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Antiplasmodial effects of *Allium sativum* extract on haematological Parameters of Albino Wistar rats infected with *Plasmodium berghei*

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Abstract

This study aimed at evaluating the antiplasmodial effects of *Allium sativum* (bulbs) on haematological parameters of albino rats infected with *Plasmodium berghei*, divided the animal model into groups, viz: the experimental group (with five different concentrations of 100, 300, 500, 800 and 1000mg/kg), the normal-control (non-inoculated), negative-control (inoculated, but untreated) and positive control (inoculated and treated with 10mg/kg Combisunate). *Allium sativum* ethanolic extracts were administered and monitored for four days before the treatment. Afterwards, the average parasitaemia was calculated and the average percentage parasite inhibition (suppressive effect) was obtained. Result showed that the parasitemia level for the treated groups decreased progressively for the five-day period. This is indicative in the mean number of the percentage parasitized red cells of 1000mg/kg doses as 9.055±2.06 on the first day post inoculation and 0.30±0.04 on the fifth day. The decrease is also observed in the 100, 300, 500 and 800mg/kg groups. Except the untreated group which showed a progressive increase in parasitemia level with average percentage parasitized red cells as 11.33±1.97 on the first day post inoculation and 18.15±1.49 on the fifth day. The haematological result showed a significant ($p<0.05$) decrease in values of RBC, PCV, Hb, and neutrophils in the inoculated groups, especially the untreated group. As compared to the treated groups, these parameters showed progressive increase as concentrations increased. Conclusively, it can be inferred that the bulbs of *Allium sativum* have antiplasmodial potentials and can therefore be purified for development of antimalarial drugs.

Keywords: Antibiotic; Resistance; *Plasmodium*; Haematological; Medicinal plants; *Allium sativum*

1. Introduction

Many scientists across the globe have reported antimicrobial properties of several medicinal plants but still a very meager portion of this tremendous potential drug-repertoire has been scientifically screened [1]. A number of medicinal plants have been screened for antimicrobial activity in recent years [2] and efforts have been made to identify their active constituents [3]. In developing countries, remedies from plants are readily used in the treatment of various kinds of diseases. This usually involves the use of medicinal plants and related products in health management. This is despite the recent revolution in medical practice as a result of technology [4]. Every culture of the world has plants and plant products used in folkloric medicine. Despite the availability of modern medicine, a great majority of people still depend on this ethno pharmacological product for health care management due to obvious reasons such as availability and affordability. In Nigeria, people of all classes depend so much on plant-based medications for the management of different illnesses although modern medication are available [5]. Different medicinal plants possess diverse therapeutic potential as no single plant has all the medicinal properties [6]. Ethno medicinal plants have been believed to have fewer side effects, this is however an erroneous impression [7, 8]. Many of the medicinal potentials of plants used in folkloric

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medicine have been subjected to scientific investigation and this has warranted their wide spread use as an alternative or complement to orthodox medicines.

Allium sativum is an under exploited plant species, although it is a common and largely available plant. This plant is used in some parts of Southern Nigeria in the treatment of malaria, hence the need to investigate its therapeutic potentials. The availability of natural products like medicinal plants will greatly help to solve the healthcare problems of these rural communities. Over the years, *Allium sativum* has been used as a spice, which have generated a lot of interest throughout human history as a medicinal panacea. This research will be particularly relevant in our society where importance of *Allium sativum* as food is neglected or under used and its cultivation is considered not important. It is more like a case of using available resources to solve a threatening health problem.

In Nigeria and the rest of the world, malaria infection continues to pose a major health challenge. In view of resistance of the parasite to antimalarial drug therapy, which leads to drug failure, new drugs or drug combinations are urgently required for the treatment of malaria infections from traditional medicinal plants [9]. This worked is aimed at evaluating the antiplasmodial and toxicological effects of *Allium sativum* extracts on albino rats infected with *Plasmodium berghei*.

2. Material and methods

2.1. Collection of Plant Samples and Extraction

The bulbs of *Allium sativum* were gotten from fruit garden D-Line in Port Harcourt and were identified by the herbarium unit of the department of Plant Science and Biotechnology University of Port Harcourt. The dried powdered bulb were extracted using absolute ethanol as a solvent in a Soxhlet extractor. The solvent was completely removed with the aid of a rotary evaporator. The crude extracts were stored at −20°C until used. Before experiments, the extracts were then dissolved in appropriate volume of water to make the respective stock solutions.

