Journal of Advances in Microbiology

8(2): 1-9, 2018; Article no.JAMB.39198
ISSN: 2456-7116

In vivo Prophylactic Evaluation of the Antiplasmodial Property of Ethanol and Hot Water Extracts of Milky Mushroom (*Calocybe indica)*

F. O. Omoya[1*] and O. Oyedirin[1]

[1]Department of Microbiology, Federal University of Technology, Akure, P.M.B. 704, Akure, Ondo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author FOO designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author OO managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2018/39198
Editor(s):
(1) Niranjala Perera, Department of Food Science & Technology, Wayamba University of Sri Lanka, Sri Lanka.
Reviewers:
(1) Nwankwo Nicodemus Emeka, Renaissance University, Nigeria.
(2) Patrick Valere Tsouh Fokou, University of Yaoundé 1, Cameroon.
Complete Peer review History: http://www.sciencedomain.org/review-history/23023

Original Research Article

Received 3rd November 2017
Accepted 25th January 2018
Published 5th February 2018

ABSTRACT

Aims: *Calocybe indica* is commonly known as the milky mushroom and is one of the protein-rich mushrooms that have high medicinal property. Malaria is endemic in Nigeria with 97% of the population of 170 million living in areas of high malaria risk. Hence, *in vivo* prophylactic antiplasmodial property of milky mushroom extracts was evaluated.
Place and Duration of Study: Department of Microbiology, Federal University of Technology, Akure between February and October, 2017.
Methodology: The experimental mice were fed for four days with different concentrations of the hot water and ethanol extracts before infecting them with *Plasmodium berghei* (NK 65 species) and observed for another three days.
Results: The results showed that the infection caused the body temperature and weight to reduce from 36.8±0.10°C to 35.1±0.45°C and 17.23±0.33 g to 16.15±0.43 g respectively. The parasitemia count 24 hours after infection with *P. berghei* on a group that were given the extracts before the infection showed that the extracts had a prophylactic effect on the parasite. Comparatively, the

**Corresponding author: E-mail: fomoya@yahoo.com;*
E-mail: ooluyhemikehinde@yahoo.com;

group that was infected without prior treatment recorded 326 parasites per field, the group treated with 400 mg/ml of the ethanol extract of the milky mushroom had two parasites per view. The *P. berghei* infection also caused a significant decrease in the packed cell volume (PCV) of the mice. Comparatively, the mushroom ethanol extract at 400 mg/ml increased the PCV to 44.50±0.67% from 35.25±1.33% in the control group. The group infected with *P. berghei* without prior treatment (group F) had less than 30% PCV.

Conclusion: *Calocybe indica* (milky mushroom) extracts used in this research exerted high prophylactic property against *Plasmodium berghei in vivo* and caused a significant increase in packed cell volume of the experimental mice. Therefore, milky mushroom could be incorporated into daily food to offer prophylaxis against malaria parasite infections that are on the increase globally.

Keywords: Milky mushroom; prophylactic; antiplasmodial; parasites.

1. INTRODUCTION

The world population is currently 7 billion, and it is increasing at a faster rate. By the year 2050, the global population is expected to reach 9 billion by the year 2100 it could be 20 billion [1]. Shortage of food and diminishing quality of human health will be a concern because of the population increase and urbanization, with a concomitant reduction in arable land. Converting lignocellulosic agricultural and forest residues into protein-rich mushrooms is one of the most economically viable and sustainable biotechnology processes to address world food demand, especially protein demand [2]. Consumption of edible fungi to fulfil human nutritional needs has been a common denominator in the history of mankind [1]. Since most of these edible mushrooms have favourable growth conditions at lower temperatures (< 25℃), a creation of infrastructure for commercial cultivation, especially in warm, humid tropical regions, is always expensive [3]. Identification and cultivation of warm-weather (30~38℃) varieties of edible mushrooms have been scientifically challenging. Milky white (*Calocybe indica* var. APK2) is one of such mushroom varieties, where complete commercial production techniques have been standardized [1]. The first ever milky white mushroom variety (*Calocybe indica* P&C) var. APK2 was released from Tamil Nadu Agricultural University, Coimbatore, Indiana 1998. Over a decade, commercial production of this mushroom variety has assumed greater impetus in India, uplifting rural livelihood.

