

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

ABSTRACT

Aim: Based on the traditional and scientific claims of intra-dermal antiparasitic 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 glutamyltransferase (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: It can be concluded that bee sting have ameliorative effect against changes caused by *P. berghei*.

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

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, 3, and 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 250-500 million 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, 8, and 9). The parasite can be transmitted via infected female Anopheles mosquito bites, by inoculating sporozoites through 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, and 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 are investigated.

MATERIALS AND METHODS

Honeybee:

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

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*.

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 blood with isotonic saline was used to infect the experimental mice

intra~~peritoneally~~. Parasitaemia was checked by Giemsa stained thin blood smears through the tail vein of infected mice (17).

Experimental Design:

Using the method described by Datta (18). All groups except group 5 (normal control) were infected with *P. berghei* parasitized erythrocytes on D₀ and treated for 4 consecutive days.

- Group I: – Parasitized and received 0.2ml normal saline daily.
- Group II: Parasitized and received aqueous solution of chloroquine (5mg/kg body weight) daily.
- Group III: Parasitized and received intradermal bee sting daily (Suppressive test i.e. treatment with bee sting before infection).
- Group IV: Parasitized and received intradermal bee sting daily (Curative test i.e. treatment commenced after 72 hours post infection) 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.

Preparation of Serum and Tissue Homogenate

Blood was collected from each mouse into a sterile centrifuge plain tube and centrifuged at 2000rpm 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.

Enzyme Analysis

Enzyme activities in 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 Tietzet *al.* (20), Aspartate transaminase (AST) and alanine transaminase (ALT) activities was assayed using the method described by Reitman and Frankel (21). Total protein concentration was determined using Biuret method described by Gornallet *al* (22) as modified by Plummer (23). Gamma glutamyltransferase (GGT) was measured by the method of Szasz and Klin. (24).

Statistical Analysis

The data were statistically analyzed using one-way analysis of variance (ANOVA) and Duncan multiple range test (25). Data from the treated or test 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).

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 significant increase ($p < 0.05$) in the standard treated when compared with other experimental groups.

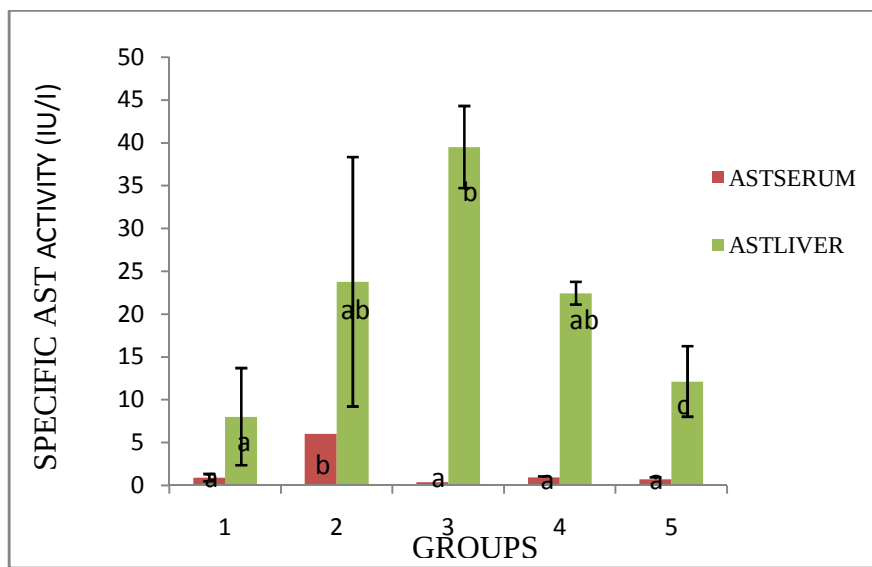


Figure 1: Specific activities of Aspartate transaminase (AST) in liver and serum of mice infected *Plasmodium berghei*. Results are mean of five determinations $\pm$ SEM. Bars carrying

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. 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.

