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Toxicological Evaluation of Bee Sting in *Plasmodium berghei* Infected Mice

Oluwatosin Kudirat Shittu[1*] and Rabiu Ibrahim[1]

[1]Tropical Disease Research Unit, Department of Biochemistry, Federal University of Technology, P.M.B. 65, Minna, Nigeria.

Authors' contributions

This work was carried out in collaboration between the authors. Author OKS did the study design and took part in the laboratory work and statistical analysis. Author RI did the literature searches and also took part in the laboratory work. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Based on the traditional and scientific claims of intra-dermal antiplasmodial activity of bee stings, its effect on liver and serum enzymes in *Plasmodium berghei*-infected mice were investigated.
Methodology: Twenty albino mice were intra-peritoneally infected with *P. berghei* and divided into four animals per group. Group I was set up as negative control (parasitized untreated), group II as parasitized treated with 5 mg chloroquine/kg body weight, group III as suppressive treated, group IV as curative treated and group five as not parasitized not treated.
Results: The results of serum alanine transaminase (ALT) and aspartate transaminase (AST) of the infected treated with chloroquine shows significant increase when compared to other experimental groups ($p< 0.05$). Whereas, there was increase in liver AST in group II, group III and group IV when compared to not parasitized not treated ($p< 0.05$). Also, there was significant decrease in liver ALT activity in all the experimental groups. The serum and liver gamma glutamyl transferase (GGT) showed no significant difference ($p<0.05$) in the curative and suppressive groups when compared to the standard drug (chloroquine). Whereas, parasitized not treated group shows significant increase ($p<0.05$) in the liver GGT and ALP when compared with other experimental groups. Therefore,

**Corresponding author: E-mail: toscueyusuf@gmail.com, toscueyusuf@futminna.edu.ng;*

these increases in specific activity of the parasitized untreated group might be due to infection.
Conclusion: It can be concluded that bee sting have ameliorative effect against changes caused by *P. berghei*.

Keywords: Malaria; bee sting; Plasmodium berghei; enzyme activities.

1. INTRODUCTION

Malaria is a vector-borne disease [1] and it is prevalent in tropical and subtropical regions including Sub-Saharan Africa, Asia, and Americas [2-4]. The disease has been eradicated in most temperate zones. But, it continues to be endemic in some areas causing human morbidity and mortality [5,6]. Malaria causes about 250-500million cases of fever and approximately one million deaths yearly. Majority of cases occur in children under five years of age and pregnant women [3,5,7-9]. The parasite can be transmitted via infected female Anopheles mosquito bites, by inoculating sporozoites through saliva into the circulatory system and eventually to the liver where they developed and replicate.

Venoms from insects of *Hymenoptera* family such as honeybees and their products play vital roles in traditional medicine in many region of the world [10-12]. It has been reported that the Honeybees produce: honey, royal jelly, pollen, propolis, bee's wax and bee venom which have extraordinary medicinal and commercial values [13,14]. Previous work has confirmed their antimalarial [15], analgesic, antimicrobial, anti-inflammatory and immunological benefits [16].

Therefore, in this study, the toxicological effects of Bee sting in *Plasmodium berghei* infected mice were investigated.

2. MATERIALS AND METHODS

2.1 Honeybee

Honeybees (*Apismellifera*) were collected from a tree near Bosso dam area, Bosso, Minna, Niger State, Nigeria.

2.2 Experimental Animal

White Swiss albino mice of both sexes with an average weight of 21g were obtained from Animal House of the Department of Biochemistry, University of Ibadan, Oyo State, Nigeria. They were kept in plastic cages and maintained on a standard pellets diet and tap water *ad-libitum.*

2.3 Parasites

A chloroquine - Sensitive strain of Plasmodium *berghei* (NK-65) was obtained from the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. A standard inoculum of $1x10^7$ parasitized erythrocytes in 0.2 ml of diluted infected blood was used to infect the experimental mice intraperitoneally. Parasitaemia was checked by Giemsa staining method [17].

2.4 Experimental Design

Using the method described by Datta [18]. All groups except group 5 (not parasitized not treated) were infected with *P. berghei* parasitized erythrocytes on D_0 and treated for 4 consecutive days.

- Group I: – Parasitized and received 0.2 ml normal saline daily.
- Group II: Parasitized and received aqueous solution of chloroquine (5 mg/kg body weight) daily.
- Group III: Parasitized and received intradermal bee sting dailybefore infection (Suppressive test).
- Group IV: Parasitized and received intradermal bee sting dailyafter 72 hours post infection (Curative test) and
- Group V: – Not parasitized and not treated.

