

Original Research Article

**TOXICOLOGICAL EVALUATION OF BEE STING IN *PLASMODIUM BERGHEI*
INFECTED MICE**

ABSTRACT

Aim: Based on 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* with four animals per group. The group I was set up as negative control (parasitized untreated), group II as parasitized treated with 5mg chloroquine/kg body weight, group III as suppressive treated, group IV as curative treated and group five as not parasitized not treated.

Results: The result for serum ALT and AST showed significant increase in infected treated with chloroquine 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, these increases in specific activity of the parasitized untreated group might be due to infection.

Conclusion: Therefore, it can be concluded that bee sting have ameliorative effect against the infection.

Key words: Malaria, bee sting, *Plasmodium berghei*, enzyme activities

INTRODUCTION

Malaria is a vector-borne disease (1). It is prevalent in tropical and subtropical regions; including Sub-Saharan Africa, Asia, and Americas (2, 3, 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. The majority of cases occur in children under five years of age and pregnant women (3, 5,7,8,9). The parasite can be transmitted when an infected female *Anopheles* mosquito bites an individual, by inoculating sporozoites via its 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, 11, 12). It has been reported that the Honeybees produce: Honey, Royal jelly, Pollen, Propolis, Beeswax and Bee venom which have extraordinary medicinal and commercial values (13, 14).

Therefore, in this study, the toxicological effects of Bee sting in *Plasmodium bergheï*infected mice areinvestigated.

MATERIALS AND METHODS

Honeybee:

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

Experimental Animal:

The laboratory animal used was White Swiss albino mice of both sexes with an average weight of 21g, obtained from Animal House, 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*.

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 1×10^7 parasitized erythrocytes from donor mice in volume of 0.2ml of diluted infected bloodwith isotonic saline was used to infect the experimental mice intraperitoneal. Parasitaemia was checked by Giemsa stained thin blood smears through tail vein cut of infected mice (15).

57 **Experimental Design:**

58 Using the same method described by Datta (16). All groups except group-5(normal control) were
59 injected with *P. berghei* parasitized erythrocytes on D₀ and treated for 4 consecutive days.

- 60 • Group I: – Parasitized and received 0.2ml normal saline daily.
- 61 • Group II: Parasitized and received the aqueous solution of chloroquine (5mg/kg body weight)
62 daily.
- 63 • Group III: Parasitized and received intradermal bee sting daily (Suppressive test i.e.
64 treatment with bee sting before infection).
- 65 • Group IV: Parasitized and received intradermal bee sting daily (Curative test i.e. treatment
66 commenced after 72 hours post infection) and
- 67 • Group V: – Not parasitized and not treated.

68

69 Treatment was withheld for 72 hours except group III, to allow the establishment of infection.
70 Blood films were made from the tail of individual mouse fixed with methanol and stained with
71 Giemsa stain, according to the method of Ryley and Peters (17). On the fifth day mice were
72 anaesthetized with chloroform and the blood and tissue (liver) were collected into sample tubes

73 **Preparation of Serum and Tissue Homogenate**

74 Blood was collected from each mouse into a sterile centrifuge tube and centrifuged at 12000rpm
75 for 5min at room temperature to obtain the serum. For the liver, 1.0g of the liver was
76 homogenized in ice-cold 4.0 ml of freshly prepared 0.25 M sucrose solution (1:4 w/v) and
77 centrifuged for 15min at 3000rpm. The homogenates were collected and stored for further
78 analysis.

79 **ENZYME ANALYSIS**

80 Enzyme activities in serum and liver samples were carried out using commercially available kits
81 of RANDOX laboratories Diagnostic(Randox, UK). Alkaline phosphatase (ALP) activity was
82 estimated by the method of Tietz *et al.* (18), Aspartate transaminase (AST) and alanine
83 transaminase (ALT) activities were assayed using the method described by Reitman and
84 Frankel(19). Total protein concentration was determined using Biuret method described by

Gornallet *al* (20) as modified by Plummer (21). Gamma GlutamylTransferase (GGT) was measured by the method of Szasz and Klin. (22).

Statistical Analysis

Values are Spresented as mean $\pm$ Standard Error of Mean (SEM). The data were statistically analyzed using one-way analysis of variance (ANOVA) and Duncan multiple range test (23). Data from the treated or test groups were compared with their respective controls and differences at $p < 0.05$ were considered significant.

