Original Research Article

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 0.2mL of $5x10^6$ parasitized blood was done before or after oral administration of the extracts of 0.1mL 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 0.4mg/mL and 0.04mg/mL were found to be most effective; producing similar effect as chloroquine (20mg/kg bw) used as control. Therefore, the optimum activity of the fungi extracts were interactive against the malaria parasite, *Plasmodium berghei berghei* in the albino mice.

Keywords: Fungi extracts, *Plasmodium s*pecies, 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. MATERIAL AND METHODS

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 (40mg/mL) were serially diluted to 4, 0.4, 0.004 and 0.0004mg/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 22grammes were used. Passaging was carried out as the albino mice were intraperitoneally injected with 0.2mL of 5 x 10^6*Plasmodium berghei berghei* infected blood sample. They were monitored for about 12days for parasitemia. Also, the packed cell volume (PCV) and weights of animals were determined.

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 twelveth 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 twelveth 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 Table 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 mushrooms possess antiplasmodial potentials. The fungi extracts reduced the parasitemic infection in the

mice in accordance with previous report of [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[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 [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; and 11]. The interactions of the Extract, and 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].

The parasitemia infection 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 [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 weight loss in the animals established the potency of the fungi extract as reported by [18].

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

		% Parasitemia					
Source of Variation	**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						

*, ** are significant and highly significant values at $p < 0.05$ and $p<0.01$ respectively.

Comment [C1]: The meaning of ns should be included and in all tables

Table 2: Interactive effects of the extract types, replicates, concentration and 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						

*, ** are significant and highly significant values 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	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						

*, ** are significant and highly significant values 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

		% Parasitemia					
Source of Variation	**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^{*}	11.61^{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						

*, ** are significant and highly significant values 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

		Packed Cell Volume (PCV)					
Source of Variation	**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	190.21^{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						

*, ** are significant and highly significant values at $p < 0.05$ and $p<0.01$ respectively.

Table 6: Therapeutic effects of extract types, replicates, concentration on 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						

*, ** are significant and highly significant values 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.

4. CONCLUSION

It is apparent from this study that the tested fungi possess prophylactic and therapeutic antiplasmodial potentials. *L. subnudus* proved as the extract with the highest prophylactic effect against the malaria parasite, *Plasmodium berghei berghei* in the experimental mice while *P. tuber-regium* had the best therapeutic effect. In both prophylactic and therapeutic experiments, concentrations 0.4mg/mL and 0.04mg/ mL produced the best effect against the malaria parasite, *P. berghei berghei* in the albino mice. Therefore, the study on interactions of the higher fungi in the prevention and treatment of malaria could be integrated in antimalarial study.

Comment [C2]: Should be recast please

COMPETING INTERESTS

Authors have no competing interest.

REFERENCES

1.Dubuex, JCB. Jr., Sollenberger, LE., Interrante, SM., Vendramini, JMB and Steward, RL., Jr. Litter decomposition ad mineralization in bahiagrass pastures managed at different intensities. Crop Sci., (2006) 46: 1303 – 1310.

2. Jonathan, S.G. Vegetative growth requirements and antimicrobial activities of some higher fungi in Nigeria. Ph.D thesis, University of Ibadan (2002).

3. Opige, M., Kateyo, E. and Olila, D. Indigenous knowledge and indigenous usage of edible and medicinal mushrooms among the Teso people of Eastern Uganda. Journal of Food Technology(2006) 4(4): 325-330.

4. WHO. Guidelines for the treatment of malaria. Fact Sheet'94 (2015)

5. Redfren J, Kinnimonth M, Burdass D, Verran J (2014) Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. *J Microb. Biol. Edu* 15(1): 45-46

6. Katsayal, UA., Abdurahman, EM., Abubakar, MS., Musa, KY., Ambah, SF., and Jahun, MB. Fungi as potential source of antimalarial agents. Nig Journal Pharma.Sci(2009)8(1): 138-142.

7. Jonathan, SG., and Olawuyi, OJ., Popoola, OO. and Aina, DA. Antibacterial activities of extracts of *Daldina concentrica*. African J. Biomed. Res. (2011)14: 57 – 61.

8. White, SR., Obradovic, T. Imeh, KM., Wheaton, MJ. The effects of methylenedioxmethamphetamine (MDMA, "Estasy") on monoaminergic neurotransmission in the central nervous system. Progress in Neurobiology (1996)49:455-479

9. Agbenin, NO., Marley, PS. *In vitro* assay of some plant extracts against *Fusarium oxysporum* F. sp lycopersici causal agent of tomato wilt. Journal of Plant Protection Research in Plant Biology (Poland) (2006) 46: 117-121

10. Babu, J., Muzafar, AD., Vinod, K. Bioefficacy of Plant Extracts to control *Fusarium solani* F. sp Melanogenae Incitant of Brinjal Wilt. Global Journal of Biotechnology and Biochemistry (2008).3(2): 56-59

11. Akanmu, AO., Olawuyi, OJ., Abiala, MA., Yaya, OS., Odebode, AC. Interactive effects of some botanicals and *Fusarium* spp on the growth of millet seedlings. Research in Plant Biology. (2013) 4(1): 01-11

12. Hasler, CM., Blumberg, JG. Symposium on phytochemicals: Biochemistry and Physiology.Journal of Nutrition (1999)129:7565-7575.
13. Smith, RA., Mettlin CJ., Davis, KJ., Eyre, H. American Cancer Society guidelines for the earlydetection of cancer. A cancer Journal for Clinicians. (2000) 50(1): 34-49.
14. Saxena, J. and Patra, AK. Dietary phytochemicals as rumen modifiers: a review of the effects of on microbial populations. Antonie van Leeuwenhoek(2009)96: 363-375
15. Gracia, EJ., Oldoni, TLC., de Alencar, SM., Reis, A., Luguerio, AD., Grande, HM. Antioxidant activity of DPPH of potential solution to be applied on bleached teeth. Branzilian Dental Journal(2012)23(1): 22-27
16. Ilondu, EM. Myco- chemical composition and efficacy of four mushroom extracts in the control of *Rhizoctania solani,* a damping-off pathogen of garden egg (*Solanum melongena* L.) seedlings. American Journal of Scientific and Industrial Research(2013) 4(5):429-437.
17. Chelela, BL., Chacha, M.,Matemu, A. O. Wild edible mushroom value chain for improved livelihoods in Southern Highlands of Tanzania. American Journal of Research Communication 2014 2(8):1-14
18. Walker, MG., Page, CP., Hoffman, BF., Curtis, M. Integrated Pharmacology. (3rd ed.). St. Louis: Mosby. 2006. ISBN 0-323-04080-2