Treatment was withheld for 72 hours except group III, to allow the establishment of infection. Blood films were made from the tail of individual mouse fixed with methanol and stained with giemsa stain, according to the method of Ryley and Peters [19]. On the fifth day mice were anaesthetized with chloroform and the blood and liver were collected.

2.5 Preparation of Serum and Tissue Homogenate

Blood was collected from each mouse into a sterile centrifuge plain tube and centrifuged at 2000 rpm for 5min at room temperature to obtain the serum. For the liver, 1.0g of the liver was homogenized in ice-cold 4.0 ml of freshly prepared 0.25 M sucrose solution (1:4 w/v) and centrifuged for 15min at 3000rpm. The homogenates were collected and stored for further analysis.

2.6 Enzyme Analysis

Enzyme activities in the serum and liver were carried out using commercially available kits of RANDOX laboratories (Randox, UK). Alkaline phosphatase (ALP) activity was estimated by the method of Tietz et al. [20], Aspartate transaminase (AST) and alanine transaminase (ALT) activities were assayed using the method described by Reitman and Frankel [21]. Total protein concentration was determined using Biuret method described by Gornall et al. [22] and modified by Plummer [23]. Gamma glutamyl transferase (GGT) was measured by the method of Szasz and Klin. [24].

2.7 Statistical Analysis

The data were statistically analyzed using one-way analysis of variance (ANOVA) and Duncan multiple range test (25). Data from the treated groups were compared with their respective controls and differences at $p<0.05$ were considered significant. Values are presented as mean $\pm$ Standard Error of Mean (SEM).

3. RESULTS

3.1 Aspartate Transaminase (AST)

The results of serum and liver specific activities of Aspartate transaminase (AST) are presented in Fig. 1. The liver specific activities were significantly increased ($p< 0.05$) in the standard, suppressive and curative treated when compared with the control group. While, the serum specific activities of AST were significant increase ($p< 0.05$) in the standard treated when compared with other experimental groups.

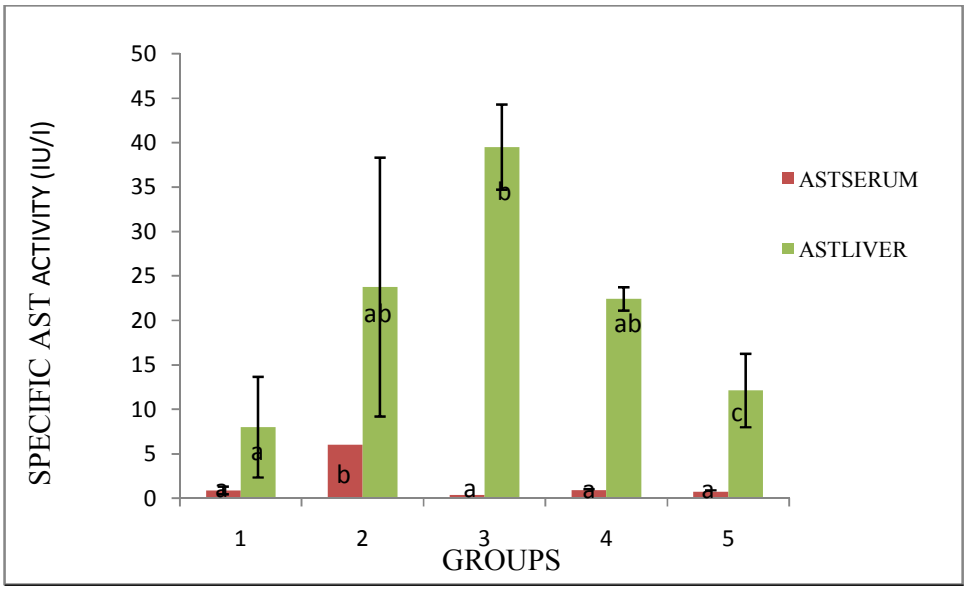

Fig. 1. Specific activities of Aspartate transaminase (AST) in liver and serum of mice infected *Plasmodium berghei*. Results are mean of five determinations ± SEM. Bars carrying different letters are significantly different at p < 0.05

Group 1 – Parasitized untreated (negative control); Group 2 – Parasitized treated after 72hrs with 5 mg/kg chloroquine phosphate (Standard); Group 3 –Suppressive treated; Group 4 – Curative treated; Group 5 – not parasitized and not treated (positive control)

3.2 Alanine Transaminase (ALT)

The results of serum and liver specific activities of Alanine transaminase (ALT) are presented in Fig. 2. The liver specific activities of ALT was significantly decreased ($p< 0.05$) in all the experimental groups when compared with the control group. While, serum specific ALT activities showed significant increase ($p< 0.05$) in standard treated when compared with the not parasitized and not treated group which shows no significant different ($p> 0.05$) from curative group.