RESULTS

Aspartate Transaminase(AST)

The results of serum and liver specific activities of Aspartate transaminase(AST) are represented in figure 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 significantincrease ($p < 0.05$) in the standard treated when compared withother experimental groups.

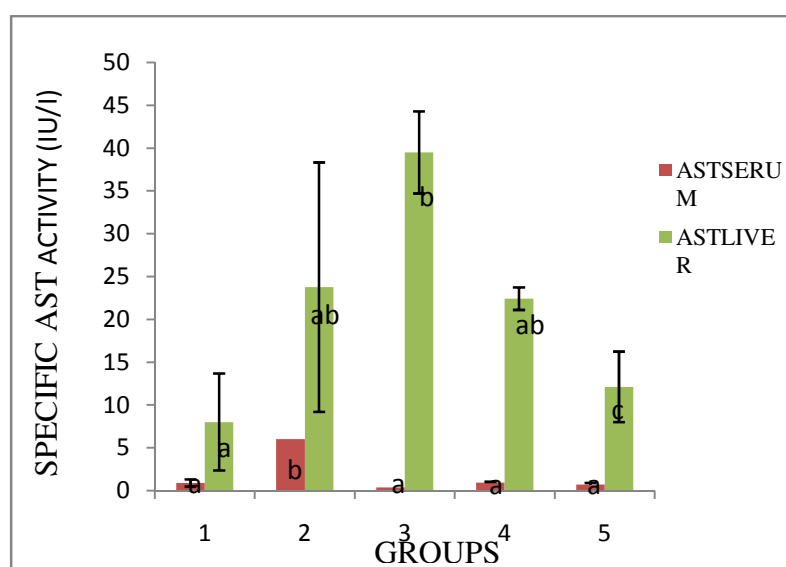


Figure 1: Specific activities of Aspartate transaminase (AST) in liver and serum of mice infected *Plasmodium berghei*. Results are mean of five determinations $\pm$ SEM. Bars carrying different letters are significantly different at $p < 0.05$.

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

Alanine transaminase(ALT)

The results of serum and liver specific activities of Alanine transaminase(ALT) are represented in figure 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 the serum specific ALT activities showed significant increase ($p < 0.05$) in standard treated when compared with the control group which was not significantly different from curative group.