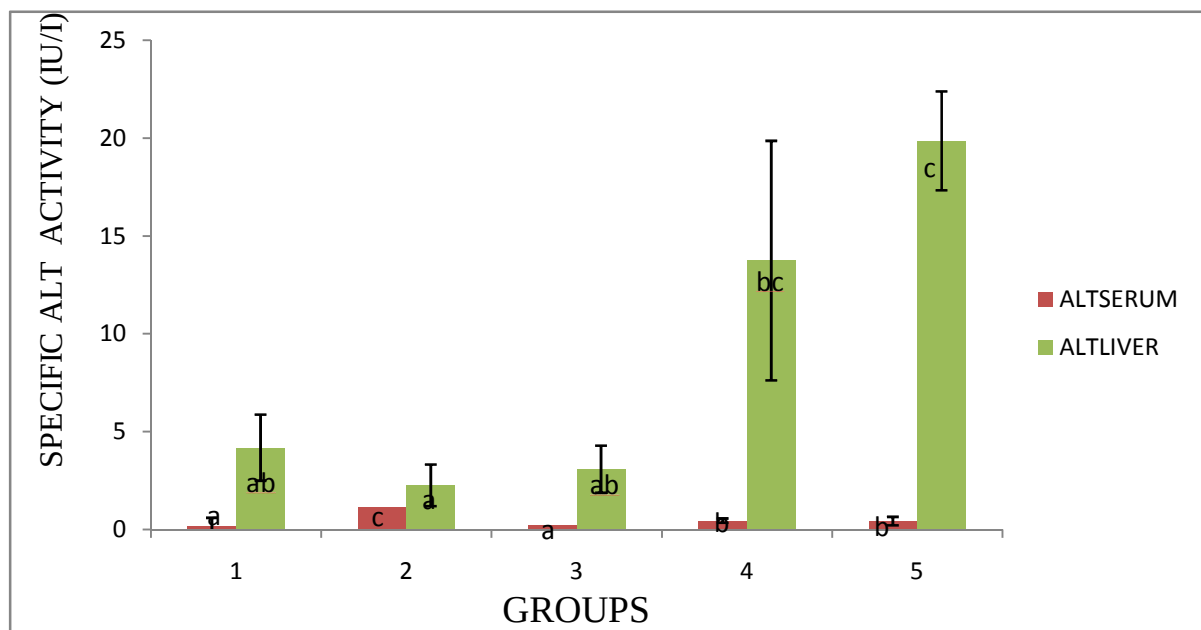


Figure 2: Specific activities of Alanine transaminase (ALT) activities in liver and serum of mice infected with *Plasmodium berghei*. Results are mean of five determinations $\pm$ sem. Bars carrying different letters are significantly different at $p < 0.05$

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 three (3) showed that there were significant increase ($p < 0.05$) in the specific activity of gamma glutamyltransferase (GGT) in the liver of parasitized untreated (PNT) group compared with other experimental groups. Whereas, 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). (Figure 3)

