NTIOXIDANT EFFECT OF *Ocimum gratissimum* EXTRACT ON MALE ALBINO WISTAR RAT INFECTED WITH *Plasmodium berghei*

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Abstract

Antioxidant effect of the ethanolic extract of *Ocimum gratissimium* was investigated on the male Wistar albino rats infected with *Plasmodium berghei*. Twenty-five (25) male albino Wistar rats were divided into five groups at random: the normal control group (rats that were neither infected nor treated), the negative control group (rats that were infected but not treated), the low-dose group (rats that were infected but treated with the extract at 2.5 mg/kg), the medium-dose group (rats that were parasitized and treated with extract at 5.0 mg/kg), and the high dose group (rats that were infected at 7.5 mg/kg). The antioxidant parameters such as superoxide dismutase, catalase, reduced glutathione, and lipid peroxidation (malondialdehyde) were measured in the serum after 14 days of therapy in the experimental rats. The findings showed that the negative control group's levels of the enzymes superoxide dismutase, catalase, and reduced glutathione were significantly lower than those of the normal control and treated groups ($p<0.05$). However, in comparism to the healthy control and treated groups and the parasitized untreated group, the MDA level significantly increased ($p<0.05$). This effect indicates that the leaf extract has antioxidative properties and the effects of *Plasmodium berghei* on antioxidant enzymes could not be completely reversed in groups treated with the ethanolic extract of *Ocimum gratissimium*, which was shown to increase superoxide dismutase, reduce glutathione and catalase activity in a dose-dependent manner, and decrease MDA levels.

Keywords: *Plasmodium, Ocimum gratissimum*, Malondialdehyde, Superoxide dismutase, Catalase, Reduced glutathione

1.0 Introduction

Malaria is a vector-borne infectious disease that predominantly targets and infects human red blood cells. It is caused by protozoan parasites from the genus Plasmodium (phylum Apicomplexa). Blood transfusions, infected needles, and mosquito bites from female Anopheles mosquitoes harboring the parasite are all ways in which they can be transmitted from one person to another. In the malaria pathophysiology, the parasite (Plasmodium) that attacks healthy red blood cells (erythrocytes) also produces additional parasites that have a variety of harmful effects on human health[1]. Some symptoms of the sickness include muscle aches, sweating, fever, headaches, coughing, exhaustion, jaundice (yellowing of the skin), and vomiting. A severe

Plasmodium falciparum infection can cause liver and renal failure, bleeding issues, shock, and bleeding disorders. A hallmark of malaria infection is an increase in oxidative stress in the human body, which is brought on by the creation of reactive oxygen species (ROS). Hydrogen peroxide production is reported in *Plasmodium berghei*-IRBC and O_2 production in *Plasmodium falciparum*-IRBC. They have been demonstrated to increase levels of lipid peroxidation. The most common method for diagnosing malaria is to microscopically examine blood and bloodfilms, although it is also possible to collect saliva and urine samples from those who are suspected of having the disease[2].

Plasmodium berghei is a kind of the genus Plasmodium. Plasmodium chabaudi, Plasmodium vinckei, and Plasmodium yoelli are the other three Plasmodium species that have been discovered in African murine mice. Due to the clinical signs of human malaria being comparable to those of rodent malaria, the Plasmodium berghei parasite is a common model organism for the research of human malaria as well as the prospective antimalarials in mice and rats[3]. The numerous controls and preventive measures against malaria and its vector (mosquitos) have become ineffective over time due to a number of factors, including the plasmodium parasite's resistance to widely available and affordable medications like fansidar and chloroquine, the vector mosquito's developed resistance mechanism to commonly available insecticides and mosquito repellants, and the abundance of suitable breeding sites for these vectors. Due to the parasites' development of drug resistance, it has become necessary to look for more potent anti-malarial drugs from natural sources. Distribution of fake and inferior anti-malarial drugs, primarily in Nigeria and other African and Asian nations, has worsened the issue of drug resistance and posed a severe threat to continuing malaria control operations. Malaria, which is endemic to most of Africa and other parts of the world, continues to pose a serious threat to global health. The alarming rate at which *P. falciparum* has gained resistance to chloroquine and other synthetic antimalarial medications makes it important to look for more effective antimalarial compounds. Over time, the use of antimalarial drugs and other methods has not been successful[5]. Malaria is not merely a sickness that is frequently linked to poverty; some data indicates that it actually contributes to poverty and is a significant barrier to economic growth. Because the poor masses lack the financial resources to prevent or treat the disease, poverty can raise the chance of contracting malaria. Additional financial consequences associated with the illness include medical expenses, lost workdays owing to illness, lost schooldays, lower productivity as a result of cerebral malarial brain

damage, and lost investment and tourism[6]. In some nations, the condition imposes a significant burden, accounting for 30–50% of hospital admissions, up to 50% of outpatient visits, and up to 40% of public health expenditures. Another clinical and public health concern is the proliferation of substandard antimalarial medicines resulting from the inappropriate concentration of ingredients, contamination with other drugs or toxic impurities, poor quality ingredients, poor stability, and inadequate packaging[7].