2.2. Phytochemical Screening of *Allium sativum* Bulb

The qualitative and quantitative phytochemistry of the bulb of *Allium sativum* were carried out.

2.3. Experimental Animals

A total of thirty-two (32) adult rats of both sexes weighing between 116 to 130g were used. They were acquired from Department of Physiology, University of Port Harcourt. The animals were housed in a specially designed plastic/wire gauze animal cage and were placed on standard feed and given access to water *ad libitum*. They were kept under observation (Acclamatization) for about 10 days before the onset of the experiment to exclude any form of infection. The chosen animals were housed in plastic well aerated cages at normal atmospheric temperature (25 ± 5 °C) and normal 12-hour light/dark cycle.

2.4. Malaria Infection of Experimental Rats

The malaria infection was carried out according to the method described by Okolie and Obiajunwa [10]. *Plasmodium berghei* ANKA (65) strain was originally obtained from the Nigerian Institute of Medical Research in Yaba, Lagos. The parasite was then maintained at the Malaria Research Laboratory of the University of Port Harcourt. Malaria infection in rat was initiated by intraperitoneal (IP) inoculation of 0.5 ml blood which contains about 2 x 10^7/ml Parasitized Red Blood Cells (PRBC) from a donor mouse infected with *P. berghei*. Controls to malaria-infected rat were given an equivalent volume and dilution of normal uninfected red blood cells.

2.5. Estimation of Percentage Parasitemia

Thin and thick blood films were prepared with blood collected from the tail of each mouse on the fourth day of inoculation. The thin films were fixed with methanol, stained with Giemsa stain and the percentage parasitemia was determined by microscopic examination using the formula:

$$\%\ Parasitemia\ = \frac{No\ of\ parasitized\ RBC}{No\ of\ total\ RBC} \times 100$$

2.6. Experimental Design

Rats were infected with *P. berghei* as described above and were divided into eight groups in a plastic wire cages. combisunate@ was used as a standard and was dissolved in distilled water and a dose of 10mg/kg (oral administration)

were given in a single daily dose at midday starting from day 1 until day 4 following four days of inoculation. Normal Control group was introduced which were exposed to the same experimental conditions as others and were allowed access to food and water only, the group is also known as the uninfected rat group. An untreated group was also created. That is, a group inoculated with *P. berghei* for four days like every other inoculated group but was not treated when others are receiving treatment for four days post inoculation process. Five categories (groups) were created with each representing the five concentrations: 100, 300, 500, 800 and 1000mg/kg ethanolic extracts administered for four days for the treatment of the parasite.

Table 1 Experimental Design for Control Groups

S/N	Group	No. of rat	Food+ water Only	Parasite induction	Treatment	100 mg/kg	300 mg/kg	500 mg/kg	800 mg/kg	1000 mg/kg	10mg/kg combisunate@
1	normal control	4	yes	no	No						
2	negative control	4	yes	yes	No						
3	positive control	4	yes	yes	Yes						yes

Table 2 Experimental Design for *A. sativum*

S/N	Group	No. of rat	Food+ water only	Parasite induction	Treatment	100 mg/kg	300 mg/kg	500 mg/kg	800 mg/kg	1000 mg/kg	10mg/kg combisunate@
1	GB1	4	yes	yes	Yes	yes					
2	GB2	4	yes	yes	Yes		yes				
3	GB3	4	yes	yes	Yes			Yes			
4	GB4	4	yes	yes	Yes				yes		
5	GB5	4	yes	yes	Yes					yes	

Where: GB1-GB5 represent 100mg/kg, 300mg/kg, 500mg/kg, 800mg/kg and 1000mg/kg ethanolic extract of A. *sativum* respectively.

2.7. Estimation of Curative Test against *P. berghei* Infection in Rat

Ethanol extracts of *Allium sativum* were assessed for *in vivo* activity in a four- day curative test against *P. berghei* infection in rat. Rats were inoculated with 0.5ml of 2 x 10^7PRBC intravenously as described above. The extracts were dissolved in 2.5% tween 80 and diluted with water (for injection) to provide doses of 100, 300, 500 and 1000 mg/kg body weight. The extracts were administered in a single daily dose orally according to their body weight from day 1 until day 4 post infection. Parasitaemia development in the infected rat was monitored on the first, third and fifth day of the treatment.