In Nigeria, malaria is responsible for 60% outpatient visits to health facilities, 30% childhood death, 25% of death in children under one year and 11% maternal death [4]. The financial loss due to malaria annually is estimated to be about 132 Billion Naira in the form of treatment costs, prevention, loss of man-hour, etc.; yet, it is a treatable and completely evitable disease.

Malaria is endemic in Nigeria with 97% of the population of 170 million living in areas of high malaria risk and an estimated 3% living in malaria-free highlands. Nigeria bears up to 25% of the malarial disease burden in Africa, conferring the country with the highest malaria mortality [5].

The Global Fund (TGF)'s response in the fight against Malaria in Nigeria is co-managed by the National Malaria Elimination Programme (NMEP). Currently, NMEP is implementing New Funding Model (NFM) of TGF which began in January 2015 [5]. Implementation of malaria control interventions is broad-based and includes Case Management; Integrated Vector Management; Special Interventions such as Intermittent Presumptive treatment with Sulphadoxine and Pyrimethamine; and other supportive interventions.

Edible mushrooms have been a source of food to a man with known health and medicinal benefits. They have served as a major source of food in several African countries including Nigeria, with different tribes having their own local name for them. However, their use as an antimalarial agent has not been documented.Hence, this study was aimed at assessing the prophylactic effect of the extracts of milky mushroom on albino mice infected with *Plasmodium berghei*.

2. MATERIALS AND METHODS

2.1 Sample Collection

Sufficient mushrooms *(Calocybe indica)* were obtained from Shoprite, Ikeja, Lagos State, Nigeria and taken to the laboratory for

confirmation by a mushroom expert at the Microbiology Department, Federal University of Technology, Akure.

2.2 Drying and Extraction

The mushroom samples were cut into pieces using a sharp knife. The pieces were air-dried properly for a period of 1 week, and to avoid denaturing it was kept in an oven at 40ºC in the night time. The air-dried mushroom sample was blended into powder using a blender [6].

Extraction of the bioactive components was done after blending the dried mushroom into powder. Two-hundred and fifty grams (250 g) of dried mushroom blend weighed into two different conical flasks. One was mixed with 750 ml 95% ethanol and covered while the second portion was soaked with 750 ml of boiled hot water. Both were shaken and left to stand for 72hours and then filtered using muslin bag. The filtrate was dried using rotary evaporator (RE-52A model Union Laboratory England) at 78ºC for the ethanol extract and 95ºC for the water extract.

2.3 Experimental Mice

Twenty three albinomice were obtained from the faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State. They weighed between 15-23g and were all male. The mice were acclimatized in the new environment for five days fed with water and commercially available feed.

2.3.1 Prophylactic test

The 7-day Peters prophylactic test according to [7] was used. After three days of treating with the extracts through oro-gastric administration (mg/Kg per body weight), they were infected with *Plasmodium berghei* intraperitoneally; the mice were then examined for another four days to observe the effect of the extract in preventing the onset of the infection in them. The mice were grouped into 7 of 3 mice per group as follows:

Group 1- Treated with 200 mg/ml of hot water extract before infection.
Group 2- Treated with 400 mg/ml of hot water extract before infection.
Group 3- Treated with 200 mg/ml of hot water extract before infection.
Group 4- Treated with 400 mg/ml of hot water extract before infection.
Group 5- Treated with 5 mg/ml of chloroquine before infection.
Group 6- Not treated before infection.
Group 7- Not infected and not treated with anything.

The mice were given the extract in the above doses daily for three days.

2.3.2 Parasitemia count

The parasitemia count was carried out daily starting from the second day after infection. This was done by a little cut on their tail and making a smear of the blood on the slide.

Parasitemia = {(Total number of parasitized red blood cell count × 100) / Total number of red blood count}

2.3.3 Temperature evaluation

A temperature of the mice was checked daily using a clinical thermometer fixed to their anus for 30 seconds after demobilizing them in a fixed container. The readings were taken daily for the period of the experiment.

2.3.4 Standard drug

Chloroquine was obtained from a Pharmacy in Akure, Ondo State and prepared to 5mg/ml for the group that served as the positive control. They were given 0.2 ml of the drug daily for the four days.