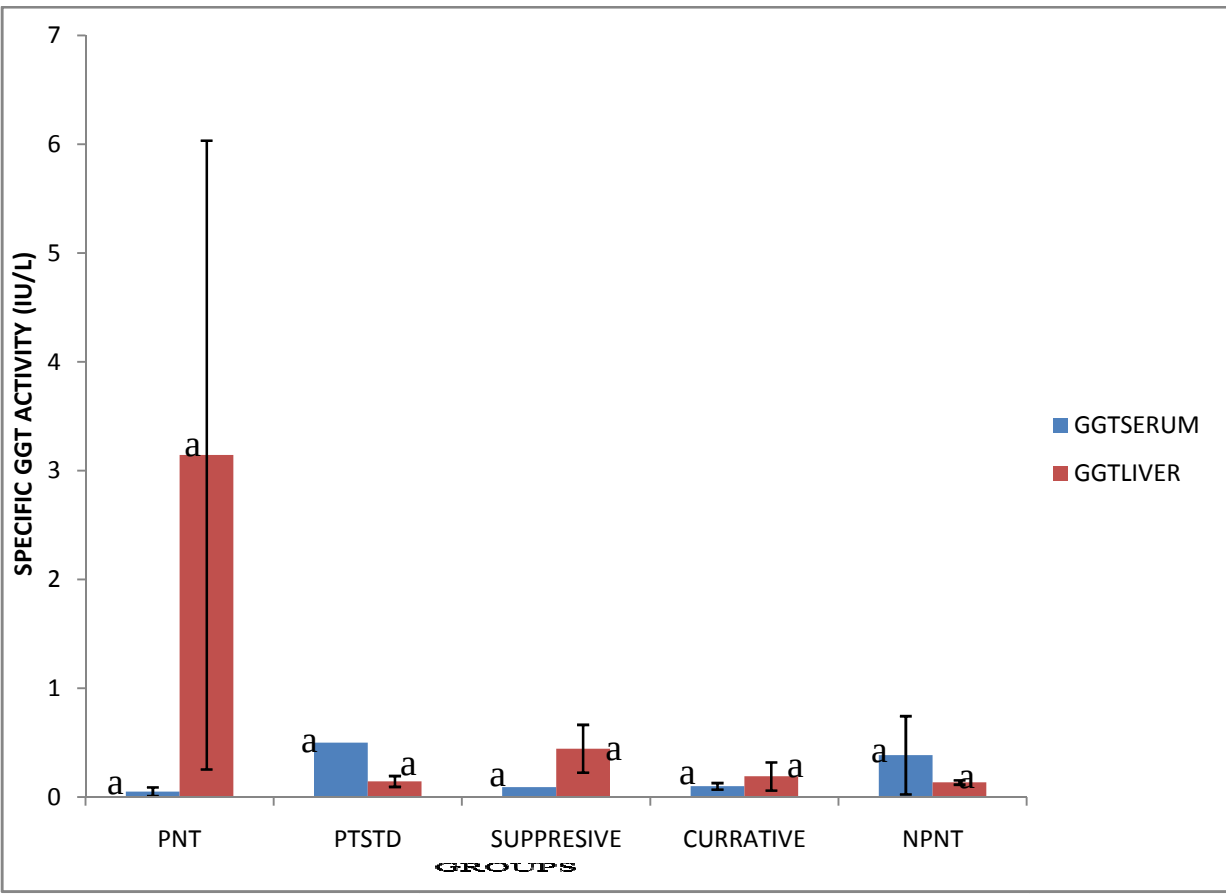


Figure 3: Changes in serum and liver Gamma glutamyltransferase (GGT) activities in *P. berghei* infected mice treated with bee sting.

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

Alkaline Phosphatase

Figure four (4) shows significant increased ($p < 0.05$) in the specific activity of alkaline phosphatase (ALP) in the liver of Parasitized untreated (PNT) group 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 reference group (PTSTD).