2.8. Blood Collection

After four days of treatment, the rats were sacrificed on the fifth day using chloroform. Blood was collected directly from the heart of the animals through 2 ml syringe into EDTA bottles for white blood cell count and packed cell volume and other haematological test. Heparinized bottles were also used to collected blood in order to obtain plasma for biochemical analysis.

2.9. Haematological Estimations

The blood samples were collected into tubes containing EDTA and were immediately used for determination of haematological parameters. Total red blood cell (RBC) and white blood cell (WBC) counts were estimated according to

the visual method of Dacie and Lewis, [11]. The percentage packed cell volume (PCV) was determined according to the haematocrit method while the blood haemoglobin (Hb) concentration in all samples was estimated according to the cyanomethaemoglobin method using Drabkin's reagent [12]. Differential white blood cell counts were estimated using the method of Osim *et al.,* [13].

2.10. Statistical Analysis

Data from this study were statistically analyzed using SPSS software version 20. Descriptive statistics were done. An independent t-test was used to show mean differences. Analysis of variance was employed to know mean differences among groups. P-values <0.05 were considered as statistically significant. Statistical analysis of the data obtained in this study was performed by one-way ANOVA followed by a single post hoc test. P<0.05 was taken as statistically significant.

3. Results

Table 1 Qualitative phytochemical composition of *Allium sativum*

S/N	Phytochemical Component	Remark
1	Alkaloids	+
2	Flavonoids	+
3	Tannins	+
4	Anthraquinones	-
5	Triterpenoid	+
6	Glycosides	+
7	Saponins	+
8	Steroids	+
9	Phytate	-

Key: + = Present; - = Absent

3.1. Quantitative phytochemical composition

The quantitative phytochemistry of showed that alkaloid, flavonoid, saponin, terpenoids, papain, oxalate, steroids, glycosides in *Allium sativum*, are 0.98mg/100g, 1.66mg/100g, 0.81mg/100g, 1.53mg/100g, 0.32mg/100g, 0.89mg/100g, 0.012mg/100g and22000000000000000 0.104mg/100g, respectively.

Table 2 Quantitative phytochemical composition of *Allium sativum*

S/N	Sample	*Allium sativum* (mg/100g)
1	Alkaloid	0.98
2	Flavonoid	1.66
3	Tannins	0.885
4	Phenols	2.108
5	Saponin	0.81
6	Glycosides	0.104
7	Terpenoids	1.53
8	Steroid	0.012
9	Papain	0.318
10	Oxalate	0.89

3.2. Curative ability of ethanolic extracts of *Allium sativum* against *P. berghei*

The parasitemia level for the treated groups decreased progressively for the five days period. This is indicative in the mean number of the percentage parasitized red cells of 1000mg/kg doses as 9.055±2.06 on the first day post inoculation and 0.30±0.04 by the fifth day. The decrease is also observed in the 100, 300, 500 and 800mg/kg groups. Except for the untreated group which showed a progressive increase in parasitemia level showing the mean number of the percentage parasitized red cells as 11.33±1.97 on the first day post inoculation and 18.15±1.49 by the fifth day. The result of plant extracts on parasitemia density in rat is presented in Table 3.

Table 3 Curative activity of control groups of ethanolic extracts of *Allium sativum* against *P. berghei*

S/N	Group	Day 1	Day 3	Day 5
1	normal control	0.00±0.00^{bc}	0.00±0.00	0.00±0.00^{c}
2	negative control	11.33±1.97^{ac}	14.95±2.65^{ac}	18.15±1.49^{ac}
3	positive control	9.17±1.39^{ab}	0.35±0.12^{b}	0.06±0.06^{a}

Data are expressed as Mean ± SEM. n=4. Values found in a column with common superscript letter a, are significantly different (p<0.05) when compared to the normal control. Values with superscript b, are significantly different (p<0.05) relative to the negative control. While values with the superscript c, are significantly different (p<0.05) compared to the positive control.