2.4 Preparation of Blood Films

2.4.1 Thick films

A drop of blood from the tail of the mice was put on a slide resting on a flat surface. The dropped blood was then spread rapidly and evenly with the edge of a clean glass slide making a circular movement till the desired diameter is achieved. The films were kept horizontally and protected from dust for best result; the slide was dried for a few minutes at 37°C then stained using Giesma stain. Microscopic examination of the stained slide was done using x100 oil immersion objective.

2.4.2 Estimation of parasite numbers/µl of blood

Parts of the thick film where the white cells are evenly distributed and the parasites are well stained were selected. Using the oil immersion

objective, 100 white blood cells (WBC) at the same time the numbers of parasites (asexual) in each field covered were systematically counted. This was repeated in two other areas of the film and averages of the three counts were taken. The number of parasites per µl of blood is calculated as follows:

Parasitemia = {(Total number of parasitized red blood cell count × 100) / Total number of red blood count}

% Parasite suppression was calculated as follows;

[{(No of parasite in infected and not treated mice-No of parasite-infected and treated) × 100} / No of parasite-infected and not treated mice]

2.5 Blood Parameters

2.5.1 Erythrocyte sedimentation rate (ESR)

A wintrobe tube was filled to the top 0 mark, and one end of it blocked with plasticine. It stood in an upright position undisturbed for 60 minutes (1 hr). The distance of the fall of red cells in it was read and expressed as the mm fall in an hour as the ESR.

2.5.1 Packed cell volume (PCV)

Blood collected into the anticoagulant bottle was mixed, and a capillary tube was filled up to 75% (3/4) of its length and placed in the micro-haematocrit centrifuge with the sealant at the outer end and centrifuged at 12,000 rpm for 5 minutes. The result was read as a percentage of packed red cells to the total volume of whole blood using a haematocrit reader.

2.5.2 Red blood cell count (RBC)

The blood sample was diluted 1:200 and mixed properly. The volume 0.02 ml of the blood was pipette into 4 ml of diluting fluid in a bijou bottle and washed thoroughly by alternately drawing up and expelling the diluting fluid. A fine Pasteur pipette was used to fill the counting chamber and counted using a counter under × 40 objectives.

2.5.3 White bloodcell count (WBC)

The blood was first diluted to ratio 1:20 and 0.05ml of the blood pipetted into 0.95 ml of diluting fluid. A little portion was charged into the counting chamber and observed using ×10 objective to count the white cells/cubic mm.

2.5.4 Haemoglobin (Hb)

Using mouthpiece, sucker and a 0.02ml pipette, blood was withdrawn and expelled into 4ml Drabkin's solution in a tube. The tube was stoppered, mixed and allowed to stand for 5 minutes for full-colour development. A standard blood sample of known haemoglobin concentration was prepared. Using a green (624) filter, the calorimeter was set to zero using plain Drabkin's solution as a blank. The readings of the sample and the standard were taken and the result calculated as follows:

Sample haemoglobin concentration = {(Reading of test × standard haemoglobin concentration) / Reading of standard}

2.5.5 Differential white cell count

- To a well-made and correctly stained thin blood film, a drop of immersion oil was dropped on the lower third of the blood film and covered with a clean cover glass.
- The film was examined microscopically using x10 objective with the condenser closed sufficiently to see the cells clearly.
- The part of the film where the red cells are just beginning to overlap was moved to and the x40objectivewas brought into place.
- The blood film was systematically examined, and different white cells were counted as seen in each field using an automatic differential cell counter [8].

2.6 Ethical Consideration

Standard consideration for animal care was followed by the infection and the killing of the experimental animals

2.7 Statistical Analysis of Result

Data obtained from the results were subjected to descriptive one-way analyses of variance, SPSS version 21 Microsoft Windows 7 and Duncan multiple range tests were used as follow up test.

3. RESULTS AND DISCUSSION

3.1 Results

Infection with *P. berghei* caused a decrease in temperature in the experimental animals. The infection caused the temperature to reduce from an average of 36.8±0.10°C to 35.1±0.45°C.

Before infection (when the mice were being fed with the extracts), all mice maintained an average temperature of 36.8±0.10°C. But after infection, the temperature began to reduce gradually on a daily basis. This result is shown in Table 1.