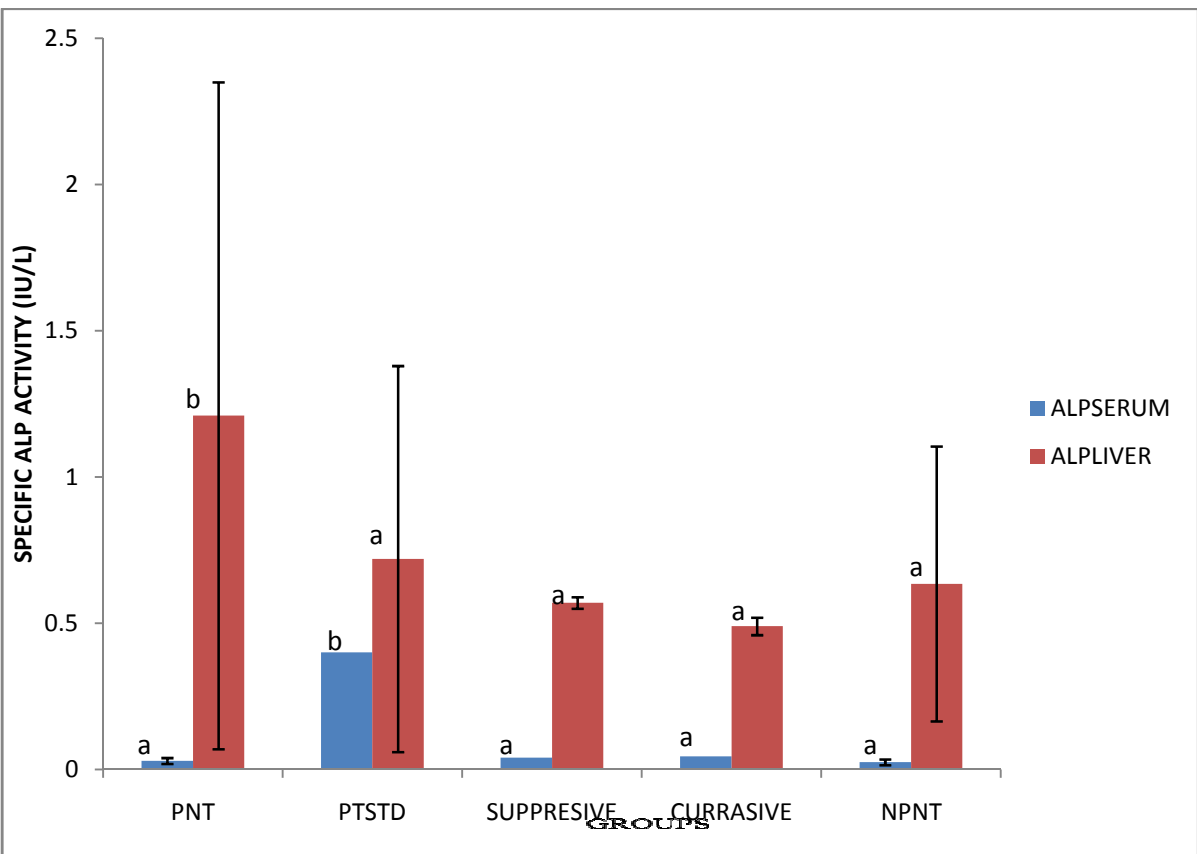


Figure 4: Changes in serum and liver alkaline phosphatase (ALP)activities in *P. berghei*infected mice treated with bee sting.

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

DISCUSSION

Liver dysfunction can affect the body metabolic processes due to the role of the liver in overall metabolism of the organism. Enzymes are necessary for normal cellular metabolism including that of the liver (24). The progressive changes in the hepatocytes due to the infection may alter the activities of the enzymes. Therefore, 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, 26, and 27).

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, there was significant ($p < 0.05$) increase in serum AST activities of the chloroquine treated when compared to not parasitized not treated group. Also, there was significant increase in serum ALT activities of the chloroquine treated when compared with parasitized untreated group. This agrees with previous studied that showed 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

observed in parasitized untreated, standard treated and suppressive groups when compared to the normal 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 tends to restore the level of liver ALT activity towards the value observed in control (normal) group, thereby reflecting the efficacy of bee stings in the treatment.

Figure 3 shows significant increase ($p < 0.05$) in liver GGT and ALP of parasitized untreated (PNT) group when compared to other groups. Which imply induction of the enzyme in the liver without cellular necrosis that causes leakage into bloodstream. This was in agreement with the report of Sowjanya *et al.* and Olorunnisola *et al.* (30, 31).

Bee venoms have been reported as potent inhibitors of the in vivo 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. The observed increased in serum ALP activities of the chloroquine treated group compared with other groups (figure 4) shows normal circumstance due to body response to a variety of drugs. Also 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 gamma glutamyl 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 function caused by *P. berghei*.

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 antimalarial drug.