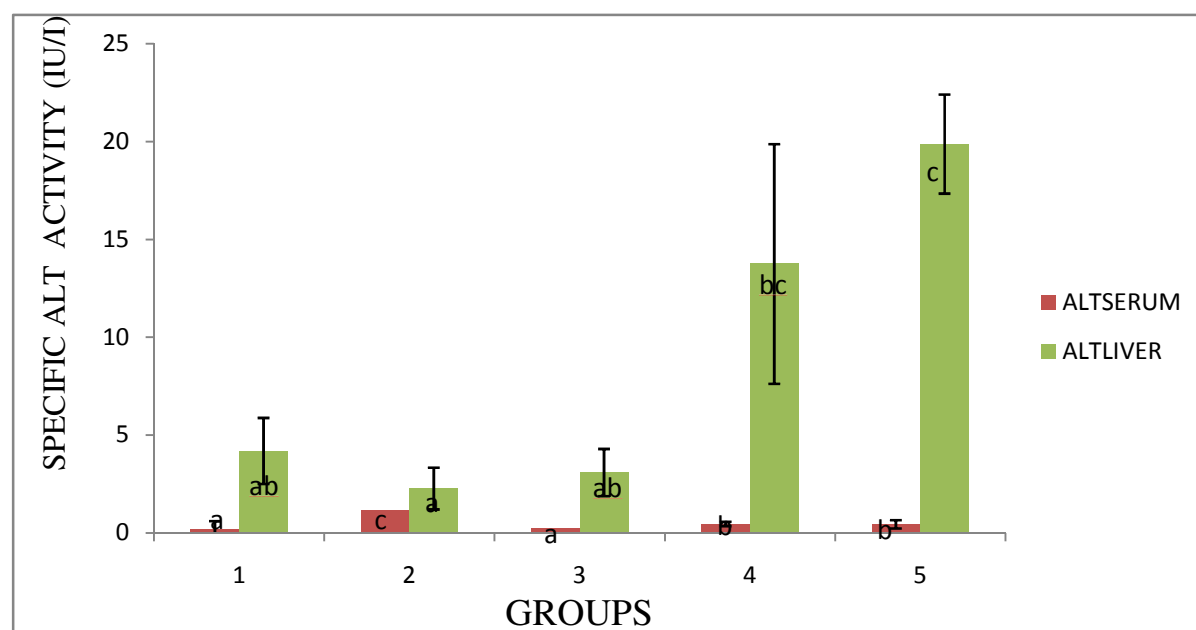


Figure 2: Specific activities of Alanine transaminase (ALT) activities in liver and serum of mice infected with *Plasmodium berghei*. Results are mean of five determinations $\pm$ sem. Bars carrying different letters are significantly different at $p < 0.05$

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

Gamma Glutamyltransferase (GGT)

The results of figure one (3) showed that there were significant increase ($p<0.05$) in the specific activity of gamma glutamyltransferase (GGT) in the liver of parasitized non-treated (PNT) mice compared with other experimental groups. And no significant difference ($p>0.05$) in the serum and liver specific activities of gamma glutamyltransferase (GGT) for suppressive and curative group when compared to parasitized treated with chloroquine (PTSTD). (Figure 3)