The infection of the mice with *P. berghei* had a significant effect on the weight of the mice. It caused a reduction in the weight of the mice. This is seen in the weight of the group infected without any prior feeding of the mice with any extract (group 6). At the beginning of the experiment (before infection) the animals recorded significant increase between days 1 and 3. Twenty four (24 hours) after infection, there was a reduction in the weight from 17.23±0.33 g to 16.15±0.43 g. The result shown in Table 2 revealed that the control group recorded a constant increase in the weight of the experimental mice, having an average weight of 17.10±0.10 which later increased to 19.31±0.19 g.

The parasitemia count 24 hours after infection with *P. berghei* to confirm if the extract has a prophylactic effect on the parasite is shown in Table 3. Comparatively, the group that was infected without prior treatment recorded 326 parasites per field, the group treated with 400 mg/ml of the ethanol extract of the milky mushroom had two parasites per view. This results result showed that the extract actually exert prophylactic antimalarial property on the infected mice. This pattern was observed after 48 hours and 72 hours. After 72 hours, the group infected without prior treatment recorded a parasitemia count of 1022 per field. This is shown in Table 5.

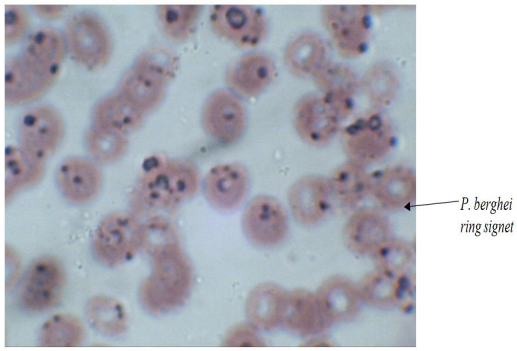

Plate 1. Photomicrograph of *P. berghei* in experimental mice' blood

The result of the haematological analysis showed that the mushroom ethanol extract at 400 mg/ml actually caused the highest effect or increase in the packed cell volume (PCV) of the mice. The *P. berghei*, on the other hand, caused a significant decrease in the PCV of the mice. Comparatively, the mushroom ethanol extract at 400 mg/ml increased the PCV to 44.50±0.67% from 35.25±1.33% in the control group. The group infected with *P. berghei* without prior treatment (group F) had less than 30% PCV. The infection caused an increase in the Erythrocyte Sedimentation Rate (ESR) of the mice from 1.33±0.10 mm/hr to 12.50±0.50 mm/hr. The mushroom extracts proved effective in reducing this ESR value below 3.00 mm/hr. The infection in the mice also caused an increase in the white blood cell count (WBC). While the control group recorded WBC count of 10.23±1.33 mm^{-3}, the infected group recorded 14.60±1.10 mm^{-3}. The extract further caused an increase in the red blood cell count in haemoglobin concentration as seen in Fig. 1.

Table 1. Effect of *P. berghei* infection on the temperature (˚C) of albino mice

S/N	1	2	3	4	5	6	7
1	36.5±0.10	37.0±0.00	36.8±0.20	36.8±0.40	36.8±0.10	36.8±0.04	36.7±0.33
2	36.9±0.30	36.9±0.33	36.8±0.20	37.2±0.10	36.8±0.04	36.9±0.33	36.8±0.30
3	36.4±0.60	36.9±0.10	36.9±0.30	36.8±0.70	36.8±0.33	36.9±0.10	36.8±0.20
4	36.5±0.50	36.7±0.33	36.9±0.69	36.9±0.22	36.9±0.10	36.8±0.10	36.8±0.40
5	36.0±0.10	36.8±0.20	37.0±0.00	36.8±0.60	36.8±0.33	36.2±0.40	36.9±0.30
6	35.9±0.30	36.7±0.30	36.5±0.10	36.7±0.10	36.7±0.31	35.6±0.22	36.9±0.50
7	35.6±0.96	36.6±0.40	36.1±0.50	36.7±0.22	36.7±031.	35.1±0.45	36.8±0.22

Keys: *Group 1- Treated with 200 mg/ml of hot water extract before infection.*
Group 2- Treated with 400 mg/ml of hot water extract before infection.
Group 3- Treated with 200 mg/ml of hot water extract before infection.
Group 4- Treated with 400 mg/ml of hot water extract before infection.
Group 5- Treated with 5 mg/ml of chloroquine before infection.
Group 6- Not treated before infection,
Group 7-Not infected and not treated with anything.

