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In Vivo Evaluation of the Antiplasmodial Property of Ethanol and Hot Water Extracts of *Pleurotus ostreatus* Mushroom

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Abstract: Mushrooms are considered as a functional food, which can provide health benefits beyond the traditional nutrients they contain and *Pleurotus ostreatus* is a common edible mushroom known for its oyster-shaped cap. The *in-vivo* evaluation of the antiplasmodial property of ethanol and hot water extracts of *P. ostreatus* mushroom was carried out using standard microbiological methods. The mice were infected with *Plasmodium berghei* NK 65 species and the 4-day Peters suppressive test was carried out using 200 and 400 mg/ml of the extracts respectively. 5mg/ml chloroquine antimalarial drug was used as control drug. The malaria parasite load, full blood counts and histopathological analysis of the liver of the mice were done at the end of the experiment. The results showed that *P. berghei* caused a reduction in the weight from 22.8±0.20 g to 17.4±0.66 g. as well as a reduction in the temperature of the mice from 36.8±1.22°C to 34.2±0.20°C. The mushroom hot water extract exerted the highest percentage suppression of 76.82% after 24 hours, 92.35% after 48 hours, 96.08% after 72 hours and 99.81% after 96 hours. The parasite caused an increase in Erythrocyte Sedimentation Rate (ESR) from 1.33±0.33 mm/hr to 11.66±0.93 mm/hr, high white blood cell (WBC) count of 275.6 mm^{-3} and low packed cell volume (PCV) of 14.00±0.57%. The WBC differential count showed that the parasites caused a reduction in the number of lymphocytes (from 64.33% to 51.67%) while causing increase in neutrophils (25.33% to 32.67%). The *P. berghei* caused some negative pathological features ranging from a liver hepatocytes with profuse haemorrhage, dilated sinusoids and hepatocellular drainage, while the control group had well-formed and thickened liver hepatocytes without distinct cells or infiltrations. Therefore, *Pleurotus ostreatus* have antiplasmodial property and the hot water extract is more potent than the ethanol extract.

Keywords: Anti-plasmodial Property, Mushroom, Malaria

1. Introduction

Mushrooms are well known all over the world and the edible ones have been considered as functional foods. They serve to enrich food as supplements and they provide health benefits beyond the traditional nutrients they contain [1]. *Pleurotus ostreatus,* is a common edible mushroom known for its oyster-shaped cap. One of the first things to look out for in identifying this mushroom is the presence of decurrent gills [2]. Edible mushrooms are the fleshy and edible fruit bodies of several species of macrofungi (fungi which bear fruiting structures that are large enough to be seen with the naked eye). They can appear either below ground (hypogenous) or above ground (epigeous) where they may be picked by hand. Edibility may be defined by criteria that include absence of poisonous effects on humans and desirable taste and aroma. Edible mushrooms are consumed for their nutritional value and they are occasionally consumed for their supposed medicinal value. Mushrooms consumed by those practicing folk medicine are known as medicinal mushrooms. While hallucinogenic mushrooms (e.g. psilocybin mushrooms) are occasionally consumed for

recreational or religious purposes, they can produce severe nausea and disorientation, and are therefore not commonly considered edible mushroom [3]. Edible mushrooms include many fungal species that are either harvested wild or cultivated.

Globally, millions of deaths attributed to malaria are being recorded. The disease constitutes a huge epidemiologic burden in Africa and continues and continues to cripple the economic development in the region [4]. In Nigeria, the disease is responsible for 60% outpatient visits to health facilities, 30% childhood death, 25% of death in children under one year and 11% maternal death [5]. The financial loss due to malaria annually is estimated to be about 132 Billion Naira in 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, making this country with the highest malaria mortality [6].

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 [6]. 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 source of food to man, even before the for-knowledge of its nutritional content. It has served as major source of food in several African countries including Nigeria. However, its use as an antimalarial agent has not been documented. There is therefore, the need to assess the suppressive effect of the extract of this mushroom on albino mice.