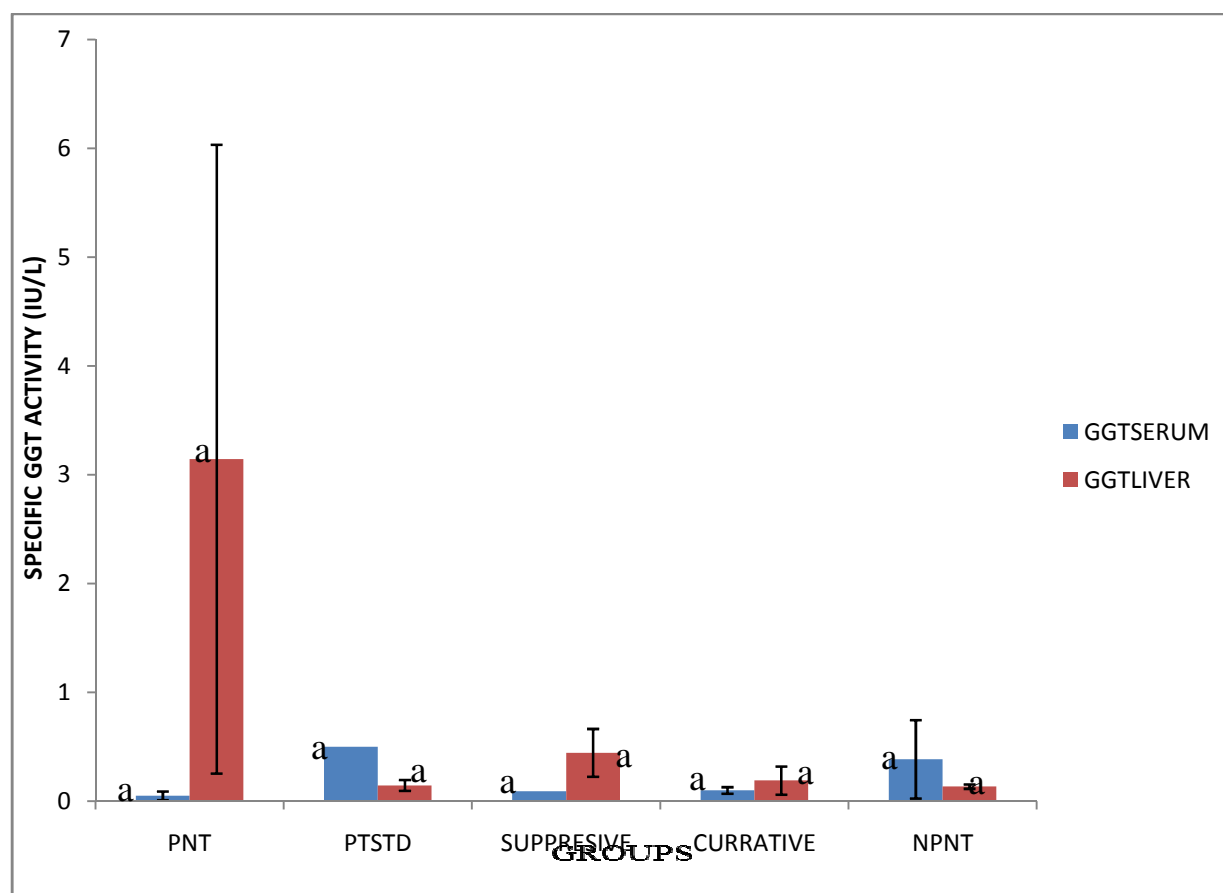


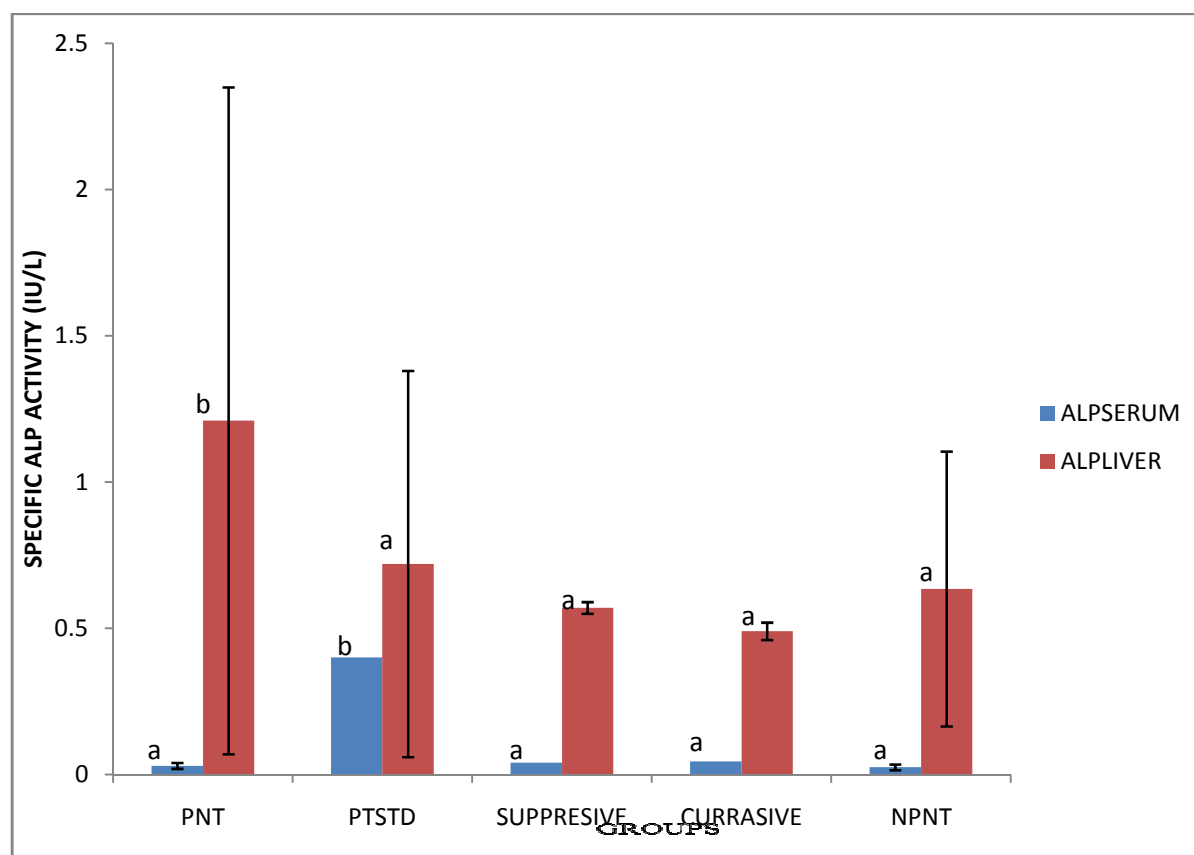
Figure 3: Changes in serum and liver Gamma glutamyltransferase (GGT) levels in *P. berghei* infected mice treated with bee sting.

129 PNT: Parasitized untreated, PTSTD: Parasitized treated with chloroquine, Group 3 –Suppressive
 130 treated; Group 4 – Curative treated; NPNT: non-parasitized non-treated. Mean $\pm$ SEM duplicate
 131 determinations. Mean values with the difference letter are significantly different ($p < 0.05$).