Table 4 Curative activity of ethanolic extracts of *Allium sativum* (leaf) against *P. berghei*

S/N	Group	Dosage of *A. sativum* in mg/kg	Day 0	Day 3	Day 5
1	GB1	100	9.23±0.83^{ac}	1.09±0.14^{b}	0.71±0.07^{ab}
2	GB2	300	15.21±0.31^{ac}	1.23±0.29^{b}	1.08±0.35^{b}
3	GB3	500	12.11±0.95^{ac}	1.15±0.16^{bc}	0.73±0.13^{b}
4	GB4	800	11.98±1.57^{ac}	1.63±0.29^{bc}	0.40±0.08^{b}
5	GB5	1000	9.055±2.06^{ac}	1.36±0.37^{abc}	0.30±0.04^{b}

Data are expressed as Mean ± SEM. n=4. Values found in a column with common superscript letter a, are significantly different (p<0.05) when compared to the normal control. Values with superscript b, are significantly different (p<0.05) relative to the negative control. While values with the superscript c, are significantly different (p<0.05) compared to the positive control.

3.3. Effects of ethanolic extracts of *Allium sativum* on some haematological indices of rats

Tables 5 and 6 show the haematological parameters in normal control, untreated and treated groups of Wistar rats. The result indicated a significant (P < 0.05) difference in the haematological parameters of normal and the untreated group. Haematological parameters as an investigating tool for cases of early malaria infections and mostly help to detect early complications associated with serious malaria infection. Reduction in red blood cells-RBC could lead to anemia which could result to death. To prevent death that may result from such complications, the need to carry out proper blood count. The report from this study shows that the untreated group tends to have significantly lower red blood cell count (RBC) 3.55±0.13 lower than that of non-infected subjects 4.82±0.11. The treated groups showed a linear and progressive increases in RBC relative with increase in dose except for the group treated with ethanolic extract of *Allium sativum* where no linearity was recorded. The PCV and Hb levels were also noted to be significantly lower in the untreated control (P<0.05) as compared with the normal control and those groups receiving treatment. There was an increased in WBC in groups receiving treatment and the inoculated but untreated group of rats compared to the normal control group. In general, the total WBC, the absolute lymphocytes and monocytes were significantly higher in the malaria infected patients than in the normal control (p<0.05). However, the PCV, Hb RBC and absolute neutrophils count were lower significantly in the malaria infected rats than in the normal control group (p<0.05).

Table 5 Effects of ethanolic extracts of *Allium sativum* on some hematological indices of rats

S/N	Group	PCV	Hb	RBC	WBC	NEU	LYM	MONO
1	normal control	43.25±1.11^{abc}	14.27±0.12^{abc}	4.82±0.11^{abc}	6.07±0.30^{abc}	76.75±0.85 ^{abc}	20.50±0.86^{abc}	0.25±0.25^{abc}
2	negative control	29.50±1.32^{abc}	10.12±0.38^{abc}	3.55±0.13^{abc}	13.42±0.56^{abc}	51.75±3.75^{abc}	38.50±3.01^{abc}	2.50±0.28^{abc}
3	positive control	45.25±1.31^{abc}	14.62±0.16^{abc}	5.02±0.11^{abc}	6.00±0.09^{abc}	77.50±1.70^{abc}	19.50±1.04^{abc}	0.25±0.25^{abc}

Data are expressed as Mean ± SEM. n=4. Values found in a column with common superscript letter a, are significantly different ($p<0.05$) When compared to the normal control. Values with superscript b, are significantly different ($p<0.05$) relative to the negative control. While values with the superscript c, are significantly different ($p<0.05$) compared to the positive control.

Table 6 Effects of ethanolic extracts of *Allium sativum* (Leaf) on some hematological indices of rats

S/N	Group	Dosage of *A. sativum* in mg/kg	PCV	Hb	RBC	WBC	Neutrophils	Lymphocytes	Monocytes
1	GB1	100	28.50±1.04^{abc}	10.07±0.17^{abc}	3.40±0.09^{abc}	10.40±0.10^{abc}	61.25±0.85^{abc}	27.25±1.10^{abc}	1.25±0.25^{abc}
2	GB2	300	31.50±0.86^{abc}	10.22±0.11^{abc}	3.72±0.10^{abc}	11.95±0.29^{abc}	65.50±1.04^{abc}	25.75±1.18^{abc}	1.25±0.25^{abc}
3	GB3	500	30.25±0.94^{abc}	10.20±0.28^{abc}	3.57±0.11^{abc}	10.32±0.15^{abc}	70.25±1.10^{abc}	22.50±0.64^{abc}	0.75±0.25^{abc}
4	GB4	800	28.75±1.31^{abc}	9.70±0.36^{abc}	3.42±0.11^{abc}	10.50±0.09^{abc}	69.50±0.64^{abc}	20.00±0.40^{abc}	0.50±0.28^{abc}
5	GB5	1000	25.25±1.65^{abc}	8.80±0.33^{abc}	3.10±0.14^{abc}	11.05±0.11^{abc}	68.50±0.95^{abc}	21.50±0.64^{abc}	0.50±0.28^{abc}

Data are expressed as Mean ± SEM. n=4. Values found in a column with common superscript letter a, are significantly different ($p<0.05$) when compared to the normal control. Values with Superscript b, are significantly different ($p<0.05$) relative to the negative control. While values with the superscript c, are significantly different ($p<0.05$) compared to the positive control.