3.3 Gamma Glutamyltransferase (GGT)

Fig. 3 showes that there was significant increase ($p<0.05$) in liver specific activity of gamma glutamyl transferase (GGT) of parasitized untreated (PNT) group when compared with other experimental groups. Where as, there was no significant difference ($p>0.05$) in the serum and liver specific activities of GGT of the suppressive and curative groups when compared to parasitized treated with chloroquine (PTSTD).

3.4 Alkaline Phosphatase

Fig. 4 shows significant increased ($p<0.05$) in liver specific activity of alkaline phosphatase (ALP) of Parasitized untreated (PNT) group when compared with other experimental groups. Whereas, there was no significant difference ($p>0.05$) in the liver ALP of the Parasitized treated with chloroquine (PTSTD), suppressive and curative groups when compared to group five (PTSTD).

4. DISCUSSION

Liver dysfunction affects the body metabolic processes due to their role in overall metabolism of the organism. Enzymes are necessary for normal cellular metabolism including that of the liver [24]. Therefore, the progressive changes in the hepatocytes due to the infection may alter the activities of the enzymes. The rapid increase or decrease in serum and liver biomarkers enzymes indicate alteration to specific organ. Hence, the changes in these biomarkers activities can be used as indicators of hepatic damage [25-27].

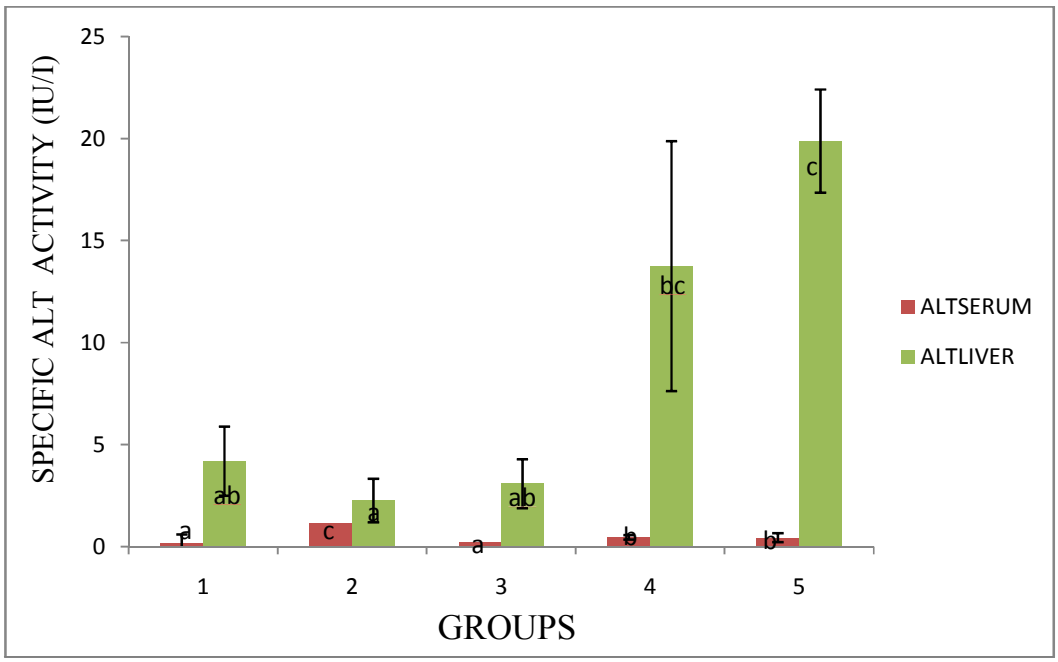

Fig. 2. Specific activities of Alanine transaminase (ALT) activities in liver and serum of mice infected with *Plasmodium berghei.* Results are mean of five determinations± SEM. Bars carrying different letters are significantly different at $p< 0.05$

Group 1 – Parasitized untreated (negative control); Group 2 – Parasitized treated after 72hrs with 5 mg/kg chloroquine phosphate (Standard); Group 3 –Suppressive treated; Group 4 – Curative treated; Group 5 – not parasitized and not treated (positive control)