192

193

REFERENCE

- 194 1. Adachi, K.,Tsutsui, H., Kashiwamura, S., Seki, E., Hiroki, N., Osamu, T.,
195 Kazuyoshi,T.,Okumura,K., Kaer, L.V., Okamura H., Akira, S. and Nakanishi,K.(2001).
196 *Plasmodium berghei* Infection in Mice Induces Liver Injury by an IL-12-and Toll-Like
197 Receptor/Myeloid Differentiation Factor 88-Dependent Mechanism. *Journal of*
198 *Immunology*; 167 (10): 5928-5934.
- 199 2. Steketee, R.W., Nahlen, B.L., Parise, M.E.,and Menendez, C. (2001): The burden Of
200 Malaria in pregnancy in malaria-endemic areas. *American Journal of Tropical Medicine*
201 *and Hygiene*, 64:28-35.
- 202 3. Alemu, A., Muluye, D., Mihret M., Adugna, M. and Gebeyaw, M. (2012).Ten year trend
203 analysis of malaria prevalence in Kola Diba, North Gondar, Northwest Ethiopia.
204 *Parasites and Vectors*, 5:173:5-173.
- 205 4. Breman, J.G (2013). Epidemiology, prevention, and control of malaria in endemic areas.
- 206 5. Uzuegbu, U.E., andEmeka, C.B.(2011). Changes in Liver Function Biomarkers among
207 Malaria Infected Patients. *Current Research Journal of Biological Sciences*. 3(3): 172-
208 174.
- 209 6. Ravikumar, S.,Inbaneson, J.S.,andSuganthi, P.(2012). In Vitro Anti *Plasmodial* Activity
210 of ethanolic Extracts of South Indian Medicinal Plants Against *Plasmodium Falciparum*.
211 *Asian Pacific Journal of Tropical Disease*. 623(409):180-183.
- 212 7. Nadjm, B.,and Behrens, R.H. (2012). Malaria: An update for physicians. *Infectious*
213 *Disease Clinics of North America*.26 (2): 243–259.
- 214 8. Nayyar, G.M.L.,Breman, J.G., Newton, P.N.,and Herrington, J.(2012).Poor quality
215 antimalarial drugs in Southeast Asia and sub-Saharan Africa. *Lancet Infectious*
216 *Diseases*12 (6): 488–496.
- 217 9. Wanga, S., Ghosha, A.K., Bongiob, N., Kevin, A., Stebbingsb, D.J.,andLampeb, M.
218 J.L.(2012). Fighting malaria with engineered symbiotic bacteria from vector
219 mosquitoes.*Proceedings of the National Academy of Sciences*; 109(31): 12734–12739.

10. [Mousavi](#), S.M., [Imani](#), S., [Haghighi](#), S., [Mousavi](#), S.E. and [Karimi](#), A(2012). Effect of Iranian Honey bee (*Apis mellifera*)Venom on Blood Glucose and Insulin in Diabetic Rats. *Juornal Arthropod Borne Disease*:6(2): 136–143.
11. Orakpo, E. (2013). The Bee Venom as HIV, Cancer Cure. Vanguard News
12. Shittu, O.K., Olayemi, I.K Omalu, I.C.J and Adeniyi, A.K (2013): Antiplasmdial properties of methanolic extract of Muscadomestica maggot on *P. berghei* infected mice *International journal of biology pharmacy and allied science* 2(5): 1064-1070
13. Lev, E. (2003). Traditional healing with animals (zootherapy): medieval to present-day Levantine practice. *Journal of Ethnopharmacology*, (86):107-118.
14. Adebayo, A. A., and Davies, B.A (2012). Apitherapy in Southwestern Nigeria: An Assessment of Therapeutic Potentials of some Honeybee Products. *Journal of Pharmaceutical and Biomedical Sciences*; 2(2): 9-15.
15. Shittu, O.K. and Abubakar, M. E.(2015). Anti-plasmodial Activity of Bee sting in *Plasmodium berghei* infected Mice. *International Journal of Tropical Disease and Health* 6(3): 80-85, 2015;
16. Tonks AJ, Dudley E, Porter NG, Parton J, Brazier J, Smith EL, Tonks A. (2007). A 5.8-kDa component of manuka honey stimulates immune cells via TLR4. *Journal of Leukocyte Biology*. 82(5):1147-1155.
17. SantiyanontR.(1985). Parasite Identification, Counting and Staining, Application of Genetic Engineering Techniques in Tropical Diseases Pathogen, With Special Reference To Plasmodia. Alaboratory Manual of Selected Techniques. Bangkok, Thailand: UNDP/World Bank/WHO special programme for research and training in tropical disease; 87(7): 413–418.
18. Datta, G.P. (1997). Proceedings of the International Workshop on Medicinal Plants: Their Bioactivity, Screening and Evaluation. Lucknow, India. L19.
19. Ryley, J.F., and Peters W. (1970).The Antimalarial Activity of Some Quinolone Esters *Annals Journal of Tropical Medicine and parasitology*;209-222.
20. Tietz NW, Rinker AD, Shaw LM (1983). International Federation of Clinical Chemistry. IFCC methods for the measurement of catalytic concentration of enzymes. Part 5.IFCC method for alkaline phosphatase (orthophosphoric-monoester phosphohydrolase, alkaline optimum, EC 3.1.3.1). *J. Clin. Chem. Clin. Biochem.*, 21:731-748.