However, several researchers have noted that using herbal remedies to treat malaria is a good option[8]. *Ocimum gratissimium* is one of the widely used traditional medicine and profound investigation has been proven its potency as a cure for various diseases[9]. *Ocimum gratissimium* is a perennial woody shrub that can be found in tropical Africa, South America, Nigeria, and Asia. It belongs to the Lamiaceae family.

The goal of this research is to determine whether Ocimum gratissimium leaf extract can effectively cure malaria. If successful, this will enable the use of this globally distributed herb and help to lower malaria-related mortality and economic burden.

2.0 MATERIALS AND METHODS

2.1 Animals Model

Twenty-five (25) male albino rats of Wistar strain purchased from a Local farm at Nsukka, Enugu State, Nigeria, weighing between 92-130 g were kept and acclimatized in a laboratory animal unit of the Brain-Phosphorylationship Scientific Solution Services for 1 week before commencement of the experiment. The animals were separated into five groups (A-E), each with five animals, and kept in well-ventilated cages at room temperature with access to food and water. The experimental rats were grouped as follows: group A(normal control) not infected/fed on animal feed and water, group B (negative control) were infected with 1x 10^7*P.berghei*/untreated, group C (low dose) were parasitized with 1x 10^7/*P.berghei* and treated with 2.5mg/kg of the extract, group D (medium dose) were parasitized with 1x 10^7/*P.berghei* and treated with 5.0mg/kg of the extract, group E (high dose) were the parasitized with 1x 10^7/*P.berghei* and treated with 7.5mg/kg of the extract.

2.2 Preparation of the extract

Fresh leaves of *Ocimum gratissimum* were purchased at Ogbete and left to dry completely for three days at room temperature, the dried leaves were ground using an electric blender, weighed and soaked on 250 ml of ethanol (extracting solvent) for 48 hrs, the extract was filtered and concentrated using a water bath.

2.3 Collection of samples for analysis

After giving the infected groups treatment for two weeks, blood samples were taken from the experimental rats using a collection tube and capillary tube through the ocular puncture, and serum was obtained by centrifuging the samples at 3000 rpm for 15 minutes to obtain antioxidant parameters (superoxide dismutase, catalase, reduced glutathione, and lipid peroxidation).

2.4 Preparation of inoculum of the strain of *Plasmodium berghei*

The parasite *Plasmodium berghei* was sourced from the Department of Veterinary Medicine Laboratory at the University of Nigeria Nsukka. *Plasmodium berghei* strain by serial blood passage from mouse to mouse was used for the study. 1.0 ml of blood was collected using heparinized capillary tubes from the auxiliary plexus of veins in the donor mouse. The blood was diluted with 9.0 ml of normal saline pH 7.2 so that each 0.5 ml approximately contains 1×10^7 infected red cells and each animal receives inocula of about ten million parasites per kilogram body weight, which is expected to produce a steadily rising infection in the rat.

2.5 Determination of Parasitemia

The animals' tails were used to create a blood smear that was transferred to microscope slides and formed into thick and thin films on both ends of the slides. Both before and after therapy, this was done. Giemsa stain and methanol were used to fix and colour the blood respectively. Under a microscope, the slides were analyzed to count the parasites.

2.6 Blood Collection and Preparation of Sample

Following the completion of the experiment, blood was collected from the experimental animals by ocular puncture using heparinized capillary tube into plain collection bottles for the biochemical analyses of serum. Blood samples were centrifuged at 3000 rpm for 15 minutes to obtain serum and this was collected into clean bottles by pipetting.

2.6 Analysis of antioxidant parameters

2.6.1 Estimation of superoxide dismutase (SOD)

This was determined following the method described[10.]

2.6.2 Estimation of Catalase (CAT)

Catalase activities of the samples were determined in erythrocyte lysate using Aebi's method[11].

2.6.3 Estimation of Reduced glutathione (GSH)

Reduced glutathione (GSH) was measured using the method of Sedlak and Lindsay[12].

2.6.4 Estimation of Lipid peroxidative (LPO) indices

Lipid peroxidation as evidenced by the formation of malondialdehyde (MDA). These parameters were measured in the serum by the method ofVarshney and Kale[13].

2.7 Data analysis:

Statistical analysis of the result was processed using the Statistical Package for Social Science (SPSS) for window 2.1 version 18. Values of the measured parameter were expressed as mean ± SEM one-way Analysis Variance (one-way ANOVA) was used to determine the effect of *Ocimum gratissimium* at different doses on Male albino rats infected with, and the significance was considered at p-value < 0.05.

3.0 RESULTS

3.1 Biochemical parameters

3.1.1 Serum SOD

The result from table 1 showed that the negative control group (Group B) had the lowest value, ($p<0.05$) (36.173 ± 1.7) but was significantly increased ($p<0.05$) (45.073 ±1.9) when treated with medium dose of extracts of *Ocimum gratssimum.* This effect indicates that the leaf extract has antioxidative properties on the serum superoxide dismutase of male albino wistar rats infected with *Plasmodium berghei.*

Table 1: Effect of the administration of *Ocimum gratssimum* extract on the SOD

Groups	Serum SOD (µ/mg)
A (Normal Control)	53.497±3.4[a]
B (Negative Control)	36.173±1.7[b]
C (Low-dose Extract)	43.263±2.7[c]
D (Medium-dose Extract)	45.073 ±1.9[c]
E (High-dose Extract)	44.192 ±0.8[c]

The mean value in a column with the same letter superscript does not differ substantially ($p < 0.05$).