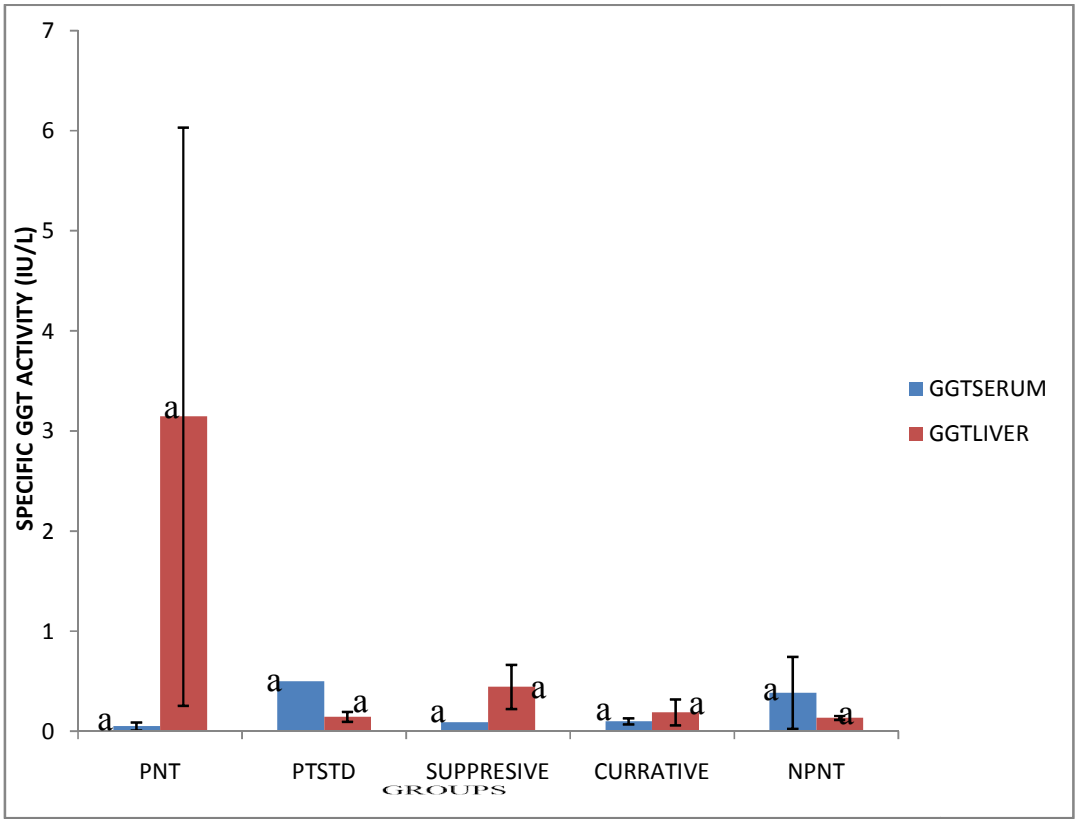

Fig. 3. Changes in serum and liver Gamma glutamyl transferase (GGT) activities in *P. berghei* infected mice treated with bee sting

PNT: Parasitized untreated, PTSTD: Parasitized treated with chloroquine, Group 3 –Suppressive treated; Group 4 – Curative treated; NPNT: non-parasitized not-treated. Mean values with the difference letter are significantly different (p<0.05)

Transaminases are responsible for amine transfer in the body metabolism. Aspartate transaminase (AST) is a peridoxal phosphate dependent and catalyzes a reversible transfer of an amino group between aspartate and glutamate. While, Alanine transaminase (ALT) catalyzes the transfer of an amino group from L-alanine to α- ketoglutarate [28]. Hence, they are very important in amino acid metabolism. In this study, significant ($p< 0.05$) increase was observed in serum AST activities of the chloroquine treated when compared to not parasitized not-treated group. Whereas, there was significant increase in serum ALT activities of the chloroquine treated when compared with parasitized untreated group. This result agrees with previous studied that shows that chloroquine affect a wide range of biochemical processes including inhibition of key metabolic enzymes [24]. The Liver AST activities of chloroquine treated, suppressive and curative treated groups was significantly increased when compared with the non-parasitized not-treated group. These may be attributed to induction of the enzyme in the presence of reactive metabolite from the treatment. Also, there was significant decrease in liver ALT activities of parasitized untreated, standard treated and suppressive groups when compared to the not-parasitized not-treated group. This agrees with the earlier report that infective condition gradually affects enzyme level [29]. However, treatment with bee sting after establishment of the parasite (curative test) tends to restore the level of liver ALT activity towards the value observed in not-parasitized not-treated group, thereby reflecting the efficacy of bee stings in the treatment.

