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Original article

EVALUATION OF ANTI-PLASMODIAL ACTIVITY OF REACTIVE OXYGEN SPECIES (ROS) DETOXIFYING ENZYME CATALASE (CAT) IN ANOPHELES MOSQUITO VECTORS

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ABSTRACT

Mosquito defense against malaria parasites involves variety of biological processes among which Reactive Oxygen Species (ROS) are important determinants of parasite invasion. *Plasmodium* parasite infection causes oxidative damage in the mosquito host cells. The mosquito host cell is often protected from such damage by numerous detoxifying enzymes including catalase. This research was designed to investigate the anti-plasmodial activity of enzyme catalase in *Anopheles* mosquitoes. Indoor resting mosquitoes were collected using Pyrethrum Spray Catch (PSC) method. *Anopheles* were sorted and identified with the aid of standard taxonomic keys, dissected and assessed for sporozoites infection. The activity of enzyme catalase in response to *Plasmodium* parasite infection in the mosquito was determined using standard methods. One way ANOVA was used to compare catalase activity in infected and uninfected mosquitoes. Findings revealed higher catalase activity (mean $\pm$ SD) 6.85 $\pm$0.49 (U/L) in infected mosquitoes as against 4.99 $\pm$ 0.87 (U/L) in uninfected mosquitoes. Moreover, anti-plasmodial activity of Catalase in the two groups showed significant difference ($P < 0.05$). The study revealed the role of enzyme Catalase in mosquito immune defence against *Plasmodium* parasite, which holds the potentials to limit *Plasmodium* development within the mosquito vector, thereby reducing the diseased transmission population.

Key words: Anti-Plasmodial, Anopheles Mosquito, Catalase, Reactive Oxygen Species (ROS).

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INTRODUCTION

Mosquitoes, like all organisms, are under constant threat of infection. For the continuation of their life cycles, females of all anautogenous species are required to take a blood meal for the production of eggs [1]. This act of blood feeding often expose mosquitoes to blood borne pathogens that aim to undergo complex developmental, reproductive and/or migrational processes inside a mosquito host before they can be transmitted during a subsequent blood meal. Consequently, the intake of a Plasmodium-infected blood meal may affect mosquito physiology and a series of trade-off may occur, in particular between immune defence, reproduction and self-maintenance [2], with majority of the parasites destroyed as a result of the lumenal and epithelial immune responses mounted by the mosquito [3].

The innate immune system of most mosquitoes (non-vectors) is able to completely clear Plasmodium infection preventing parasite transmission to humans. Mosquito defence against malaria parasites involves variety of biological processes, among which reactive oxygen species (ROS) are important determinants of parasite invasion to mosquitoes [4]. ROS are toxic by-product of cellular metabolism in all living beings. During mosquito response to infection, reactive oxygen species (ROS) are generated to limit Plasmodium infection. In spite of these active intermediates being beneficial for parasite clearance, they are potentially toxic to the host itself. Therefore it is important that they are generated transiently and kept well localized. This task is often accomplished through the action of antioxidant enzymes and other proteins that participate in redox metabolism [5]. Mosquito host cells are protected from oxidative damage by numerous detoxifying enzymes in a precise manner including Superoxide dismutase (SOD), Peroxidases e.g. Catalase.

These enzymes exert their anti- plasmodial activity by inducing oxidative imbalance in the mosquito midgut. This oxidative imbalance can overstress the malaria parasite, leading to arrested development in the mosquito midgut and reduced transmission [6]. Considering the anti-plasmodial and detoxifying potentials of these enzyme, it is expected that the enzyme – induced oxidative stress can be exploited as a transmission – blocking strategy against human malaria parasite.

MATERIALS AND METHODS

Adult Mosquito Collection and Preservation

Indoor resting mosquitoes were collected using the Pyrethrum Spray Catch (PSC) between the hours 06:00 and 09:00 am in the study areas. Food items were covered properly and moveable furniture were taken care of before spraying. Large white sheets of cloths was spread wall to wall to cover the floors of the room while all doors and windows were shut. All cracks and openings in walls were stocked with rag papers to prevent mosquitoes from escaping. After about 20 minutes, the spread cloths were carefully folded starting from the corners. Knock down mosquitoes were collected with forceps into a damp petri dish. Collections were done according to the standard described by [7].

Identification

Morphological identification was carried out using a trinocular dissecting microscope (Amscope SZMT2/MU100010APTINA COLOR CMOS) with the aid of standard keys [8][9].The mosquitoes were identified using the gross morphology of the species, external morphology of the head, mouthparts,

antennae, proboscis, patches of pale and black scales on the wings and legs and the terminal abdominal segments [10].

