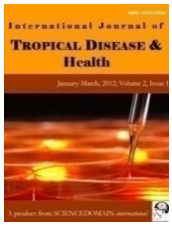

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Anti-plasmodial Activity of Bee Sting in *Plasmodium berghei* Infected Mice

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Authors' contributions

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

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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: Sixteen albino mice were intraperitoneally infected with chloroquine sensitive *P. berghei* strain and divided into four groups each consisted of four animals. Group I was set up as negative control of 0.2 ml normal Saline/kg body weight, group II as 5 mg chloroquine/kg body weight, group III had suppressive treatment and group IV was administered curative treatment. The thin blood smear was used to determine the parasiteamia counts and the haematological parameters were 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 groups show 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 groups, where as chloroquine treated

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group shows significant 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: Anti-plasmodial; bee sting; P. berghei; Mice.

1. INTRODUCTION

Malaria is important cause of morbidity and mortality in tropical countries. The increase in resistance of the malaria parasite to the currently available antimalarial drugs result in the death of millions of people over the past 30 years [1]. For many years, traditional medicines have been used for treatment of malaria and they are the source of artemissinin and quinine derivatives. They are of plant source and are effectively used in malaria treatment [2]. The high cost and difficulties encountered by the rural settlers in obtaining these drugs make them go for available traditional medicine such as herbal remedy which are sometimes perceived as being more effective than conventional antimalaria drugs including artemissinin 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 [3].

Bee (*Apis melliferal*) sting is obtained from the tip of the abdomen of honey bee of the class insect. It is believes that the venom releases 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 bee dies soon after it has stung and released its venom [4]. This 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 acts as strong anti-inflammatory agent that induce production of cortisol in the body [5]. *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 [4]. Bee sting has been reported traditionally for antimalaria 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.

2. MATERIALS AND METHODS

2.1 Experimental Animals

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

2.2 Parasite

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

2.3 Honey Bee

Life healthy honey bees were obtained from bee hive in Bosso Dam, Minna, Niger state. They were house in wooden cage and were constantly given honey and fruits.

2.4 Animals Grouping

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

2.5 Innoculation of Parasite

A donor mouse which had been infected with *Plasmodium berghei* was anesthetized and the blood was immediately diluted with normal saline and passage intraperitoneally to healthy mice

with 0.2 ml of the blood containing about 1 x 10^7 parasitized red blood cells.

2.6 Parasitaemia Count

The method of Giemsa blood smear was used to count the number of the parasite red blood cells. The blood was taken from the tail of mice and thin blood smear was prepared using Giemsa stain and the parasitemia was calculated by determining the average of red blood cells infected divided by total red blood cells times 100.

2.7 Treatment

The experimental mice were treated as follows:

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

Group 2: infected and received 5 mg per kg body weight chloroquine intraperitoneally (treatment commenced after 72 hours infection).

Group 3: Infected and received intradermal bee sting daily (Suppressive test: this was done according to the methods of [6]. The treatment commenced immediately after infecting the animals and the parasitaemia was determined from drop of blood collected from the tail of the mice for smeared on to the slide to make thin film. The film were then stained with Giemsa and view microscopically at magnification of x 100 to determine the average percentage (%) parasitemia.

Average % suppression =

[(Average % parasitemia of infected untreated - Average % parasitemia of infected treated x 100)/ Average parasitemia of infected untreated]

Group 4: Infected and received intradermal bee sting daily for 5 days. (Curative test i.e. treatment commenced after 72 hours of *P. berghei* infection. On each day, drops of blood were collected from the tail each mouse smeared on the slide to make a thin film. The film were stained with Giemsa stain and view microscopically at magnification of x 100.

2.8 Mean Survival Time

The mean survival time of the mice in each group over a period of 28 days inoculations (Do – D28) was calculated. The deaths occurring within this period were recorded. The blood smear of the mice that survive beyond 28 days was also monitored. The Mean Survival Time (MST) for each group was calculated as

MST = [(Sum of survival time of all mice in a group (days)/ Total number of mice in that group]

2.9 Hematological Parameters

Automated haematologic analyzer SYSMEX KX21, a product of SYSMEX Corporation, Japan, was used to determine the haematological indices including the packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC) and Haemoglobin concentration (Hb) as described by [7].

2.10 Ethical Clearance

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

2.11 Statistical Analysis

The data obtained were subjected to Analysis of Variance (ANOVA) using statistical package (SPSS) version 16. Means were separated using Duncan's Multiple Range Test (DMRT). Significance was accepted at $P < 0.05$. The data is given as mean±SEM.

3. RESULTS

3.1 Curative

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

3.2 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	Parasitaemia count	% suppression
Infected not treated	53.00±0.00	-
Infected chloroquine treated	17.00±0.20	67.90
Infected suppressive treated	23.10±0.10	56.60

Values are expressed as Mean ±SEM.

3.3 Mean survival Period (M.S.P)

The mean survival period of all the experimental groups 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.

3.4 Haematological Parameters

The results of haematological studies of *P. berghei* infected mice are presented in Table 3. The level of PCV and HB of infected untreated group was significantly (11.70±1.05 and 3.90±0.55) lower when compare with all other experimental group and chloroquine treated group shows significant ($p<0.05$) increase when compared to the bee treated group. The R.B.C count was significantly ($p<0.05$) lowered in infected untreated group when compare with curative and chloroquine treated groups. However the WBC counts was significantly ($p<0.05$) higher in infected bee sting treated groups when compare to the infected untreated and infected chloroquine treated mice.