The significant increase ($p<0.05$) observed in liver GGT and ALP of parasitized untreated (PNT) group when compared to other groups implies induction of the enzyme in the liver without cellular necrosis that causes leakage into blood stream. This is also agreement with the report of Sowjanya et al. [30] and Olorunnisola et al. [31].

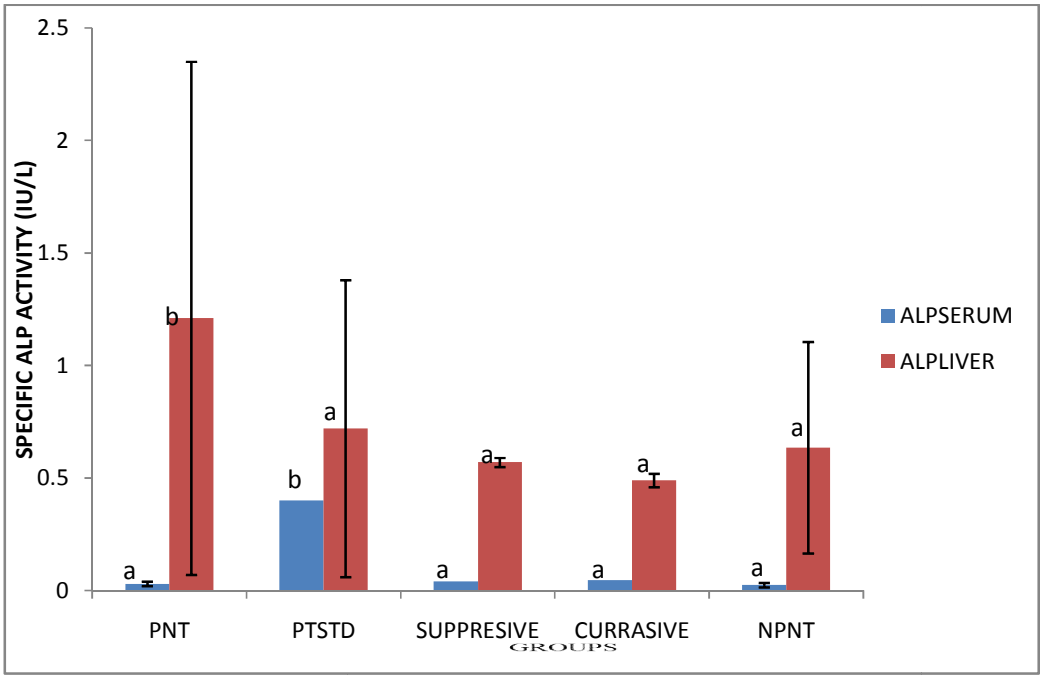

Fig. 4. Changes in serum and liver alkaline phosphatase (ALP) activities in *P. berghei* infected mice treated with bee sting

PNT: Parasitized untreated, PTSTD: Parasitized treated with chloroquine, Group 3 –Suppressive treated; Group 4 – Curative treated; NPNT: not-parasitized not-treated. Mean values with the difference letter are significantly different ($p<0.05$)

Bee venoms have been reported as potent inhibitors of the intra erythrocytic development of *plasmodium* [32]. Therefore, the significant reduction in liver GGT and ALP activities of suppressive and curative groups may be attributed to stings constituents. Whereas, the observed increased in serum ALP activities of the chloroquine treated group compared with other groups (Fig. 4.) shows normal circumstance due to body response to a variety of drugs. This confirm previous studies that chloroquine affects a wide range of biochemical processes including inhibition of key metabolic enzymes such as alcohol dehydrogenase, succinate dehydrogenase and glucose-6-phosphate dehydrogenase [24,33].

The normalization of liver and serum alkaline phosphatase (ALP) and gammaglutamyl transferase (GGT) of the suppressive and curative groups suggests that bee sting is able to change the condition of the hepatocytes by protecting the membrane integrity against *P. berghei*. Therefore, the result infers a protective effect of bee sting on impaired changes caused by *P. berghei.*

5. CONCLUSION

This study has shown that Bee sting has ability to ameliorate the effect caused by *Plasmodium berghei* infection, thereby making it a potential agent for anti-malarial drug.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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