

ANTI PLASMODIAL ACTIVITY OF BEE STING IN *PLASMODIUM BERGHEI* INFECTED MICE

ABSTRACT

Aim: Based on traditional claims and practice, the antiplasmodial activity of bee stings and its effect on haematological indices was investigated in *P. berghei*, infected mice.

Methodology: Twenty albino mice were intraperitoneally infected with chloroquine sensitive *P. berghei* stain and divided into four groups. Group I was set up as negative control of 0.2ml normal Saline/kg body weight, group II as 5mg chloroquine/kg body weight, group III as suppressive (suppressive) treated and group IV as curative (curative). The thin blood smear was used to determine the parasiteamia counts and the haematological parameters was estimated on day 7.

Results: The result of percentage chemosuppression shows that bee stings suppress the parasitaemia to 56.6%. Also, the suppressive and curative group shows longer mean survival period of 15.0 and 20.0 respectively. The haematological studies show that the level of packed cell volume (PCV) and haemoglobin concentration (HB) of infected untreated group was significantly ($p < 0.05$) lower when compare with all other experimental group, whereas chloroquine treated group shows significantly increase compared to the bee treated groups. The Red blood cell (R.B.C.) counts was significantly ($p < 0.05$) lowered in infected untreated group when compare with suppressive and chloroquine treated groups. However the white blood cell (WBC) counts was significantly ($p < 0.05$) higher in infected bee sting treated when compare to the infected untreated and infected chloroquine treated groups.

Conclusion: Based on the result obtained, this study confirms the antiplasmodial activity of bee stings and suggests its potential as drug agent or lead against malaria.

Keywords: Malaria, *Plasmodium berghei*, Bee Sting, Antiplasmodial

INTRODUCTION

Malaria is important cause of mortality and illness in tropical countries. The increase in resistance of the malaria parasite to the currently available antimalaria drug results in death of over millions of people over the past 30 years (Gessler *et al.*, 1995). For many years, traditional medicine have been use for malaria treatment and they are the source of artemisinin and quinine derivatives. They are of plant source and are effectively used in malaria treatment (Bodeker and Willcox, 2000). The high cost and difficulties encountered by the rural settlers in obtaining these drugs make them go for available traditional medicine such as herb traditional medicine. Which are sometimes perceived as being more effective than conventional antimalaria drugs including artemisinin combination therapy (ACT). In view of the problem associated with antimalaria drug resistance, new drug or drug combination are urgently required for the treatment of malaria. Preferably, the new drugs should have novel mechanism of action or be chemically different from drug in current use (Tijani *et al.*, 2008).

Bee sting is obtained from honey bee, class insect. It is believes that the venom release by bee during stings boost the immune system's in order to fight many disease including arthritis, malaria and so on. Bee sting are painful and are therefore avoided by many people. The bees die soon after it has stung and release its venom (Luciano *et al.*, 2002). These is due to the destruction of it abdomen, digestive tract, muscle and nerve. Mellitin is the most important and abundant component of bee sting (about 52%) and it act as strong anti-inflammatory agent that induce production of cortisol in the body (Meier and White, 1995). In vitro intra erythrocytic development of malaria parasite is strongly inhibited by phospholipase A₂ from bee sting. This inhibition depends on enzyme activities that require the presence of serum lipoprotein in the parasitic culture medium (Luciano *et al.*, 2002). Bee sting has been reported traditionally for antimalarial activities but scientific information is limited or absent on the therapeutic efficacy and safety for clinical application. Therefore, in this study the anti plasmodial activities of bee sting in *P. berghei* infected mice was investigated.

MATERIAL AND METHOD

Experimental animals

58 Swiss adult albino mice weighing 20-24g were obtained from animals breeding unit of the
59 Department of Biochemistry, University of Ibadan, Oyo State. They were housed in plastic cages
60 and  maintained under standard laboratory conditions.

61 **Parasite**

62 Chloroquine – sensitive strain of *Plasmodium berghei* (NK-65) was obtained from the National
63 Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria.

64 **Honey bee**

65 Life healthy honey bees were obtained from bee hive in Bosso Dam  nna, Niger state. They
66 were house in wooden cage and were constantly given honey and fruit.