Table 2. Effect of infection on the weight (g) of experimental mice

Day	1	2	3	4	5	6	7
1	15.40±0.20	15.33±0.15	15.80±0.40	16.10±0.33	16.20±0.40	16.50±0.10	17.10±0.10
2	15.90±0.21	15.75±0.05	16.40±0.90	16.84±0.50	16.65±0.22	16.66±0.20	17.38±0.29
3	16.22±0.34	16.06±0.21	17.33±0.33	17.26±0.00	17.36±0.20	16.91±0.37	17.77±0.33
4	16.81±0.99	16.69±0.33	17.97±0.31	18.11±0.10	18.04±0.10	17.23±0.33	18.21±0.11
5	17.10±0.40	17.11±0.10	18.20±0.05	18.90±0.25	18.92±0.03	17.02±0.50	18.60±0.40
6	17.10±0.41	17.20±0.10	18.20±0.60	19.25±0.88	19.23±0.33	16.15±0.43	18.96±0.10
7	17.20±0.01	17.40±0.20	18.30±0.50	20.04±0.01	19.99±0.33	15.80±0.11	19.31±0.19

Keys: *Group 1- Treated with 200mg/ml of hot water extract before infection.*
Group 2- Treated with 400mg/ml of hot water extract before infection.
Group 3- Treated with 200mg/ml of hot water extract before infection.
Group 4- Treated with 400mg/ml of hot water extract before infection.
Group 5- Treated with 5mg/ml of chloroquine before infection.
Group 6- Not treated before infection,
Group 7-Not infected and not treated with anything.

Table 3. Parasitemia (Count per field) count from infected mice after 24 hours

Day	Extract	Concentration(mg/ml)	Count per field
1	Water	200	20
1	Water	400	29
1	Ethanol	200	10
1	Ethanol	400	02
1	Chloroquine	5	0
1	Infected	326	326

Keys: *Group A- Treated with 200 mg/ml of hot water extract before infection.*
Group B- Treated with 400 mg/ml of hot water extract before infection.
Group C- Treated with 200 mg/ml of hot water extract before infection.
Group D- Treated with 400 mg/ml of hot water extract before infection.
Group E- Treated with 5 mg/ml of chloroquine before infection,
Group F- Not treated before infection.

Table 4. Parasitemia count from infected mice after 48 hours

Day	Extract	Concentration	Count per field
2	Water	200	108
2	Water	400	82
2	Ethanol	200	69
2	Ethanol	400	12
2	Chloroquine	5	0
2	Infected	326	844

Keys: *Group A- Treated with 200 mg/ml of hot water extract before infection.*
Group B- Treated with 400 mg/ml of hot water extract before infection.
Group C- Treated with 200 mg/ml of hot water extract before infection.
Group D- Treated with 400 mg/ml of hot water extract before infection.
Group E- Treated with 5 mg/ml of chloroquine before infection,
Group F- Not treated before infection.

Fig. 2, which is the white blood cell differential count, shows that the extracts caused an increase in the lymphocyte counts of the test groups compared to the control group. The most significant increase was caused by the control group of ethanol extract of the milky mushroom which increased from 60.05±0.10% to 65.40±0.20%. The infection with the *P. berghei* on the other hand without prior treatment with the extract caused a significant decrease in the lymphocyte count from 59.63±0.67% to 48.30±0.50%. The WBC differential counts for neutrophils showed that there was increase in the percentage of the neutrophils from 22.10±0.50% to 33.65±1.39%, the extracts caused significant reduction of it to 16.30±0.90%. This effect was caused by the ethanol extract of the milky mushroom thereby showing that the ethanol extract exerted the highest effect on the malaria parasites.

Table 5. Parasitemia count from infected mice after 72 hours

Day	Extract	Concentration	Count per field
3	Water	200	349
3	Water	400	85
3	Ethanol	200	73
3	Ethanol	400	13
3	Chloroquine	5	2
3	Infected	326	1022

Keys: *Group A- Treated with 200 mg/ml of hot water extract before infection.*
Group B- Treated with 400 mg/ml of hot water extract before infection.
Group C- Treated with 200 mg/ml of hot water extract before infection.
Group D- Treated with 400 mg/ml of hot water extract before infection.
Group E- Treated with 5 mg/ml of chloroquine before infection,
Group F- Not treated before infection.