2. Materials and Methods

2.1. Sample Collection

Sufficient mushrooms *(Pleurotus ostreatus)* was gotten from Afe Babalola University, Ekiti 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 rot it was kept in an oven at 40°C in the night time. The air-dried mushroom sample was blended into powder using a blender with the model [7]. Extraction of the bioactive components was done after blending the dried mushroom into powder. 250 g of it was weighed into two different conical flask. One was mixed with 750ml 95% ethanol and covered while the second portion was soaked with 750ml 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 at 78°C for the ethanol extract and 95°C for the water extract.

2.3. Experimental Mice

Twenty one white mice were obtained from faculty of Pharmacy, Obafemi Awolowo University, Ile-ife, Osun State. They weighed between 15-23 ± 2g and were all male.

2.3.1. Infection of the Mice

The mice were infected intraperitoneally with *Plasmodium berghei* (NK 65 species) gotten from the National Institute of Medical Research (NIMER), Lagos, Nigeria. They were given 0.2 ml of 1.67×10^6 parasitised red blood cells.

2.3.2. Suppressive Test

The 4-day Peters suppressive test according to [8] was used. After 2-3hours of infection without waiting physical signs of established infection, the first dose of the extract was given to the mice after dividing them into the following groups of 3 mice per group as follows:

Group 1- Infected and treated with 200mg/ml of hot water extract; Group 2- Infected and treated with 400mg/ml of hot water extract; Group 3- Infected and treated with 200mg/ml of ethanol extract; Group 4- Infected and treated with 400mg/ml ofethanol extract; Group 5- Infected and treated with 5mg/ml of chloroquine; Group 6- Infected and not treated at all and Group 7-Not infected and not treated with anything. The mice were given the extract in the above doses daily for 4 days.

2.3.3. Parasitemia Count

The parasitaemia count was done daily starting from after the first oral treatment. This was done by a little cut on their tail and making a smear of the blood on the slide.

$$\text{Parasitaemia} = \frac{\text{Total number of parasitized red blood cell count} \times 100}{\text{Total number of red blood count}}$$

2.3.4. Temperature Evaluation

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.

2.3.5. 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.

2.3.6. Making of Blood 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 were dried for a few minutes at 37°C then stained using Giemsa stain. Microscopic examination of stained slide was done using x100 oil immersion objective.

2.3.7. 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 is 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:

$$\text{Parasitaemia} = \frac{\text{Total number of parasitized red blood cell count} \times 100}{\text{Total number of red blood count}}$$

% 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

2.4. Full Blood Counts

The full blood count analysis was done using the method of [9] as follows:

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 cover 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 removed and taken to the x40 objective for clear viewing.
- The blood film was systematically examined and different white cells was counted as seen in each field using an automatic differential cell counter.

2.5. Histopathological Analysis

Histopathologic tests were carried out on the organs of the laboratory animals as follows: the organs of the animals were collected and fixed in 10% formalin to prevent decay. They were dehydrated in different percentage (50%, 70%, 80% and 100%) of alcohol 1 ½ hours each. After dehydration they were cleared with 100% xylene and left for 2hours to remove any remnant alcohol and impregnated in liquid wax for 2 hours for embedding. The embedded organs were sectioned using microtome and were stained with haematoxylin-eosin [9]. Excess stain was removed with tap water. After clearing in xylene, Canada balsam was added and cover slips placed on the slides. The preparations were left in the oven at 40°C and then placed under the microscope with a digital camera connected to a computer system to be examined by an expert and take the photographs [10].

2.6. Statistical Analysis of Result

Result obtain will be subjected to descriptive one way analyses of variance, SPSS version 21 Microsoft windows 7 and Duncan multiple range test was used as follow up test.

3. Results

The results of the effect of the suppressive tests and treatments on the weight of the mice (table 1) showed that the infection with the *P. berghei* caused a reduction in the weight of the albino mice. For instance, it caused the reduction in the weight of the mice in group 6 (the group infected and not treated at all) caused the reduction of their weight from 22.8±0.20 g to 17.4±0.66 g. This is about 24% reduction in their weight; when compared with the weight of the control (the group that is neither infected nor treated) that increased from 19.8±0.44 g to 22.7±0.65 g; about 13% increase in the weight within the period of the experiment.