67 **Animals Grouping:**

68 The mice were group into four (4) groups of four (4) mice each. The group were labeled group 1
69 (infected not treated), group 2 (infected treated with standard), group 3 (infected suppressive
70 treated) and group4 (infected curative treated).

71 **Innocation of parasite:**

72 A donor mouse with plasmodium infected erythrocyte was anathesized and the blood was
73 immediately diluted with normal saline and passage to healthy mice with 0.2ml of the *P.*  ghei
74 parasite intraperitoneally.

75 **Parasitaemia count**

76 The method of Giemsa blood smear was use to count the number of the parasite in the blood.
77 The blood was taken from the tail of mice and prepared by thin and thick smeared blood method
78 with Giemsa colouring and the number of parasitemia was calculated by determining the average
79 of red blood cells infected by *Plasmodium berghei*.

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81 **Treatment**

82 The treatments were given to the experimental mice as follows:

83 **Group 1:** infected and received 0.2ml of normal saline daily.

84 **Group 2:** infected and received chloroquine (5mg) per kg body weight.

85 **Group 3:** infected and received intradermal bee sting daily (Suppressive test i.e. treatment with
86 bee sting before infection).



87 **Group 4:** infected and received intradermal bee sting daily (Curative test i.e. treatment for 72
88 hours after infection, with bee sting) and

89 **Group 5:** Uninfected Untreated.

90 **Suppressive test**

91 Treatment was withheld for 72 hours except group III to allow the establishment of infection.
92 Four (4) days Suppressive test against chloroquine sensitive *P. berghei* infected mice were done
93 according to the methods Knight and Peters (1980). Group 1 act as the negative control and
94 received 0.2ml of normal saline daily, Group 2 serve as the positive control and received 0.2ml
95 of 5mg chloroquine/kg body weight daily and Group 3 received intradermal bee sting daily. A
96 drop of blood were collected from the tail of the mice and smeared on to the slide to make thick
97 film. The film were stained with Giemsa and view microscopically to determine the average
98 percentage (%) parasitemia

99 Average % suppression =

100
$$\frac{\text{Average parasitemia of infected untreated} - \text{Average parasitemia of infected treated}}{\text{Average parasitemia of infected untreated}} \times 100$$

101
$$\frac{\text{Average parasitemia of infected untreated} - \text{Average parasitemia of infected treated}}{\text{Average parasitemia of infected untreated}} \times 100$$

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103 **Curative Test**

104 There are three groups for the experiment. Group1 (negative control) received 0.2ml of normal
105 saline daily, group 2 (positive control) received 0.2ml of 5 mg chloroquine/kg body weight and
106 group 3 received intradermal bee sting daily for 5 days. The treatment commenced after 72hrs
107 of *P.berghei* infected. On each day, drops of blood were collected from the tail each mouse

108 smeared on the slide to make a thick film. The film were stained with Giemsa stain and view
109 microscopically at magnification of x40

110 **Mean Survival Time**

111 The mean survival time of the mice in each group over a period of 28 days inoculations (Do –
112 D28) was calculated .The death occurring within this period are noted and recorded. The blood
113 smear of the mice that survive beyond 28 days was also monitored.

114 **Hematological Parameters**

115 Automated haematologic analyzer SYSMEX KX21, a product of SYSMEX Corporation,
116 Japan, was used to determine the haematological indices including the packed cell volume
117 (PCV), red blood cell count (RBC), white blood cell count (WBC) and Haemoglobin
118 concentration (Hb) as described by **Dacie and Le** (1991).

120 **Ethical Clearance**

121 Ethical Clearance was given by Federal University of Technology, Minna/Nigerian Ethical
122 Review Board (CUERB) in accordance with International standard on care and use of
123 experimental animal

124 **Consent**

125 Not Applicable

126 **Competing of Interests**

127 Authors have declared no competing interest.

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132 Statistical analysis

133 The data obtained were subjected to Analysis of Variance (ANOVA) using SAS statistical
 134 package. Means were separated using Duncan's Multiple Range Test (DMRT). Significance
 135 was accepted at $P < 0.05$. The data is given as mean $\pm$ SEM.