21. Reitman S, Frankel S (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvate transaminases. *American Journal of Clinical Pathology* 28: 56–63.
22. Gornall, A. C., Bardawill, C. J. and David, M. M. (1949). Determination of serum protein by means of biuret reaction. *Journal of Biological Chemistry*, 177, 751-756.
23. Plummer, D.T. (1978). *An Introduction to Practical Biochemistry*, 2nd Edn., McGraw-Hill, London
24. Szasz, G., and Klin, M.Z. (1972). Serum Gamma glutamyl Transferase determination. *Chemical Biochem*; 12:228.
25. Mahajan BK, (1997). Significance of difference in mean. In: *Methods in Biostatistics for medical and research workers*, 6th Ed., Jaypee Brothers Medical Publi., New Delhi, 130-155.
26. Srivastava, T., and Chosdol, K. (2007). *Clinical Enzymology and Its Applications. Clinical Biochemistry. New Delhi India.*, 6 – 8.
27. Akanji , M.A., Olagoke, O.A. & Oloyede, O.B. (1993). Effect of chronic consumption of metabisulphite on the integrity of the kidney cellular system. *Toxicology*, 81: 173-179
28. Alqutub, A. N., Masoodi, I., Alsayari, K. and Alomair, A. (2011). Bee sting therapy-induced hepatotoxicity: A case report. *World Journal Hepatology* 3(10): 268–270.
29. Hyder, M. A., Hasan, M., and Mohieldein, A. H. (2013). Comparative Levels of ALT, AST, ALP and GGT in Liver associated Diseases. *European Journal of Experimental Biology*, 3(2):280-284
30. Ghouri, N., Preiss, S., David, W., Sattar, A. and Naveed, N. (2010). Liver Enzymes, Nonalcoholic Fatty Liver Disease and Incident Cardiovascular Disease: A Narrative Review and Clinical Perspective of Prospective Data. *Hepatology* 52 (3): 1156–61
31. Kennedy, G.E.P. (2004). Human african trypanosomiasis of the CNS, current issue and challenges. *Clinical Investigation* (113) 496-504.
32. Sowjanya M., Kalyan Kumar K., and Sunita K. (2013). Assessment of Biochemical Variations and Splenomegaly during Falciparum Malaria in Mice Model. *International*

- 279 33. Olorunnisola, O.S., and Afolayan, A.J. (2013). *In Vivo* Antioxidant and Biochemical
280 Evaluation of *Sphenocentrum jollyanum* Leaf Extract in *P. Berghei* Infected Mice. *Pak.*
281 *Journal of Pharmaceutical and Biomedical Sciences*: 26(3): 445-450.
- 282 34. Guillaume, C., Calzada, C., Lagarde, M., Schrével, J., and Deregnaucourt, C. (2006).
283 Interplay between lipoproteins and bee venom phospholipase A2 in relation to their anti-
284 plasmodium toxicity. *Journal of Lipid Research* 47: 1493–1506.
- 285 35. Bagai U., Rajan A., and Kaur S. (2012). Antimalarial potential of Nosode 30 and 200
286 against *Plasmodium berghei* infection in BALB/c mice. *Journal of Vector Borne Disease*
287 49: 72–77.