutic effects on the parasitemia, PCV, and weights of BALB/c albino mice. Extract and concentration produced high significant ($p< 0.01$) prophylactic effects on parasitemia except on the first and twelfth days of infection. The third order of interaction; concentration and replicates was significant only on the second day. The fungi extract types, concentration and their first order of interaction (extract x concentration) had prophylactic effects on the packed cell volume of albino mice on the first and third days of infection, while only Concentration produced significant effect on the twelfth day after infection (Table 2).

The result shown in Table 3 reveals that the extracts produced higher prophylactic effect on the weight of the experimental animals. Due to the effect of the extracts, weight loss in the animals was minimal on the first and second days of infection. The results in Tables 4 and 5 show the effects of the extracts, concentrations, interactions of the extracts and concentrations were highly significant (therapeutically) on the parasitemia and PCV in the animals throughout the period of the experiment. The effect of the concentrations, extracts and concentrations was highly significant ($P<0.01$) on the seventh day of parasitic infection, while the interactive effect of the extracts and replicates was significant (Table

6). The interactions of the parameters on the parasitemia, PCV, weight showed highly significant (P<0.01) therapeutic effect for Extract x Concentration. Similar results were obtained in the therapeutic experiments. This reveals the efficacy of the fungi extracts for both prophylactic and therapeutic experiments.

The findings from this study show that higher fungi especially mushroom possess antiplasmodial potentials. The fungi extracts reduced the parasitemic infection in the mice in accordance with previous report of Katsayal et al. [6]. The evaluation of *in-vivo* single and interactive effects of the fungi extracts at different concentration levels against the malaria parasite, *Plasmodium berghei berghei* was observed for a period of time was established as previously confirmed by Jonathan et al. [7]. The single interactive effects of the extract types, concentrations, as well as the combination of extract and concentrations increased prophylactic effect on the parasitemia with the exception of the day of infection of the plasmodium on the albino mice. This is in accordance with the report of White et al. [8].

The prophylactic and therapeutic effects of the fungi extracts was enhanced except in the replicate and in the co-interaction of the Extracts X Replicate at all levels of interaction in parasitemia, PCV and weights of the experimental animals. This was in agreement with the findings on inhibitory effects of some botanicals against *Fusarium* species [9,10,11]. The interactions of the extract by concentration increased the preventive and curative potentials of the fungi. This could be attributed to the pharmacological compounds and bioactive components of the fungi extracts. They evidenced the biological and medicinal qualities of the higher fungi. These are naturally-occurring chemical compounds play the roles of protecting human health [12,13,14,15,16].

**Table 1. Interactive effects of extract types, replicates, concentration on parasitemia in albino mice for the days of infection**

| Source of variation | % Parasitemia | | | | | | |
|---|---|---|---|---|---|---|---|
| | df | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 12 |
| **Extract types** | 5 | $6.9^{ns}$ | $56.54^{**}$ | $57.46^{**}$ | $54.25^{**}$ | $18.79^{*}$ | $137.96^{**}$ |
| **Replicate** | 2 | $7.17^{ns}$ | $0.48^{ns}$ | $3.90^{ns}$ | $2.53^{ns}$ | $2.05^{ns}$ | $68.07^{ns}$ |
| **Concentration** | 5 | $9.65^{ns}$ | $94.40^{**}$ | $88.01^{**}$ | $98.98^{**}$ | $95.44^{**}$ | $329.26^{**}$ |
| **Extract x replicate** | 10 | $8.46^{ns}$ | $2.43^{ns}$ | $3.61^{ns}$ | $6.11^{ns}$ | $2.94^{ns}$ | $27.01^{ns}$ |
| **Extract x concentration** | 25 | $8.62^{ns}$ | $19.46^{**}$ | $15.18^{**}$ | $22.56^{**}$ | $26.67^{**}$ | $68.50^{ns}$ |
| **Concentration x replicate** | 10 | $8.65^{ns}$ | $8.01^{*}$ | $5.42^{ns}$ | $3.36^{ns}$ | $8.87^{ns}$ | $30.01^{ns}$ |
| **Error** | 50 | | | | | | |
| **Total** | 108 | | | | | | |
| **Corrected total** | 107 | | | | | | |

*ns, *, and ** are not significant, and highly significant at p < 0.05; and p<0.01 respectively*