4. Discussion

The results of the standard 4-day curative test against *P. berghei* infected rats were summarized in Tables 3 and 4. Several studies have attributed the antiplasmodial properties of plants to their alkaloids, flavonoids, terpenoids, anthraquinones, and glycosides contents [14]. The phytochemical analysis reviewed in this study confirmed the existence of alkaloids, flavonoids, glycosides, and terpenoids in *Allium sativum.*

This study showed a reduction in the average percentage parasitemia in a correlated pattern with the various doses administered for *Allium sativum* recorded an average percentage parasitemia of 9.23±0.83, 15.21±0.31, 12.11±0.95, 11.98±1.57, 9.055±2.06 on the first day and 0.71±0.07, 1.08±0.35, 0.73±0.13, 0.40±0.08, 0.30±0.04 on the fifth day post inoculation, corresponding with the ethanolic extract doses ranging from 100, 300, 500, 800 and 1000mg/kg body weight. Unlike other groups, the untreated (negative) control group had 11.33±1.97 average percentage parasitemia value on day 1 post inoculation and 18.15±1.49 on the fifth day indicating an increase in the parasitemia level compared with the group treated with combisunate as showed in table 3. Judging from the results displayed in tables 3 and 4, no single group in all the groups inoculated with *P. berghei* had total clearance.

This study showed a lower RBC count in *P. berghei* infections compared to the normal non-inoculated control. The cause and effect of malaria and anemia is complex and not fully understood. Infected RBCs display a reduced deformability and altered surface characteristics, which usually would lead to them being filtered and cleared by the spleen. However, the malaria parasite *P. berghei* has found a way to counter this protective measure. They modify their host cell membrane, which ultimately results to the cytoadherence of RBCs onto the endothelium. Anaemia in acute malaria is due to increase in haemolysis and decrease in the rate of production of red blood cells, increased destruction of parasitized red blood cells and accelerated removal of both parasitized and non-parasitized red blood cells. Other factors contributing to anaemia in malaria include increased red blood cell deformability, splenic phagocytosis and/or pooling as reported by Angus *et al.,* [15]. In addition to anaemia, a reduction in the number of PCV is another one of the more well-known haematologic changes observed in rats with malaria. This study supported that lower PCV among rats infected with *P. berghei* in comparison to the control group were notably important. Trends between increasing parasite density and an increase in the level of haematological parameters were observed in this study. Leukocyte counts, especially lymphocytes and monocytes, were significantly higher in rats with high parasitemias compared to those with low and moderate parasitemias. However, lymphocyte and monocyte numbers were significantly higher in patients with low parasitemias compared to those with moderate and high parasitemias. The trend of decreasing Hb concentration with increasing levels of parasitemia was observed in this study. Low Hb concentrations were associated with increased parasite density.

5. Conclusion

The investigation of the antiplasmodial property of the ethanolic leaf extract of *Allium sativum* showed that the extract at all levels of dose do not have curative effects after four day administrations, but possessed antimalarial activity as revealed by the significant percentage parasitemia reduction. The extract also showed haematopoietic potential, increasing the levels of haemoglobin, packed cell volume and red blood cells. These further established the antimalarial potential of the plant extract considering that antiplasmodial activity is closely related to haematopoietic activity and the lowering of liver enzymes concentrations. The result of the finding did not demonstrate a curative effect against plasmodial infection in the treated groups. The antiplasmodial, haematopoietic, hepatic-enhancing and renalgesic effects of *Allium sativum* might therefore be as a result of any one or combination of the phytochemicals present in the plants. The use of the plant material in folkloric medicine is thus verified by this study.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

Statement of ethical approval

Ethical Approval for the use of adult wistar rats in this research work was obtained from Imo State Ministry of Agriculture and Natural Resources.

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