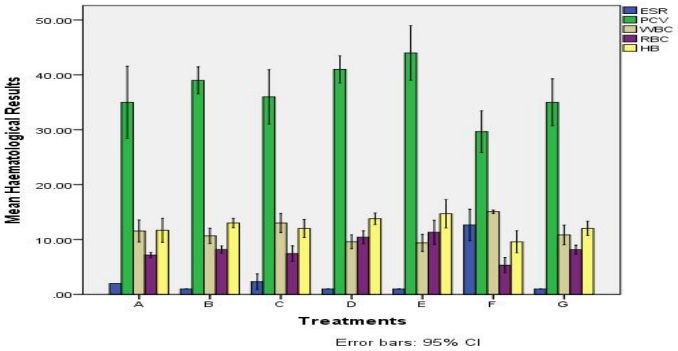

Fig. 1. Mean haematological indices of mice infected with*. Beghei* and treated with a mushroom extract

Legends: *Group A- Treated with 200 mg/ml of hot water extract before infection.*
Group B- Treated with 400 mg/ml of hot water extract before infection.
Group C- Treated with 200 mg/ml of hot water extract before infection.
Group D- Treated with 400 mg/ml of hot water extract before infection.
Group E- Treated with 5 mg/ml of chloroquine before infection,
Group F- Not treated before infection.

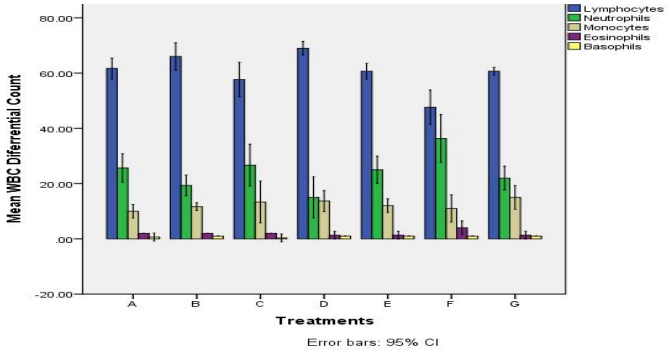

Fig. 2. Mean white blood cell differential counts in mice infected with *P. beghei* and treated with mushroom extract

Legends: *Group A- Treated with 200 mg/ml of hot water extract before infection.*
Group B- Treated with 400 mg/ml of hot water extract before infection.
Group C- Treated with 200 mg/ml of hot water extract before infection.
Group D- Treated with 400 mg/ml of hot water extract before infection.
Group E- Treated with 5 mg/ml of chloroquine before infection,
Group F- Not treated before infection.

3.2 Discussion

The results obtained in this research have shown that the two milky mushroom extracts exerted antiplasmodial activities against *Plasmodium bergheiin vivo*. The decrease in temperature from an average of 36.8±0.10˚C to 35.1±0.45˚C in the group infected without any prior treatment with any of the extracts was evidence that infection was actually established in the mice. According to [9], the establishment of malaria in mice is usually accompanied by a decrease in their body temperature. However, critical look at the groups that were given the extracts before the infection did not show a sudden decrease in their body temperature. According to [8], any substance, drug or medicinal supplement that exerts prophylactic property will prevent the primary signs and symptoms of infection. [10] listed temperature rise or fall (in case of some animals like mice) as one of the primary sign of infection.

The infection of the mice with *P. berghei* had a significant effect on the weight of the mice. It caused a reduction in the weight of the mice. This is seen in the weight of the group infected without any prior feeding of the mice with any extract (group 6). At the beginning of the experiment (before infection) the animals recorded a significant increase in between day 1 and day 3. This result agrees with a result obtained by [7], in which infected mice that had malaria all showed a decrease in body weight. The prophylactic effect of the extracts, which was more in the ethanol extract of the milky mushroom was observed in the level of weight loss.

