Journal of Experimental Research

DECEMBER 2023, Vol 11 No 4

Email: editorinchief.erjournal@gmail.com
editorialsecretary.erjournal@gmail.com

Received: Oct., 2023
Accepted for Publication: Dec., 2023

ANTIOXIDANT 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 *Ocimum gratissimium*. 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 and treated at 7.5 mg/kg). The antioxidant parameters such as superoxide dismutase, catalase, reduced glutathione, and lipid peroxidation product (malondialdehyde) were measured in the serum after 14 days of therapy in the experimental rats. The findings showed that the negative control group's activities of the enzyme's superoxide dismutase, catalase, and reduced glutathione were significantly lower than those of the normal control and treated groups ($p<0.05$). However, upon the administration of *Ocimum gratissimium* the parameters enzymes activities of the infected and treated groups significantly increased ($p<0.05$). This effect affirmed that the extract has antioxidative properties that reversed oxidative potentials caused by *Plasmodium berghei infection.*

Keywords: *Plasmodium*, *Ocimum gratissimum*, Malondialdehyde, Superoxide dismutase.

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 (Webb, 2009). 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 caused by the generation of reactive oxygen species (ROS). Hydrogen peroxide production is reported in *Plasmodium berghei*-IRBC and O_2 production in *Plasmodium falciparum*-IRBC (Rahbari *et al.,* 2017). They have been demonstrated to increase levels of lipid peroxidation. The most common method for diagnosing malaria is to microscopically examine blood and blood films, although it is also possible to collect saliva and urine samples from those who are suspected of having the disease (Sutherland and Hallett, 2009).

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 anti-malarial in mice and rats (Pedronic *et al.,* 2006). The numerous controls and preventive measures against malaria and its vector (mosquitos) have become ineffective over time due to several factors, including the plasmodium parasite's resistance to widely available and affordable medications like fansidar and chloroquine, the vector'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 anti-malarial medications makes it important to look for more effective antimalarial compounds. Over time, the use of anti-malarial drugs and other methods has not been successful (Bhat and Surolia, 2001). 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 because of cerebral malarial brain damage, and lost investment and tourism (Greenwood *et al.,* 2005). 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 (Caudron *et al.,* 2008).

However, several researchers have noted that using herbal remedies to treat malaria is a good option (WHO, 2002). *Occimum* ***gratissimum*** is one of the several used traditional herb that has been used to cure various diseases (Bhatia *et al.,* 2008). *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.

MATERIALS AND METHODS

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 1.0 x 10^7*P.berghei*/untreated, group C (Low Dose) were parasitized with 1.0 x 10^7/*P.berghei* and treated with 2.5 mg/kg of the extract, group D (Medium Dose) were parasitized with 1.0 x 10^7/*P.berghei* and treated with 5.0 mg/kg of the extract, group E (High Dose) were parasitized with 1x 10^7/*P.berghei* and treated with 7.5 mg/kg of the extract.

Preparation of the extract

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

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. The serum samples were obtained by centrifuging the samples at 3000 rpm for 15 minutes to obtain the antioxidant parameters (superoxide dismutase, catalase, reduced glutathione, and lipid peroxidation).

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 x 10^7 infected red cells, and the experimental animal receives inoculate of about ten million parasites per kilogram body weight, which is expected to produce a steadily rising infection in the rat.

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. Giemsa stain and methanol were used to fix and stain the blood respectively. Under a microscope, the slides were observed by x40 objective lens.

Blood Collection and Preparation of Sample

Following the completion of the experiment, blood was collected from the experimental animals by ocular puncture using 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 sample, and this was collected into clean bottles by pipetting.

Anaatilysis of antioxidant parameters

Estimon of superoxide dismutase (SOD)

This was determined following the method described (McCord and Fridovich, 1969).

Estimation of Catalase (CAT)

Catalase activities of the samples were determined in erythrocyte lysate using Aebi's method (Aebi, 1984).

Estimation of Reduced glutathione (GSH)

Reduced glutathione (GSH) was measured using the method of Sedlak and Lindsay (Sedlak and Lindsay, 1968).

Estimation of Lipid Peroxidative (LPO) indices

Lipid peroxidation as evidenced by the formation of malondialdehyde (MDA) was measured in the serum by the method of Varshney and Kale (Varshney and Kale, 1990).

Table 1: Effect of the administration of *Ocimum gratssimum* extract on the serum enzymes activities.

SOD (µ/mg)	Catalase (µ/mg)	Reduced Glutathione (µ/mg)	MDA (µ/mg)
53.497±3.4[a]	10.784±1.2[a]	26.818±1.1[a]	3.5487±0.0629[a]
36.173±1.7[b]	7.870±0.6[b]	22.238±0.4[b]	6.2483±0.0476[b]
43.263±2.7[c]	9.629±1.6[c]	24.562±0.7[c]	5.6170±0.0685[c]
45.073 ±1.9[c]	9.903±0.5[c]	24.339±0.7[c]	5.6617±0.0586[c]
44.192 ±0.8[c]	10.377±0.7[c]	24.366±0.9[c]	5.8513±0.0586[c]

The mean value in a column with the same letter superscript is not significantly different (p< 0.05).

DISCUSSION AND CONCLUSION

Discussion

The result obtained from the serum analysis showed decrease in the activities of superoxide dismutase and catalase, as well as the level of glutathione in the infected groups. this result aligns with the report of Oyewole *et al.* (2011), who also recorded a decrease in superoxide, catalase and glutathione levels with increased infectivity of plasmodial parasite. This finding is also consistent with the report from Kulkarni *et al.* (2003) and Guha *et al.* (2006), who also recorded a decrease in superoxide dismutase, catalase, and reduced glutathione levels in their study on malaria infection in rats. These decreases 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 product – MDA, 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 (2012), who also noted a rise in MDA levels in *Plasmodium berghei*-infected mice. They also noted an increase in parasitaemia at the same time as the MDA level increase. Several disease conditions have been known to be characterized by increased lipid peroxidation product, 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 product activities in the treated group could be the result of increased superoxide activity which protects cells from the damaging effect of reactive oxygen species.

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 which reversed the oxidation occasioned by the infection.

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