Table 2. Average Mean survival period of *Plasmodium berghei* infected mice treated with chloroquine and bee sting

Group	Survival days
Infected 0.2 ml of normal saline	12.0
Infected 0.2 ml of chloroquine	17.0
Infected suppressive treated	15.0
Infected curative treated	20.0

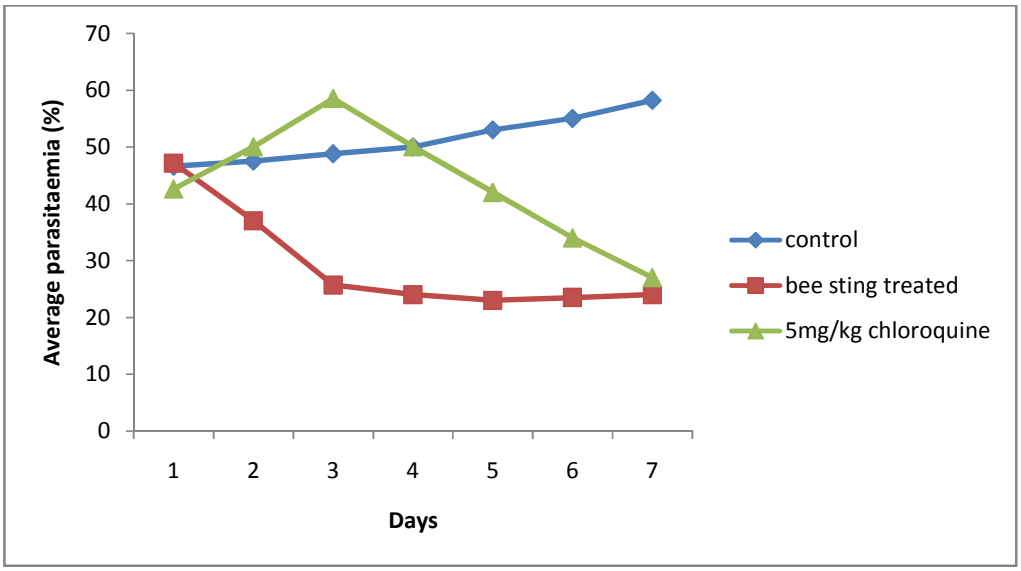

Fig. 1. Changes in *in vivo* anti-plasmodial activity of honey bee sting against *P. berghei* infected mice

Table 3. Effects of bee stings on haematological parameter of *P. berghei* infected mice

	PCV (%)	Hb (g/dL)	RBC (x10^6 cells/µL)	WBC (x10^3 cells/µL)
INT	11.70±1.05[a]	3.90±0.55[a]	6.90±0.61[a]	9.02±1.23[b]
IQT	25.23±2.10[c]	8.41±0.70[c]	7.80±0.34[b]	7.95±0.96[a]
IST	22.61±2.68[b]	7.53±0.88[b]	6.50±0.56[a]	10.21±1.04[c]
ICT	23.11±3.01[b]	7.70±0.14[b]	12.01±1.98[c]	10.09±1.21[c]

Values are expressed as Mean ±SEM. Each mean is an average of four replicate (n=4).Values with different superscript (a, b, or c) are significantly different (p<0.05). INT: infected not treated, IQT: infected chloroquine treated, IST: infected suppressive treated, ICT: infected curative treated.

4. DISCUSSION

Many studies have been carried out in recent years on the pharmacological effects of bee products such as honey, propolis, bee pollen and bee venom [8,9]. They have been reported to have analgesic, antimicrobial, anti-inflammatory and immunological benefits [10]. But little or no scientific data exist to validate the activities of traditional medicine of bee stings against malaria. Bhattacharya has reported that plasmodium parasites are susceptible to toxins and alcohol [11]. The current anti malarial drugs derived from plants or synthesized (such as chloroquine and artemisinin) are beset with the problem of resistance and toxicity from the use of single compounds. It has also been reported that the chloroquine resistance was as a result of a single point mutation in the transporter gene Pf*crt* [12] and that artemisinin resistance in *P. falciparum* has also been detected in Cambodia [13]. Therefore, it is important that their claimed for antimalaria activities are investigated in order to established their 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 as phospolipase A2, the enzyme that inhibit the intraerythrocytic development of malaria parasite [4].

The significant ($P<0.05$) decrease in parasitaemia of both the chloroquine and the bee sting treated groups compare to the negative control reported in this study could be attributed to the potency of bee sting against *Plasmodium berghei*. Although, the initial increase in the parasitaemia observe 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 further showed the efficacy of the bee sting as an antimalaria agent.

Haematological parameters like anaemia, thrombocytopenia and leukocytosis have been documented in malaria infection [14]. Reactive oxygen species are produce by the body during the activation of immune system there by resulting in haemoglobin degradation [15]. Therefore, haematologial 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 anaemic condition caused by the malarial infection. However, the infected suppressive and infected curative treated shows increase 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.

5. CONCLUSION

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

CONSENT

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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