Dissection of Salivary Gland for Determination of Sporozoite Infection

This is intended to incriminate mosquito vectors and establish Sporozoite rates. The anterior part of the same mosquito was placed on a slide with the head pointing to the right. A drop of saline was added to keep the specimen fresh. Meanwhile, the left dissecting needle was placed gently on the thorax, just below the region where the glands lie. The right needle was pulled towards the right direction to bring out the head with the salivary glands attached. Some salivary glands may not come out with the head of the mosquito but these were located by carefully teasing the lower part of the thorax. The glands were detached from the head and then placed on another microscope slide with little drop of saline and covered with a cover slip. A gentle pressure was exerted on the cover slip to rupture the gland cells. The sporozoites were seen (if present) and identified as minute needle like forms.

Measurement of Catalase activity

The method of [11] was used to determine catalase (CAT) activity.

Principle: This method relies on the reduction of dichromate in acetic acid to chromic acetate when heated in the presence of hydrogen peroxide. The chromic acetate generated is then quantified colorimetrically at 570 – 610 nm. The CAT in the mosquito is allowed to split hydrogen peroxide over specific minutes after which the reaction is terminated by the addition of dichromate/acetic acid mixture. The hydrogen peroxide left unsplit is quantified by measuring chromic acetate colorimetrically after heating the reaction mixture.

Procedure: The assay mixture is made up of 1 ml of H2O2 solution (800μmoles) and 1.25 ml of phosphate buffer (0.1M, pH 7.4). 1ml of appropriately diluted enzyme preparation (1:5) was rapidly mixed with the reaction mixture by gentle swirling. A 1 ml aliquot of the reaction mixture was withdrawn and added to 2ml dichromate/acetic acid reagent and the absorbance read at 60 sec interval for 3 min at 570 nm.

Catalase activity = Δ OD/min x volume of assay

Volume of enzyme x protein conc (mg)

Where: OD = optical density

Δ OD = change in optical density

RESULTS

The antioxidants represents the main pathway to detoxify free radicals produced during oxidative stress. Exposure to *Plasmodium* infection induces oxidative stress which contributes to antioxidant enzymes to prevent and repair the damage caused by reactive oxygen species (ROS). The activity of detoxifying enzyme catalase have been measured in both *Plasmodium* infected and uninfected *Anopheles* mosquitoes. In general, the activity of catalase was observed in both infected and uninfected *Anopheles* mosquitoes. However, catalase activity in infected *Anopheles* mosquitoes showed varied elevation patterns when compared with uninfected *Anopheles* vectors. Thus, compared with uninfected females (4.99 ± 0.87), catalase level was significantly higher in infected mosquitoes (6.85 ± 0.49) at ($P<0.05$).

Table 1: Effect of *Plasmodium* parasite infection on the activity of detoxifying enzyme Catalase (CAT) in Anopheles mosquitoes.

Infections status	Infected	Uninfected
Mean CAT activity	6.85±0.49	4.99±0.87

DISCUSSION

The results of this study implied that catalase activity was induced by oxidative stress caused by Plasmodium parasite infection in Anopheles mosquitoes as [12] reported that anti-oxidants defence is one among the survival mechanisms against oxidative injury.

Oxidative stress induces a rapid production of ROS and their elimination by anti-oxidant system is essential for the survival of the animal [13]. Exposure to Plasmodium parasite as observed in this study is responsible for the induction of oxidative stress in Anopheles mosquitoes which consequently induced a significant increase in the activity of catalase (CAT) to clear the parasites and prevent damage caused by ROS.

An earlier study by [14] revealed that oxidation resistance 1 (OXR1) gene regulates the basal levels of catalase (CAT) and glutathione peroxidase (GPx) expression in in *Anopheles gambiae,* this gene silencing decreases *Plasmodium* infection in the mosquito.

In haematophagous insects, catalase (CAT) has been detected among transcripts that are up regulated after ingestion of blood, indicating its possible role in response against oxidative stress caused by blood meal [15][16]. Further silencing of *Anopheles gambiae* catalase (CAT) increase mosquito mortality after a blood meal [17]. Several studies have indicated an induction of the peroxidase catalase (CAT) activity and glutathione peroxidase (GPx) post blood meal. However, the induction of catalase (CAT) activity in infected Anopheles mosquitoes in this study is likely a part of a defence response triggered by *Plasmodium* parasite invasion.

CONCLUSION

Immune and antioxidant defense as a function of Plasmodium parasite infection showed that the activities of catalase as detoxifying enzyme was significantly enhanced by Plasmodium parasite infection of the female Anopheles mosquitoes. The exposure to Plasmodium parasite infection therefore induced the activities of detoxifying enzyme catalase.

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