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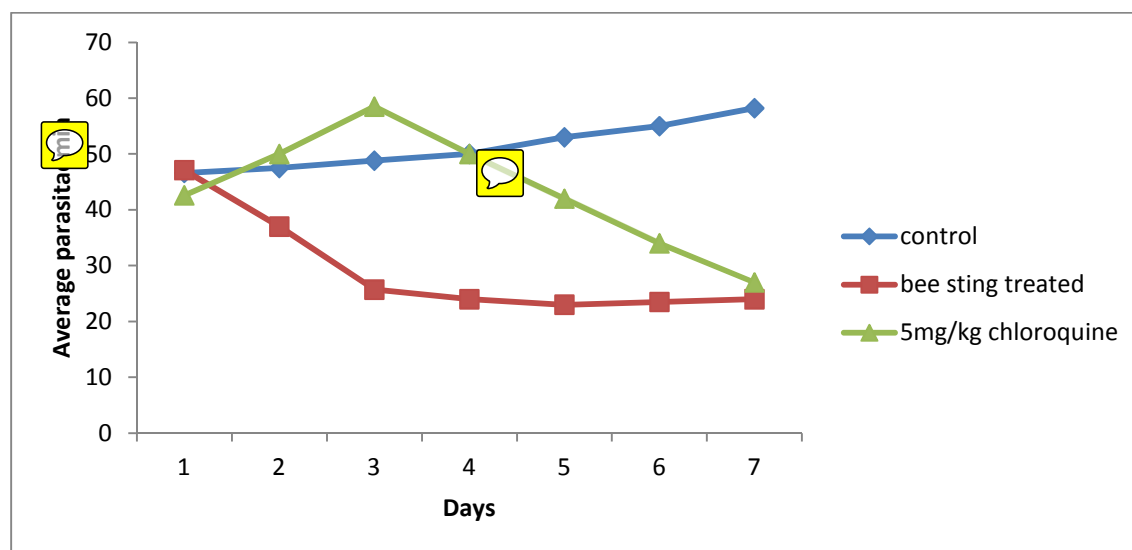
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139 RESULTS

140 CURATIVE

141 The average parasitemia of the infected untreated group, infected treated with 5mg of
 142 chloroquine and the bee sting, early treated group mice (prophylactic) are shown in figure 1. The
 143 negative control group (infected untreated) show a gradual increase in the parasitemia count
 144 when compare to the bee sting treated group which show decrease in the level of parasitaemia up
 145 to day 7. The chloroquine treated group on the other hand show gradual increase in the
 146 parasitaemia till day 3 when thereafter continue to decline.



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148 **Figure 1:** **In-vivo** Antiplasmodial Activity of Honey bee sting Against *P. berghei* infected
 149 mice

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SUPPRESSIVE

The percentage chemosuppression by bee sting and chloroquine treated group is shown in Table 1. The percentage chemosuppression by bee stung and chloroquine was 56.60 and 67.90 respectively.

Table 1 Percentage Chemosuppression by Bee sting and Chloroquine

Treatment	Parasitemia Count	% Supression
Infected Not Treated	53.00 ± 0.00	-
Infected Treated With chloroquine	17.00 ± 0.20	67.90
Bee Sting Treatment	23.10 ± 0.10	56.60

Mean survival period (M.S.P)

The mean survival period of all the experimental group are shown in the Table 2. The infected untreated group shows the lowest M.S.P of 12 days while the infected treated with standard ,suppressive and curative show survival days of 17, 15 and 20 days respectively.

Table 2 : Average Mean Survival Period of the Experimental

GROUP	SURVIVAL DAYS
Infected 0.2ml of normal saline	12.0
Infected 0.2ml of chloroquine	17.0
Infected bee stings suppressive	15.0

173 Haematological parameters

174 The results of haematological studies of *P. berghei* infected mice are presented in Table 3. The
 175 level of PCV and HB of infected untreated group was significantly ($P<0.05$) lower when
 176 compared with all other experimental group and chloroquine treated group shows significantly
 177 increase when compared to the bee treated group. The R.B.C count was significantly ($p<0.05$)
 178 lowered in infected untreated group when compare with suppressive, curative and chloroquine
 179 treated groups. However the WBC counts was significantly ($p<0.05$) higher in infected bee sting
 180 treated groups when compare to the infected untreated and infected chloroquine treated mice.