**Table 2. Interactive effects of the extract types, replicates, concentration and on PCV of albino mice for the days of infection**

| Source of variation | Packed Cell Volume (PCV) | | | | | | |
|---|---|---|---|---|---|---|---|
| | df | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 12 |
| **Extract types** | 5 | $164.25^{*}$ | $2794.49^{ns}$ | $205.12^{ns}$ | $534.52^{ns}$ | $300.6^{ns}$ | $307.16^{ns}$ |
| **Replicates** | 1 | $4.01^{ns}$ | $3200.00^{ns}$ | $490.89^{*}$ | $193.39^{ns}$ | $1530.89^{ns}$ | $196.68^{ns}$ |
| **Concentration** | 5 | $272.98^{**}$ | $3246.85^{ns}$ | $225.39^{ns}$ | $522.12^{ns}$ | $1041.00^{ns}$ | $1489.22^{**}$ |
| **Concentration x Replicate** | 5 | $63.31^{ns}$ | $2599.90^{ns}$ | $79.99^{ns}$ | $39.29^{ns}$ | $405.06^{ns}$ | $134.71^{ns}$ |
| **Extract x replicate** | 5 | $106.51^{ns}$ | $2482.43^{ns}$ | $169.19^{**}$ | $66.42^{ns}$ | $90.32^{ns}$ | $369.71^{ns}$ |
| **Extract x Conc.** | 25 | $129.15^{*}$ | $2480.06^{ns}$ | $208.89^{*}$ | $283.45^{ns}$ | $407.18^{ns}$ | $688.42^{ns}$ |
| **Error** | 25 | | | | | | |
| **Total** | 72 | | | | | | |
| **Corrected total** | 71 | | | | | | |

*ns, *, and ** are not significant, and highly significant at p < 0.05; and p<0.01 respectively*

**Table 3. Interactive effects of extract types, replicates, and concentration on weights of albino mice during the period of infection**

| Source of variation | Weight | | | | | | |
|---|---|---|---|---|---|---|---|
| | df | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 12 |
| **Extract types** | 5 | 21.72** | 23.42** | 23.06ns | 19.59ns | 46.71* | 50.08ns |
| **Replicate** | 2 | 21.84** | 48.51** | 32.78* | 44.53* | 76.44** | 152.12** |
| **Concentration** | 5 | 3.08** | 3.17ns | 4.30ns | 15.19ns | 90.96** | 510.56** |
| **Extract x replicate** | 10 | 2.93ns | 6.24ns | 10.42ns | 7.14ns | 15.02ns | 34.95ns |
| **Extract x concentration** | 21 | 9.95** | 18.99* | 16.18ns | 20.87** | 44.25** | 44.36ns |
| **Concentration x replicate** | 10 | 5.39ns | 28.36** | 27.33* | 33.41** | 41.80** | 29.11ns |
| **Error** | 50 | | | | | | |
| **Total** | 108 | | | | | | |
| **Corrected total** | 107 | | | | | | |

*ns, *, and ** are not significant, and highly significant at p < 0.05; and p<0.01 respectively*

**Table 4.Therapeutic effects of extract types, replicates, concentration on parasitemia during the period of infection in albino mice**

| Source of variation | % Parasitemia | | | | | | |
|---|---|---|---|---|---|---|---|
| | df | Day 7 | Day 8 | Day 9 | Day 10 | Day 11 | Day 14 |
| **Extract types** | 5 | 26.44** | 14.44** | 11.62** | 24.15* | 31.84** | 20.32** |
| **Replicate** | 2 | 14.01* | 11.61ns | 5.50ns | 3.52ns | 5.68ns | 0.67ns |
| **Concentration** | 5 | 30.80** | 29.91** | 20.59** | 26.60** | 22.39** | 23.61** |
| **Extract x replicate** | 10 | 5.69ns | 2.49ns | 3.90ns | 2.06ns | 1.83ns | 2.82ns |
| **Extract x concentration** | 21 | 11.29** | 15.83** | 23.68** | 18.21** | 21.31** | 23.60** |
| **Concentration x replicate** | 10 | 3.30ns | 4.55ns | 1.39ns | 2.99ns | 4.11ns | 3.17ns |
| **Error** | 42 | | | | | | |
| **Total** | 96 | | | | | | |
| **Corrected total** | 95 | | | | | | |

*ns, *, and ** are not significant, and highly significant at p < 0.05; and p<0.01 respectively*

**Table 5. Therapeutic effects of extract types, replicates, concentration on PCV during the period of infection in albino mice**

| Source of variation | Packed Cell Volume (PCV) | | | | | | |
|---|---|---|---|---|---|---|---|
| | df | Day 7 | Day 8 | Day 9 | Day 10 | Day 11 | Day 14 |
| **Extract types** | 5 | 1294.51** | 3336.35** | 3815.00** | 4539.48** | 4282.39** | 3245.45** |
| **Replicate** | 2 | 190.21ns | 285.18** | 24.83ns | 95.30ns | 120.02ns | 85.06ns |
| **Concentration** | 5 | 399.71** | 518.46** | 443.72ns | 804.79** | 992.81** | 2357.93** |
| **Extract x replicate** | 10 | 52.86ns | 69.87ns | 199.78ns | 52.40ns | 42.64ns | 61.18ns |
| **Extract x Concentration** | 21 | 427.47** | 329.13** | 521.30** | 423.41** | 438.71** | 281.41** |
| **Concentration x Replicate** | 10 | 46.11ns | 86.61ns | 92.77ns | 115.27ns | 114.11ns | 130.06ns |
| **Error** | 42 | | | | | | |
| **Total** | 96 | | | | | | |
| **Concentrated total** | 95 | | | | | | |