The temperature of the mice as the experiment progressed showed that the infection of the mice caused a reduction in the temperature of the mice. Most of the mice had an average temperature of 36.8±1.22°C before the commencement of the experiment. However, as the experiment proceeds, there was decrease in their body temperature. The group that was infected and not treated had a temperature as low as 34.2±0.20°C. The result of the temperatures recorded is shown in table 2.

Table 3 shows the result of parasitemia count and the percentage suppression exerted by the mushroom extracts on the mice after 24 hours. The result showed that group 5 (the group infected and treated with chloroquine) recorded the highest percentage suppression of 81.55% at end of the experiment. Among the treated group, the group given 200 mg/ml of the ethanol extract of the mushroom recorded the least percentage suppression of 68.66%. comparatively, the hot water extract exerted a higher percentage suppression of 76.82% than the ethanol extract which exerted 76.39% at 400 mg/ml; thereby making the hot water extract more potent than the ethanol extract.

The same trend was observed for the experiment after 48 hours and 72 hours respectively, except that there was increase in percentage suppression, at 72 hours of the experiment in which only two parasites was seen on the smear of the group given chloroquine, that is, it was able to exert 98.80% suppression on the *P. berghei*. However, at the end of the experiment (96 hours), no parasite was seen at all for the group infected and treated with chloroquine.

Table 1. *Weight (g) of experimental mice.*

	Group 1	2	3	4	5	6	7
1	18.5±0.33	20.6±0.20	20.5±0.00	23.1±0.11	20.0±0.00	22.8±0.20	19.8±0.44
2	18.9±1.20	21.4±0.10	20.6±0.10	21.9±0.90	21.0±1.10	20.9±0.33	20.5±020
3	17.8±0.40	21.0±0.00	20.5±0.55	21.3±0.93	22.4±1.16	19.7±0.45	21.2±0.76
4	17.5±0.50	20.6±1.67	20.1±0.10	21.8±1.05	23.1±0.11	19.2±0.25	21.9±0.33
5	18.1±0.10	21.0±0.20	19.8±0.40	22.6±0.33	24.5±0.50	17.4±0.66	22.7±0.65
6	18.4±0.61	21.2±0.10	19.8±0.40	22.6±0.33	24.5±0.50	17.4±0.66	22.7±0.65

Legend:

Group 1- Infected and treated with 200mg/ml of hot water extract; Group 2- Infected and treated with 400mg/ml of hot water extract; Group 3- Infected and treated with 200mg/ml of ethanol extract; Group 4- Infected and treated with 400mg/ml of ethanol extract; Group 5- Infected and treated with 5mg/ml of chloroquine; Group 6- Infected and not treated at all; Group 7-Not infected and not treated with anything.

Table 2. *Temperature of experimental mice (°C).*

DAY/TEMP	1	2	3	4	5	6	7
1	36.8±1.22	36.5±0.90	36.5±0.92	36.5±0.92	36.7±0.88	36.7±0.96	36.6±1.02
2	35.0±0.05	35.4±0.08	34.9±0.95	35.0±0.05	36.2±0.50	35.3±0.33	36.8±0.45
3	35.3±0.11	35.9±0.50	35.2±0.80	35.7±0.10	36.5±0.20	34.0±0.00	36.9±0.33
4	35.5±0.05	36.5±0.50	35.3±0.33	36.1±0.11	36.8±0.00	34.00±0.00	36.8±0.60
5	35.5±1.11	36.8±0.04	35.2±0.30	36.4±0.10	36.8±0.40	34.2±0.2	036.9±0.50

Legend:

Group 1- Infected and treated with 200mg/ml of hot water extract; Group 2- Infected and treated with 400mg/ml of hot water extract; Group 3- Infected and treated with 200mg/ml of ethanol extract; Group 4- Infected and treated with 400mg/ml of ethanol extract; Group 5- Infected and treated with 5mg/ml of chloroquine; Group 6- Infected and not treated at all; Group 7-Not infected and not treated with anything.