181 **Table 3: Effects of Bee Stings on Haematological parameter of *P. berghei* infected mice**

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	PCV(%)	Hb(g/dL)	RBC($\times 10^6$ cells/ μ L)	WBC($\times 10^3$ cells/ μ L)
INT	11.70 $\pm$ 1.05 ^a	3.90 $\pm$ 0.55 ^a	6.90 $\pm$ 0.61 ^a	9.02 $\pm$ 1.23 ^b
IQT	25.23 $\pm$ 2.10 ^c	8.41 $\pm$ 0.70 ^c	7.80 $\pm$ 0.34 ^b	7.95 $\pm$ 0.96 ^a
IST	22.61 $\pm$ 2.68 ^b	7.53 $\pm$ 0.88 ^b	6.50 $\pm$ 0.56 ^a	10.21 $\pm$ 1.04 ^c
ICT	23.11 $\pm$ 3.01 ^b	7.70 $\pm$ 0.14 ^b	12.01 $\pm$ 1.98 ^c	10.09 $\pm$ 1.21 ^c

183 Values are expressed as Mean $\pm$ SEM. Each mean is an average of four replicate (n=4). Values
 184 with different superscript are significantly different ($p<0.05$). INT: infected not treated, IQT:
 185 infected chloroquine treated, IST: suppressive, ICT: curative.

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187 DISCUSSION

188 Many studies have been carried out in recent years on the pharmacological effects of bee
 189 products such as honey, propolis, bee pollen and bee venom (Gheldof and Engeseth, 2002;
 190 Taormina *et al.*, 2001). They have been reported to have analgesic, antimicrobial, anti-
 191 inflammatory and immunological benefits (Tonks *et al.*, 2007). But little or no scientific data
 192 exist to validate the activities of traditional medicine of bee stings against malaria. It is therefore
 193 important that their claimed for antimalaria activities are investigated in order to establish and their
 194 efficacy and determine the potential as a source of new antimalaria drug.

Some studies have reported that bee sting poses some therapeutic properties such as boosters of the immune system's ability to fight many disease including arthritics and so on. The most destructive component of bee sting has been reported has phospholipase A2, the enzyme that inhibit the intraerythrocytic development of malaria parasite (Luciano *et al.*, 2002).

The result obtained from this study shows significant decrease in parasitaemia of both the chloroquine and the bee sting treated groups compare to the negative control group over the period of the experiment is an indication that bee sting have positive activity against *Plasmodium berghei* infection. Although, the initial increase in the parasitaemia observed in the bee sting prophylactic group may be attributed to the accumulation of the venom before it inhibit the development of the parasite. The longer mean survival period observed in the bee sting treated group compare to the negative control group is an additional indication of the bee sting efficacy as an antimalaria agent.

Several changes in the hematological parameters like anemia, thrombocytopenia and leukocytosis have been documented in malaria infection (Fleming, 1981). One of the major reasons for the development of anemia was reported to be oxidative stress (Kremsner *et al.*, 2000). Reactive oxygen species are produce by the body during the activation of immune system there by resulting in haemoglobin degradation (Loria *et al.*, 1999). Therefore, haematological parameters were always used to assess the effect of malarial parasite on blood component.

The significant decrease in the level of PCV of the infected untreated group is an indication of anemic condition caused by the malarial infection. However, the intradermal administrations of bee stings increase the decrease in the packed cell volume level. Thereby, indicating the ameliorative potentials of bee sting on effect caused by malaria on haematological parameters. White blood cell (WBC) play active role in the immune system. Thus, the significant increase in the WBC counts in the bee sting treated group when compare with other experimental groups suggest that bee sting stimulate immune response. Therefore, from the result obtained, it can be concluded that bee sting component has potential in antimalarial drugs and bave different mechanism of action or target differ from chloroquine.

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CONCLUSION

226 In conclusion, the result of this study indicate that bee sting possess some antimalaria property
 227 which is  by significant plasmodial parasite clearance compared to chloroquine and
 228 ameliorative effect on symptoms cause by the infection.

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