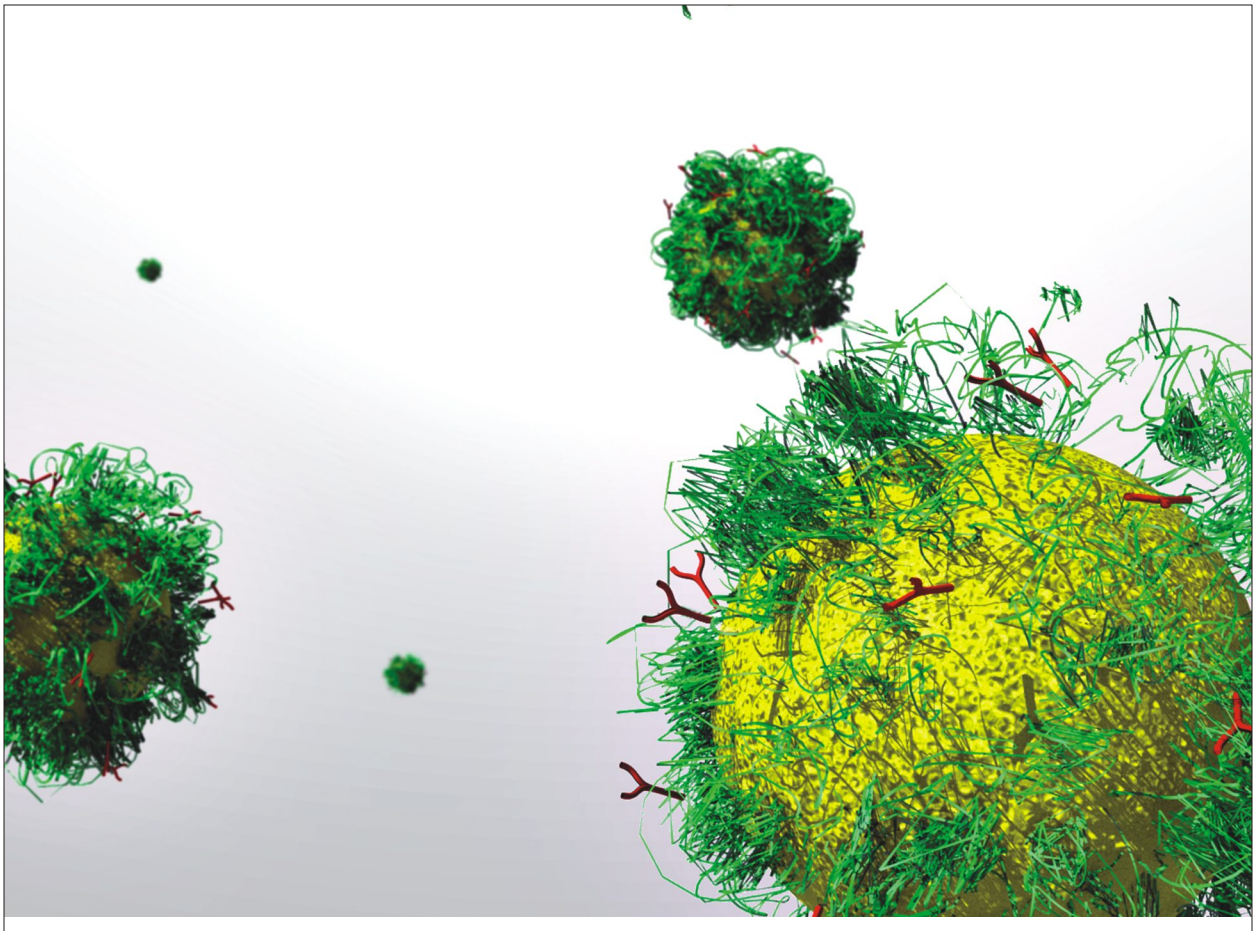

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Therapeutic Potentials of Nigerian Insect-propolis Against the Malarial Parasite, *Plasmodium berghei* (Haemosporida: Plasmodidae)

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ABSTRACT

This study reports the results of bio-assay of extract of Nigerian honey-bee propolis against *Plasmodium berghei*. Extract was tested for acute oral toxicity anti-plasmodial efficacy following standard protocols, using15 healthy mice experimentally infected with *P. berghei*. The mice were randomized into three groups and intra-peritoneally injected daily with 0.2 mL kg^{-1} body weight saline solution (Negative Control); 600 mg kg^{-1} (Test experiment) and 5 mg kg^{-1} Chloroquine diphosphate (Positive Control). Thin blood smear was used to determine levels of parasitaemia while, Erythrocyte Packed Cell Volume (PCV) was estimated Days 0, 4 and 7. The results showed that the extract significantly ($p<0.05$) reduced level of parasitaemia in the treated mice, with peak activity recorded on the last day of observation; a pattern distinctly different from the positive Control. There was a general increase in PCV till Day 4 before dropping significantly, especially. Survivorship of mice treated with extract was not significantly different from those treated Chloroquine.

Key words: Anti-plasmodial, drug, honey bee, oral toxicity, packed cell volume, parasitaemia

INTRODUCTION

Despite more than a decade-long of sustained roll-back-malaria global campaign (Roll-Back-Malaria, 2002; WHO, 2010) the disease remains the fore-most cause of morbidity and mortality, particularly among the vulnerable groups, i.e., pregnant women and infants below the age of 5 years (WHO, 2010). As much as 500 million new malaria infections, resulting in over one million deaths, are recorded world-wide per annum (Muturi *et al*., 2008). More than 90% of the global burdens of malaria are felt in Africa, with the disease accounting for at-least 10% of the continent's overall disease burden. Malaria is holo-endemic in many African countries and may be responsible for 50% out-patient attendance in their health facilities (Schwartlander, 1997; USAID, 2005).