Table 3. *Parasitemia count and percentage suppression after 24hour.*

Day	Groups	Concentration (mg/ml)	Parasitaemia Per View	Percentage (%) Suppression
Monday	Group A	200	120	74.24
Monday	Group B	400	108	76.82
Monday	Group C	200	146	68.66
Monday	Group D	400	110	76.39
Monday	Group E	5	86	81.55
Monday	Group F	466	466	0
Monday	Group G	Not treated	Not treated	

Legend:

Group A- Infected and treated with 200mg/ml of hot water extract; Group B- Infected and treated with 400mg/ml of hot water extract; Group C- Infected and treated with 200mg/ml of ethanol extract; Group D- Infected and treated with 400mg/ml of ethanol extract; Group E- Infected and treated with 5mg/ml of chloroquine; Group F- Infected and not treated at all; Group G-Not infected and not treated with anything.

Table 4. *Parasitemia count and percentage suppression after 48Hours.*

Day	Groups	Concentration (mg/ml)	Parasitemia count per view	Percentage Suppression (%)
Tuesday	Group A	200	109	81.89
Tuesday	Group B	400	46	92.35
Tuesday	Group C	200	142	76.41
Tuesday	Group D	400	106	82.39
Tuesday	Group E	5	12	98.00
Tuesday	Group F	602	602	0
Tuesday	Group G	Not treated	-	Not treated

Legend:

Group A- Infected and treated with 200mg/ml of hot water extract; Group B- Infected and treated with 400mg/ml of hot water extract; Group C- Infected and treated with 200mg/ml of ethanol extract; Group D- Infected and treated with 400mg/ml of ethanol extract; Group E- Infected and treated with 5mg/ml of chloroquine; Group F- Infected and not treated at all; Group G-Not infected and not treated with anything.

Table 5. *Parasitemia count and percentage suppression after 72Hours.*

Days	Groups	Concentration (mg/ml)	Parasitemia count per view	Percentage Suppression (%)
Wednesday	Group A	200	62	93.92
Wednesday	Group B	400	40	96.08
Wednesday	Group C	200	209	79.52
Wednesday	Group D	400	100	90.20
Wednesday	Group E	5	2	99.80
Wednesday	Group F	Not treated	1021	0
Wednesday	Group G	-		--

Legend:

Group A- Infected and treated with 200mg/ml of hot water extract; Group B- Infected and treated with 400mg/ml of hot water extract; Group C- Infected and treated with 200mg/ml of ethanol extract; Group D- Infected and treated with 400mg/ml of ethanol extract; Group E- Infected and treated with 5mg/ml of chloroquine; Group F- Infected and not treated at all; Group G-Not infected and not treated with anything.

Table 6. *Parasitemia count and percentage suppression after 96 hours.*

Days	Groups	Concentrations (mg/ml)	Parasitemia count per view	Percentage Suppression (%)
Thursday	Group A	200	45	95.92
Thursday	Group B	400	2	99.81
Thursday	Group C	200	221	79.98
Thursday	Group D	400	42	96.19
Thursday	Group E	5	None seen	-
Thursday	Group F	Not treated	1104	0
Thursday	Group G	-	-	--

Legend:

Group A- Infected and treated with 200mg/ml of hot water extract; Group B- Infected and treated with 400mg/ml of hot water extract; Group C- Infected and treated with 200mg/ml of ethanol extract; Group D- Infected and treated with 400mg/ml of ethanol extract; Group E- Infected and treated with 5mg/ml of chloroquine; Group F- Infected and not treated at all; Group G-Not infected and not treated with anything.

The haematology results (table 7) showed the effect of the infection and suppression by the extract on the mice. The group that was infected without treatment had a very high erythrocyte sedimentation rate (ESR) of 11.66±0.93 mm/hr when compared to the control which had 1.33±0.33 mm/hr. The PCV of the mice showed that the mushroom extracts caused significant increase in the blood volume of the mice. The control group had 29.66% while the ones given extract especially 400 mg/ml of the hot water extract had 37.67%. The results obtained for WBC count showed that the infected group had high counts, as high as 275.6 mm^{-3} which is also a sign of infection in the mice. The WBC differential count showed that the parasites caused a reduction in the number of lymphocytes (from 64.33% to 51.67%) while causing increase in neutrophils (25.33% to 32.67%) respectively. Other parameters assessed followed the same pattern as seen in tables 7 and 8 respectively.