3.1.2 Serum catalase

From table 2, it can be deduced that the negative control group (Group B) had the lowest value, ($p<0.05$) (7.870 ± 0.6) but was significantly increased ($p<0.05$) (10.377 ± 0.7) when treated with high - dose extract of *Ocimum gratssimum.* This indicates that the leaf extract has an amilorating effect on the serum catalase of male albino wistar rats infected with *Plasmodium berghei.*

Table 2: Effect of the administration of *Ocimum gratssimum* extract on the Catalase

Groups	Serum Catalase (µ/mg)
A (Normal Control)	10.784±1.2[a]
B (Negative Control)	7.870±0.6[b]

C (Low-dose Extract)	9.629±1.6 [c]
D (Medium-dose Extract)	9.903±0.5 [c]
E (High-dose Extract)	10.377±0.7 [c]

The mean value in a column with the same letter superscript does not differ substantially ($p < 0.05$).

3.1.3 Serum Reduced Glutathione

The negative control (Group B) recorded the lowest value of serum reduced glutathione at ($p<0.05$) (22.238 ± 0.4) but was significantly increased at ($p<0.05$) (24.562 ± 0.7) when treated with low - dose extract of *Ocimum gratssimum* (table 3). This indicates that the leaf extract has an effect on the serum reduced glutathione of male albino wistar rats infected with *Plasmodium berghei.*

Table 3: Effect of the administration of *Ocimum gratssimum* extract on the reduced glutathione

Groups	Serum Reduced Glutathione (µ/mg)
A (Normal Control)	26.818±1.1 [a]
B (Negative Control)	22.238±0.4 [b]
C (Low-dose Extract)	24.562±0.7 [c]
D (Medium-dose Extract)	24.339±0.7 [c]
E (High-dose Extract)	24.366±0.9 [c]

The mean value in a column with the same letter superscript does not differ substantially ($p < 0.05$).

3.1.4 Lipid Peroxidation

The negative control (Group B) recorded the lowest value of serum lipid peroxidation at ($p<0.05$) (3.5487 ± 0.0629) but was significantly increased at ($p<0.05$) (5.6170 ± 0.0685) when treated with low - dose extract of *Ocimum gratssimum* (table 4). This indicates that the leaf extract has an effect on the serum lipid peroxidation of male albino wistar rats infected with *Plasmodium berghei.*

Table 4: Effect of the administration of *Ocimum gratssimum* extract on the lipid peroxidation

Groups	Serum lipid peroxidation (μ/mg)
A (Normal Control)	3.5487 ± 0.0629^{a}
B (Negative Control)	6.2483 ± 0.0476^{b}
C (Low-dose Extract)	5.6170 ± 0.0685^{c}
D (Medium-dose Extract)	5.6617 ± 0.0586^{c}
E (High-dose Extract)	5.8513 ± 0.0586^{c}

The mean value in a column with the same letter superscript does not differ substantially ($p< 0.05$).

4.0 DISCUSSION AND CONCLUSION

4.1 Discussion

The result obtained from the serum analysis showed a decrease in the level of superoxide dismutase, catalase, glutathionein the infected groups, this result aligns with the report of Oyewole[14] who also recorded a decrease in superoxide, catalase and gluthatione level with increase infectivity of plasmodial parasite, this finding is also consistent with the report from Kulkarni *et al.*[15] and Guha *et al*.[16] who also recorded a decrease in superoxide dismutase, catalase and reduced glutathione level in their study on malaria infection in rats, this decrease could be as a result of the

increased utilization of the enzymes to balance the reactions triggered off by increased infectivity. Similarly the result obtained revealed that lipid peroxidation in the *Plasmodium berghei* infected groups were significantly increased, it was also drawn from the result that infectivity had an increasing effect on lipid peroxidation as the control group A recorded the lowest level of lipid peroxidation. This result is in line with the findings of Henriques and de Dominguez[17], who also noted a rise in MDA levels in *Plasmodium berghei*-infected mice. They also noted an increase in parasitemia at the same time as the MDA level increase. Several disease conditions have been known to be characterized by increases lipid peroxidation, and the reduction of this activity by the extract as shown in the treated groups infected with the disease may be due to the depletion of defense system and further reduction of the lipid peroxidation level in the treated group could be the result of increased superoxide activity which protects cells from the damaging effect of reactive oxygen species.

4.2 Conclusion

The results of the study showed that the ethanolic leaf extract of Ocimum gratissimium significantly raised the levels of antioxidant enzymes in the Plasmodium berghei-infected groups. This effect indicates that the leaf extract has antioxidative properties, but treatment with the extract was unable to fully reverse the oxidative stress brought on by *Plasmodium berghei* and counteract it.

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