The parasitemia count 24 hours after infection with *P. berghei* to check if the extract has a prophylactic effect on the parasite is shown in table 3. Comparatively, the group that was infected without prior treatment recorded 326 parasites per field, the group treated with 400 mg/ml of the ethanol extract of the milky mushroom had 326 parasites per view. This results result shows that the extract actually exertedrophylactic antimalarial property on the infected mice. This result is in agreement with the result obtained by [2] in which the parasitemia count was highly reduced in the group given chloroquine than the other group in an assay to evaluate the prophylactic effect of chloroquine syrup. The ethanol extract also exerted the highest prophylactic effect in this research. This probably may because ethanol solvent may be more potent in extracting the active ingredients than the hot water. According to [11], ethanol may be more potent in the extraction of certain active ingredients of some plants that may contain volatile active ingredients like salicin. [12], has suggested that milky mushroom (*Calocybe indica)* is either eaten raw or extracted with cold water and taken like cold tea to prevent losing its volatile ingredients.

The result of the haematological analysis showed that the mushroom ethanol extract at 400 mg/ml actually caused the highest effect or increase in the packed cell volume (PCV) of the mice. The *P. berghei*, on the other hand, caused a significant decrease in the PCV of the mice. This result is similar to the results obtained by [9] in which infection of mice with *P. berghei* caused a decrease in the packed cell volume of the mice. According to [5], *Plasmodium* species causes lysis of red blood cells (haemolysis) that consequently leads to decrease in blood volume which if not checked will eventually cause anaemia. This may be responsible for the decrease in the PCV result obtained in this research. The group treated with the extract before the infection of the mice did not show much decrease in their PCV; evidence that the mushroom extract actually had prophylactic property. According to [10] any substance or extract that could cause a reduction in erythrocyte sedimentation rate would have both prophylactic and therapeutic property. Hence, the use of mushrooms as a food supplement and in cooking soup in Africa and in Nigeria, in particular, has a scientific justification. Therefore, based on the result obtained in this research, the milky mushroom has prophylactic property and can be used to reduce the incidence of malaria when incorporated into food.

4. CONCLUSION

Calocybe indica (milky mushroom) extract used in this research exerted high prophylactic property against *Plasmodium berghei in vivo*. The ethanol extract of the milky mushroom exerted a higher prophylactic effect than the hot water extract. These extracts caused a significant increase in packed cell volume of the experimental albino mice.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Alam N, Amin R, Khair A, Lee TS. Influence of different supplements on the commercial cultivation of milky white mushroom. Mycobiology. 2010;38:184-188.
2. Anju RP, Ukkuru MP. Health impact and medicinal properties of edible mushrooms. International Journal of Advanced Engineering Research and Science. 2016;5(10):45-55.
3. Amin R, Khair A, Alam N, Lee TS. Effect of different substrates and casing materials on the growth and yield of *Calocybe indica.* Mycobiology. 2010;38:97–101.
4. Phillips R. Mushrooms. Pub. McMillan, 2006; ISBN 0-330-44237-6.P.266
5. World Health Organization. Tasty Mushrooms from Dirty Diapers. Discovery Communication inc. World malaria day 2016 fact sheet, Geneva; 2016.
6. Fawole MO, Oso BA. Characterization of bacteria: Laboratory manual of microbiology. 4th Edition, Spectrum Book Ltd., Ibadan, Nigeria. 2004;24-33.
7. Ebiloma GU, Omale J, Aminu RO. Suppressive, currative and prophylactic potentials of *Morinda lucida* (Benth) against erythrocytic stage of mice infective chloroquine sensitive *Plasmodium berghei* NK-65. British Journal of Applied Science and Technology. 2011;1(3):131-140.
8. Cheesbrough M. District laboratory practice in tropical countries. 2nd Edition, Cambridge University Press, Cambridge, UK., ISBN-13:9781139449298. 2006;50: 165-176.
9. Omoya FO, Momoh AO. The efficacy of *Andrographis paniculata* and *Chromolaena odorata* plant extracts against malaria parasite. International Journal of Biological, Life Science and Engineering (France). 2014;8(5):134-137.
10. Baker JF, Breach MR, Chris P. Medical laboratory science. Chris Publisher, United Kingdom. 2014;487.
11. Owolabi BJ, Olarinoye NO. Organic chemistry, the Fundamentals. Bohan; 2014.
12. Pushpa H, Purushothama. Nutritional analysis of wild and cultivated edible medicinal mushrooms. World J. of Diary & Food Scie. 2010;5(2):140-144.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/23023