Table 7. *Haematololgy Result of Experimental Mice.*

Group	ESR (mm/hr)	PCV (%)	WBC (mm^{-3})	RBC (mm^{-3})	HB (mm^{-3})
1	2.83±0.44	31.33±1.76	163.0±13.57	644.0±21.8	10.46±0.60
2	1.00±0.00	37.67±1.45	13.3±3.53	745.6±15.98	12.57±0.49
3	5.00±1.15	22.67±3.71	211.6±13.86	617.6±63.52	8.39±0.86
4	2.33±0.33	32.00±1.15	136.3±9.53	710.7±18.04	11.07±0.45
5	1.00±0.00	38.33±0.33	108.0±2.31	749.3±10.97	12.63±0.20
6	11.66±0.93	14.00±0.57	275.6±6.57	1018.5±34.66	4.88±0.12
7	1.33±0.33	29.66±1.45	173.0±11.15	456.0±26.63	9.87±0.47

Legend:

Group 1- Infected and treated with 200mg/ml of hot water extract; Group 2- Infected and treated with 400mg/ml of hot water extract; Group 3- Infected and treated with 200mg/ml of ethanol extract; Group 4- Infected and treated with 400mg/ml of ethanol extract; Group 5- Infected and treated with 5mg/ml of chloroquine; Group 6- Infected and not treated at all; Group 7-Not infected and not treated with anything.

Table 8. *Differential count of white blood cells.*

Groups	Lymphocyte (%)	Neutrophil (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
1	63.67±1.45	25.67±1.45	8.66±0.67	2.33±0.33	0.67±0.33
2	54.67±1.45	30.3±0.88	8.33±0.67	1.00±0.00	0.67±0.33
3	66.00±1.15	24.67±0.88	6.00±0.58	3.00±0.00	0.67±0.33
4	61.66±1.20	28.33±0.88	7.00±1.53	2.00±0.58	1.00±0.00
5	64.33±1.85	24.66±0.88	9.00±1.00	1.33±0.33	0.67±0.33
6	51.67±2.18	32.67±1.76	10.00±1.15	5.00±0.58	0.67±0.33
7	64.0±1.15	25.3±0.88	8.33±0.67	1.33±0.33	1.00±0.00

Legend:

Group 1- Infected and treated with 200mg/ml of hot water extract; Group 2- Infected and treated with 400mg/ml of hot water extract; Group 3- Infected and treated with 200mg/ml of ethanol extract; Group 4- Infected and treated with 400mg/ml of ethanol extract; Group 5- Infected and treated with 5mg/ml of chloroquine; Group 6- Infected and not treated at all; Group 7-Not infected and not treated with anything.

Histopathology Results of the liver of experimental mice

The hepatocytes are intact, no necrosis or haemorrhage seen in the group that were given the hot water extract. There was however, a washing away of hepatic wall without necrosis or haemorrhage seen as well as the presence of distinct pigmentation of nuclear materials that was without necrosis or haemorrhage in the group treated with ethanol extract. The liver of the group treated with chloroquine antimalarial had well-formed liver hepatocytes without distinct cells or infiltrations from both ends. Contrary to all these features, the group that was infected with *P. berghei* and was not treated at all showed negative pathological features ranging from a liver hepatocytes with profuse haemorrhage, dilated sinusoids and hepatocellular drainage, while the control group had well-formed and thickened liver hepatocytes without distinct cells or infiltrations. There was equally no trace of necrosis or haemorrhage in the control group. These results are shown in plate 2A-2G.