*ns, *, and ** are not significant, and highly significant at p < 0.05; and p<0.01 respectively*

The parasitemia infections in the mice were effectively suppressed by the interactions of the fungi extracts. This indicates the efficacy of the extracts against the malaria parasite as earlier reported by Chelela et al. [17]. As a result of the potency, moderate percentage of parasitemia was recorded for the extracts administered at different concentration levels throughout the period of infection. The results of the interactions of extract and replicate, concentration and replicate could be due to the non-significance of the replicates. The efficacy of the extracts and the prompt activities in reducing the parasitemia of the mice, stabilizing the PCV and reducing

**Table 6. Therapeutic effects of extract types, replicates, concentration on weights in albino mice during the period of infection**

| Source of variation | | Weight | | | | | |
|---|---|---|---|---|---|---|---|
| | df | Day 7 | Day 8 | Day 9 | Day 10 | Day 11 | Day 14 |
| **Extract types** | 5 | $53.46^{**}$ | $46.34^{**}$ | $65.55^{*}$ | $73.31^{*}$ | $54.21^{ns}$ | $44.17^{ns}$ |
| **Replicate** | 2 | $0.99^{ns}$ | $5.26^{ns}$ | $23.99^{ns}$ | $39.46^{ns}$ | $1.37^{ns}$ | $5.88^{ns}$ |
| **Concentration** | 5 | $27.40^{**}$ | $19.68^{*}$ | $40.05^{ns}$ | $76.33^{*}$ | $110.19^{ns}$ | $75.81^{**}$ |
| **Extract x replicate** | 10 | $10.17^{*}$ | $18.82^{*}$ | $54.99^{ns}$ | $49.95^{*}$ | $34.38^{ns}$ | $28.32^{ns}$ |
| **Extract x concentration** | 21 | $18.57^{**}$ | $33.67^{**}$ | $50.16^{**}$ | $63.91^{*}$ | $76.80^{**}$ | $66.84^{**}$ |
| **Concentration x replicate** | 10 | $13.65^{**}$ | $21.09^{**}$ | $52.63^{ns}$ | $51.93^{*}$ | $34.38^{ns}$ | $28.32^{ns}$ |
| **Error** | 42 | | | | | | |
| **Total** | 96 | | | | | | |
| **Corrected total** | 95 | | | | | | |

*ns, *, and ** are not significant, and highly significant at p < 0.05; and p<0.01 respectively*

**Table 7. Quantitative phytochemical components of the fungi extracts**

| Phytochemicals | Lent | Mix | Fom | PP | PT | Term |
|---|---|---|---|---|---|---|
| **Tannin** | 0.52 | 0.02 | 0.53 | 0.17 | 0.67 | 0.50 |
| **Steroid** | 0.64 | 3.35 | 1.06 | 1.24 | 0.91 | 1.76 |
| **Oxalate** | nd | 0.01 | nd | nd | 0.01 | 0.01 |
| **Saponin** | nd | nd | nd | 0.12 | nd | nd |
| **Flavonoid** | nd | nd | nd | 0.72 | nd | 0.58 |
| **Alkaloid** | nd | 0.01 | nd | nd | nd | nd |
| **Cyanogenic glucoside** | 0.15 | 0.01 | 0.10 | 0.20 | 0.15 | 4.00 |
| **Phenol** | 0.28 | 0.005 | 0.28 | 0.72 | 0.45 | 0.02 |
| **DPPH (Antioxidant)** | 73.40 | 85.34 | 89.30 | 85.20 | 83.20 | 69.08 |

*DPPH- 2, 2–diphenyl-1-picrylhydrazyl; nd- not detected; FOM - Fomes lignosus; PT - Pleurotus tuber-regium; PP - Pleurotus pulmonarius; Term- Termitomyces robustus; Lent - Lentinus subnudus; Mix - Mixture of all the fungi samples in equal proportion*

weight loss in the animals established the potency of the fungi extract as reported by Walker et al. [18].

## 4. CONCLUSION

It is apparent from this study that the tested fungi possess prophylactic and therapeutic antiplasmodial potentials. *L. subnudus* and *P. tuber-regium* gave the best prophylactic and therapeutic effect against the malaria parasite, *Plasmodium berghei berghei* in the albino mice. Concentrations 0.4 mg/mL and 0.04 mg/mL produced the best effect against the malaria parasite. Therefore, the study on interactions of the higher fungi in the prevention and treatment of malaria could be integrated in antimalarial study.

## ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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