132 Alkaline Phosphatase

133 Figure four (4) shows significant increased ($p < 0.05$) in the specific activity of alkaline
 134 phosphatase (ALP) in the liver of Parasitized non-treated (PNT) mice compared with other
 135 experimental groups. Whereas there was no significant difference ($p > 0.05$) in the liver ALP of
 136 the Parasitized treated with chloroquine (PTSTD), suppressive and curative groups compared to
 137 reference group (PTSTD).

138



139

140 Figure 4: Changes in serum and liver alkaline phosphatase (ALP) levels in *P. berghei* infected
 141 mice treated with bee sting.

142 PNT: Parasitized non-treated, PTSTD: Parasitized treated with chloroquine, Group 3 –
 143 Suppressive treated; Group 4 – Curative treated; NPNT: non-parasitized non-treated. Mean $\pm$
 144 SEM duplicate determinations. Mean values with the difference letter are significantly different
 145 ($p < 0.05$).

146 DISCUSSION

147 Liver dysfunction can affect the body metabolic processes due to the role of the liver in overall
 148 metabolism of the organism. Enzymes are necessary for normal cellular metabolism including
 149 that of the liver (24). The progressive changes in the hepatocytes due to the infection of
 150 *Plasmodium* may alter the activities of the enzymes, therefore rapid increase or decrease in
 151 serum and liver biomarkers enzymes, indicate damage to specific organ. Hence, changes in
 152 enzyme activities are used as indicators of hepatic damage (25, 26, 27).

153 Transaminases are responsible for amine transfer in the body metabolism. Aspartate
 154 transaminase (AST) is a peridoxal phosphate dependent transaminase enzyme. It catalyzes a
 155 reversible transfer of an amino group between aspartate and glutamate and therefore it is an
 156 important enzyme in amino acid metabolism. Alanine transaminase (ALT) catalyzes two parts of
 157 alanine cycle; it catalyzes the transfer of an amino group from L- alanine to α - ketoglutarate(28).
 158 In this study, there was significant ($p < 0.05$) increase in serum AST activities of the
 159 chloroquinetreated when compared tonotparasitized not treated group. Also, there was significant
 160 increase in serum ALT activities of the chloroquine treated when compared with parasitized
 161 untreated group; this agrees with other studied that showed chloroquine to affect a wide range of
 162 biochemical processes including inhibition of key metabolic enzymes (24). Liver AST activities
 163 of chloroquine treated, suppressive and curative treated groups increased when compared with
 164 the non-parasitized non-treated group, these may be attributed to induction of the enzyme in the
 165 presence of reactive metabolite from the treatment. The significant decrease in the level of liver
 166 ALT activities observed in infected not treated, standard treated and suppressive groups when

167 compared to the normal group probably confirms to the earlier report that infective condition
168 gradually affect enzyme level (29). However, treatment with bee sting after establishment of the
169 parasite tends to restore the level of liver ALT towards the normal value, thereby reflecting the
170 efficacy of bee stings in the treatment.

171 The result in figure 3 shows increase in liver GGT and ALP of parasitized non-treated (PNT)
172 group compared to other groups, which imply induction of the enzyme in the liver without
173 cellular necrosis that causes leakage from the liver into bloodstream which may eventually
174 increase serum GGT level and this was in agreement with the report of Sowjanya *et al.* and
175 Olorunnisola *et al* (30, 31).

176 The suppressive and curative groups show that intradermal administration of bee sting
177 significantly reduced in liver GGT and ALP which may be as a result of stings constituents. Bee
178 venoms have been reported as potent inhibitors of the in vivo intraerythrocytic development of
179 *plasmodium* (32).

180 The observed increased serum ALP in group treated with chloroquine (group II) compared with
181 other groups (figure 4) shows normal circumstance due to body response to a variety of drugs.
182 Also confirm previous studies that shown that chloroquine affects a wide range of biochemical
183 processes including inhibition of key metabolic enzymes such as alcohol dehydrogenase,
184 succinate dehydrogenase and glucose-6-phosphate dehydrogenase. (24, 33).

185 The normalization of liver and serum alkaline phosphatase (ALP) and
186 gammaglutamyltransferase (GGT) of the suppressive and curative groups suggests that bee sting
187 is able to change the condition of the hepatocytes so as to protect the membrane integrity against
188 *P. berghei*. Thus, the result infers a protective effect of bee sting on impaired function caused by
189 *P. berghei*.

190 CONCLUSION

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

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