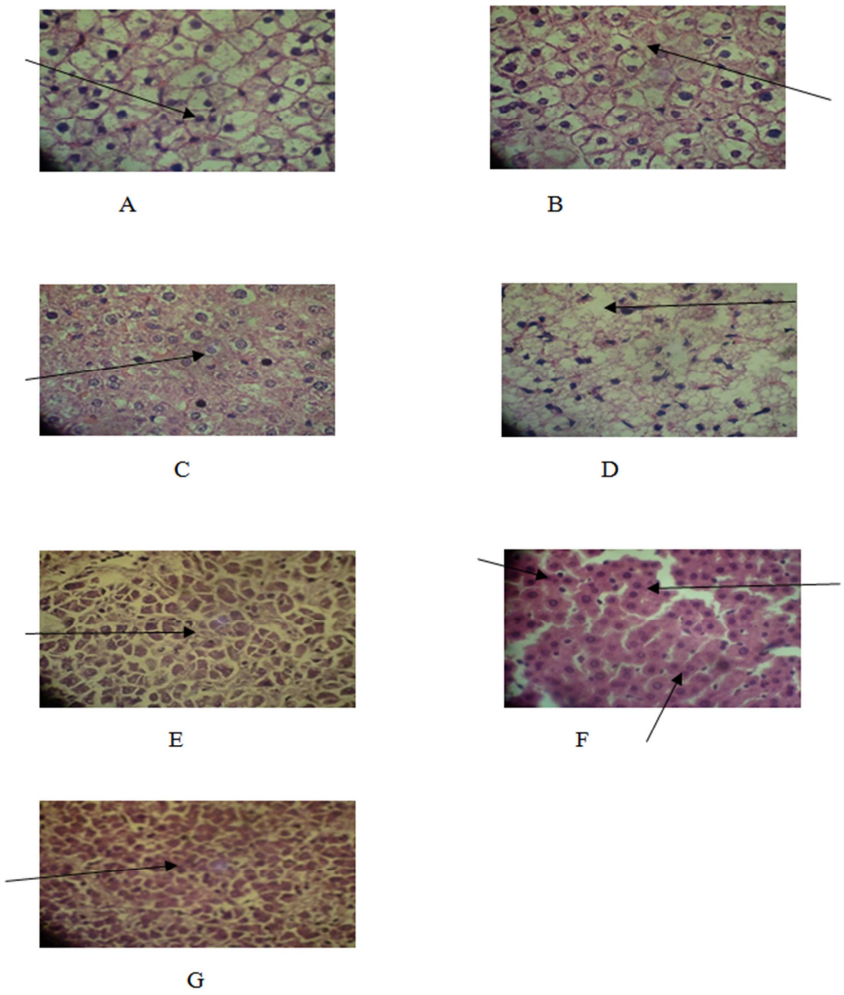

Figure 1. *Photomicrographs of the liver of infected and treated albino mice.*

Interpretation

Figure 1a= The hepatocytes are intact, no necrosis or haemorrhage seen

Figure 1b = The hepatocytes are intact, no necrosis or haemorrhage seen

Figure 1c = There is washing away of hepatic wall without necrosis or haemorrhage seen

Figure 1d = There is distinct pigmentation of nuclear materials without necrosis or haemorrhage

Figure 1e = Well-formed liver hepatocytes without distinct cells or infiltrations

Figure 1f = Liver hepatocytes with profuse haemorrhage, dilated sinusoids and hepatocellular drainage

Figure 1g = Well-formed and thickened liver hepatocytes without distinct cells or infiltrations

Legend:

Group 1- Infected and treated with 200mg/ml of hot water extract; Group 2- Infected and treated with 400mg/ml of hot water extract; Group 3- Infected and treated with 200mg/ml of ethanol extract; Group 4- Infected and treated with 400mg/ml of ethanol extract; Group 5- Infected and treated with 5mg/ml of chloroquine; Group 6- Infected and not treated at all; Group 7-Not infected and not treated with anything.

4. Discussion

The results of the effect of the suppressive tests and treatments on the weight of the mice shown in table 1 confirmed the effect of infection *P. berghei*. The reduction in the weight of the albino mice, according to [8] is one of the most important factors that shows sign of infection or that infection has set in. For instance, the fact that the infection caused the reduction in the weight of the mice in group 6 drastically beyond doubt (the group infected and not treated at all) when compared to the group not infected at all is a confirmation of this fact. This result is also in accordance with the result obtained by [11], in which the major sign that the albino rats used for the experiment were infected with *Escherichia coli* was the reduction of their weight. Comparatively, while the weight of the control (the group that is neither infected nor treated) that increased from 19.8±0.44 g to 22.7±0.65 g; about 13% increase in the weight within the period of the experiment.

