

INTERACTIONS OF EXTRACTS OF SELECTED MACROFUNGI AND
MALARIA PARASITE, *Plasmodium berghei berghei* IN BALB/C STRAIN
ALBINO MICE

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 5×10^6 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 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 (20mg/kg bw) 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

49 selected higher fungi against malaria parasite, *Plasmodium berghei berghei* in
50 albino mice.

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52 **2. MATERIAL AND METHODS**

53 **Sources of fungi extracts, experimental animals and malaria parasite**

54 Fungi samples (*Pleurotus tuber-regium*, *P. pulmonarius*, *Termitomyces robustus*,
55 *Fomes lignosus* and *Lentinus subnudus*) were collected from different locations.
56 Extraction of the five fungi were done separately with ethanol using soxhlet
57 apparatus [5]. The extracts (40 mg/mL) were serially diluted to 4, 0.4, 0.004 and
58 0.0004 mg/mL before administering orally to the mice. The malaria parasite,
59 *Plasmodium berghei berghei*; and BALB/C strain albino mice (*Mus musculus*) of 4-5
60 weeks old of an average weight of 22 grammes were used. Passaging was carried
61 out as the albino mice were intraperitoneally injected with 0.2 mL of 5×10^6
62 *Plasmodium berghei berghei* infected blood sample. They were monitored for about
63 12 days for parasitemia. Also, the packed cell volume (PCV) and weights of animals
64 were determined.

65 **Statistical Analysis**

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

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72 **3. RESULTS AND DISCUSSION**

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

84 The result shown in Table 3 reveals that the extracts produced higher prophylactic
85 effect on the weight of the experimental animals. Due to the effect of the extracts,
86 weight loss in the animals was minimal on the first and second days of infection.
87 The results in Tables 4 and 5 show the effects of the extracts, concentrations,
88 interactions of the extracts and concentrations were highly significant
89 (therapeutically) on the parasitemia and PCV in the animals throughout the period of
90 the experiment. The effect of the concentrations, extracts and concentrations was
91 highly significant ($P < 0.01$) on the seventh day of parasitic infection, while the
92 interactive effect of the extracts and replicates was significant (Table 6). The
93 interactions of the parameters on the parasitemia, PCV, weight showed highly
94 significant ($P < 0.01$) therapeutic effect for Extract x Concentration. Similar results
95 were obtained in the therapeutic experiments. This reveals the efficacy of the fungi
96 extracts for both prophylactic and therapeutic experiments.

97 The findings from this study show that higher fungi especially mushroom possess
antiplasmodial potentials. The fungi extracts reduced the parasitemic infection in the

98 mice in accordance with previous report of Katsayal et al.[6]. The evaluation of *in-*
 99 *vivo* single and interactive effects of the fungi extracts at different concentration
 100 levels against the malaria parasite, *Plasmodium berghei berghei* was observed for a
 101 period of time was established as previously confirmed by Jonathan et al. [7]. The
 102 single interactive effects of the extract types, concentrations, as well as the
 103 combination of extract and concentrations increased prophylactic effect on the
 104 parasitemia with the exception of the day of infection of the plasmodium on the
 105 albino mice. This is in accordance with the report of White et al. [8].

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

116 The parasitemia infections in the mice were effectively suppressed by the
 117 interactions of the fungi extracts. This indicates the efficacy of the extracts against
 118 the malaria parasite as earlier reported by Chelela et al. [17]. As a result of the
 119 potency, moderate percentage of parasitemia was recorded for the extracts
 120 administered at different concentration levels throughout the period of infection. The
 121 results of the interactions of extract and replicate, concentration and replicate could
 122 be due to the non-significance of the replicates. The efficacy of the extracts and the
 123 prompt activities in reducing the parasitemia of the mice, stabilizing the PCV and
 124 reducing weight loss in the animals established the potency of the fungi extract as
 125 reported by Walker et al. [18].

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

Source of Variation	df	% Parasitemia					
		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						

130 ns, *, and ** are not significant, and highly significant at $p < 0.05$; and $p < 0.01$
 131 respectively.

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

Source of Variation	df	Packed Cell Volume (PCV)					
		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						

138 ns, *, and ** are not significant, and highly significant at $p < 0.05$; and $p < 0.01$
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145 **Table 3: Interactive effects of extract types, replicates, and concentration on**
 146 **weights of albino mice during the period of infection**

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

147 ns, *, and ** are not significant, and highly significant at $p < 0.05$; and $p < 0.01$
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161 **Table 4 :** Therapeutic effects of extract types, replicates, concentration on
 162 parasitemia during the period of infection in albino mice

Source of Variation	df	% Parasitemia					
		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*	11.61 ^{ns}	5.50 ^{ns}	3.52 ^{ns}	5.68 ^{ns}	0.67 ^{ns}
Concentration	5	30.80**	29.91**	20.59**	26.60**	22.39**	23.61**
Extract x Replicate	10	5.69 ^{ns}	2.49 ^{ns}	3.90 ^{ns}	2.06 ^{ns}	1.83 ^{ns}	2.82 ^{ns}
Extract x Concentration	21	11.29**	15.83**	23.68**	18.21**	21.31**	23.60**
Concentration x Replicate	10	3.30 ^{ns}	4.55 ^{ns}	1.39 ^{ns}	2.99 ^{ns}	4.11 ^{ns}	3.17 ^{ns}
Error	42						
Total	96						
Corrected Total	95						

163 ns, *, and ** are not significant, and highly significant at $p < 0.05$; and $p < 0.01$
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168 **Table 5:** Therapeutic effects of extract types, replicates, concentration on
 169 PCV during the period of infection in albino mice

Source of Variation	df	Packed Cell Volume (PCV)					
		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	190.21 ^{ns}	285.18**	24.83 ^{ns}	95.30 ^{ns}	120.02 ^{ns}	85.06 ^{ns}
Concentration	5	399.71**	518.46**	443.72 ^{ns}	804.79**	992.81**	2357.93**
Extract x Replicate	10	52.86 ^{ns}	69.87 ^{ns}	199.78 ^{ns}	52.40 ^{ns}	42.64 ^{ns}	61.18 ^{ns}
Extract x Concentration	21	427.47**	329.13**	521.30**	423.41**	438.71**	281.41**
Concentration x Replicate	10	46.11 ^{ns}	86.61 ^{ns}	92.77 ^{ns}	115.27 ^{ns}	114.11 ^{ns}	130.06 ^{ns}
Error	42						
Total	96						
Concentrated Total	95						

170 ns, *, and ** are not significant, and highly significant at $p < 0.05$; and $p < 0.01$
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184 **Table 6: Therapeutic effects of extract types, replicates, concentration on**
185 **weights in albino mice during the period of infection**

Source of Variation	df	Weight					
		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						

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

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

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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 0.0 4mg/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.

COMPETING INTERESTS

Authors have no competing interest.

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UNDER PEER REVIEW