The temperature of the mice as the experiment progressed showed that the infection of the mice caused a reduction in the temperature of the mice. According to [12], malaria in mice causes the temperature to reduce in mice. This is the complete opposite this phenomenon in man in which malaria causes a rise in temperature. Most of the mice had an average temperature of 36.8±1.22°C before the commencement of the experiment. This shows that they were apparently healthy and that they were previously not having malaria symptoms. Another confirmation of the effect of the parasites in the mice was the group that the control group had almost constant temperature all through the experiment.

The effect of the treatments on the parasitemia load of the experimental mice after 24 hours of administration of samples showed that the hot water extract of *Pleurotus* mushroom was most effective. This probably indicates that the local people cooking it with water get sufficient nutrients according to [13]. The effect of chloroquine may be attributed to the level of purity and extraction [8], hence the 81.55% suppression attained in the group infected and treated with the drug. Although, the ethanol extract was equally active in suppressing the parasitemia level, but not as the water extract. The ability of the ethanol to extract active components of the mushroom may be due to its polar nature which is similar to water. [14] Reported that water and ethanol have a very close proximity in terms of polarity and as such, both will extract any substance at a very close rate. Hence, the result of their percentage suppression was almost the same.

The percentage suppression between 72 and 96 hours of administration of chloroquine was 100%, which means that the parasites were completely wiped out. This result shows the reason why chloroquine is still a drug of choice in the treatment of bacteria today. [15] Stated that chloroquine is one of the antimalarial drug that have stood the test of time in not being resisted by *Plasmodium* species. Equally the result of the variation of concentration of the mushroom extracts revealed that the higher the concentration, the higher the effectiveness of the extract as antiplasmodium substance.

The effect of the parasites on the mice blood revealed that erythrocyte sedimentation rate was greatly increased above what was obtained in the control. According to [9], high parasitemia level in the blood often cause a sudden rise in ESR of animals. The parasites caused a reduction in the Packed Cell Volume (PCV) of the mice. [10] Stated that one effect of malaria parasite is the haemolysis of red blood cells that that culminate in pernicious anaemia for the animal if not treated on time and properly.

The results of the histopathology which showed that the hepatocytes were intact and that no necrosis or haemorrhage was seen in the liver of the group that were given the hot water extract showed that the therapeutic potential of the hot water extract of the mushroom. [11] Concluded that once an animal is fed constantly with any substance for a period of 96 hours without showing any of these negative pathological signs and symptoms has therapeutic property. Comparatively, there was however, a washing away of hepatic wall without necrosis or haemorrhage seen as well as the presence of distinct pigmentation of nuclear materials that was without necrosis or haemorrhage in the group treated with ethanol extract. A washing away of these hepatic materials signify the presence of a substance that has acidic or bleaching (oxidizing) property according to the assay [16]. The liver of the group treated with chloroquine antimalarial had well-formed liver hepatocytes without distinct cells or infiltrations from both ends. [9] Stated that chloroquine have been subjected proper purification and equally exert selective toxicity. Contrary to all these features, the group that was

infected with *P. berghei* and was not treated at all showed negative pathological features ranging from a liver hepatocytes with profuse haemorrhage, dilated sinusoids and hepatocellular drainage, while the control group had well-formed and thickened liver hepatocytes without distinct cells or infiltrations. There was equally no trace of necrosis or haemorrhage in the control group.

Pleurotus ostreatus used in this research have been shown to have medicinal property that could suppress malaria parasites *in-vivo* and that the hot water extract of the mushroom exerted a higher percentage suppression. Therefore, the consumption of *P. ostreatus* is highly encouraged as it will help suppress or prevent malaria parasites. Further analysis on the active components in *P. ostreatus* that displayed antiplasmodial property should be extracted, purified and incorporated into antimalarial drugs, thereby reducing the menace of *Plasmodium* resistance.

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