Malaria Control, particularly in Africa, is anchored primarily on chemotherapy; as poor understanding of the biology and ecology of anopheline mosquitoes has rendered vector control strategy unsustainably effective, in addition to the lack of viable vaccine candidates (Matuschewski, 2006). Chemotherapy is popular for malaria control, due to its relative accessibility and potent functionality in the usual critical clinical conditions. However, the success of this strategy has been seriously constrained by the challenge of wide-spread plasmodial parasite-resistance to available drugs (Bankova, 2005; Pemberton, 1999; Sherman *et al*., 2000; Costa-Neto, 2005; Chakravorty *et al*., 2011).

To this end, the extracts of honey-bee Propolis have been credited with significant clinical anti-pathogenic microbial efficacies (Sforcin *et al.*, 2000; Freitas *et al.*, 2006; Pena, 2008; Farnesi *et al.*, 2009; Koru *et al.*, 2007). The medicinal efficacies of Propolis have equally being attributed to inherent composition of a diversity of physiologically active compounds including, Ketones, Flavonoids, Phenols, Triterpenes, Cinnamic acids, etc. (Marcucci, 1995; Komericki and Kranker, 2009). Therefore, in order to explore the potentials of Propolis for malarial chemotherapeutic control, this study bio-assayed methanolic extract of Nigeria honey bee Propolis against *Plasmodium berghei* in infected mice.

This development has elicited a global search for alternative anti-plasmodia lead-agents, especially, of non-botanical origins, to fore-stall the weakness of vulnerability to parasite resistance, commonly associated with phytochemical-based drugs. A viable source of such potential resistance insulated antimalarial lead-agents is entomo-pharmacology, as many pathogenic human ailments have been successfully treated with insect product-based drugs (Bankova *et al.*, 1999; Pemberton, 1999; Sherman *et al.*, 2000; Costa-Neto, 2005; Chakravorty *et al.*, 2011).

MATERIALS AND METHODS

Source of propolis: Propolis material was collected from an apiary in Akure, Ondo State, Nigeria. The identity of the Propolis was authenticated by an Entomologist in the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria, where a voucher specimen was deposited. The Propolis material was chopped in to small pieces and air dried in the Shade at room temperature for two weeks.

Sources of mice and *P. berghei* parasites: Swiss Albino mice, 21.00±3.00 mean weight, were obtained from the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria. The Mice were maintained in plastic cages under ambient Laboratory conditions, following standard protocols. The animals were fed with rat pellets and tap water *ad libitum*.

For parasite collection, a Chloroquine-sensitive strain of *P. berghei* (NK-65) was obtained from National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria. The *Plasmodium parasite* was perpetuated in the Laboratory, through Intra-peritoneal re-infection of clean mice with 0.20 mL *P. berghei*-infected blood suspension, after every 6 days.

Preparation of propolis extract: Preparation of the extract of Propolis material followed standard procedures (Najafi *et al.*, 2007; Adebayo *et al.*, 2003). Two hundrad grams of Propolis pellets were percolated in 1600 mL of absolute methanol and subsequently allowed to stand in the shade for 48 h before filtration, using Whatman No. 1 filter paper. The extract concentrate obtained was stored in air-tight vials in the refrigerator at 4°C, until needed for bio-assay.

Acute oral toxicity test: Mammalian toxicity assay followed the techniques of Jigam *et al.* (2009) Six groups of 5 mice each were starved over-night prior to the administration of Propolis-extract. Then, the 6 groups of mice were orally administered with extract concentration doses of 400, 600, 1200 and 1600 kg^{-1} b.wt., respectively. The mice were subsequently monitored for a period of 72 h for clinical signs of morbidity or mortality.

Anti-malarial efficacy assays

Plasmodial parasite clearance test: Fifteen mice were inoculated with 0.20 mL of *P. berghei*-infected blood suspension and left untreated for three days, for the parasites to be established in the mice. On the expiration of the third day, the establishment of plasmodial parasites, in each of the inoculated mice, was confirmed by thin blood smear test. Then, the three infected mice groups were, each, intra-peritoneally administered with three different test treatments, respectively: Group I mice were given 0.20 mL normal saline kg^{-1} body weight (i.e., Negative Control); Group II mice were offered 600 mg of the extract kg^{-1} body weight (i.e., Test experiment) and Group III received 5 mg Chloroquine kg^{-1} body weight (Positive Control). These dose treatments were administered, respectively, to the group of mice, for five consecutive days. On each treatment day, thin blood smears, stained with 10% Giemsa, was prepared from a cut in the tail of each mouse and examined for parasitaemia level, under a compound microscope using x100 objective lens.

Estimation of packed cell volume (PCV): Following standard procedures (Dacie and Lewis, 1991) Packed Cell Volume (PCV) was determined in the three groups of mice prior to infection (day 0); 72 h post-infection (day 4) and four days post-confirmation, i.e., post-commencement of treatment (day 7). This was done by collecting blood in a heamatocrit capillary tube (up to about three-quarters of its length), from a cut at the end of the tail of each mouse. The capillary tubes were then spinned in a haematocrit centrifuge, before further haematological analysis.

Data analysis: Data collected for variables investigated were processed as Mean±SE and statistically analysed for significance of differences in means among treatments, using one-way ANOVA and Duncan Multiple Range test (Mahajan, 1997; Shittu *et al.*, 2013). Relationships among the effects of the extract and Chloroquine were determined using Linear Correlation Coefficient.

RESULTS

The *in vitro* anti-plasmodial activity of the extract of honey-bee propolis relative to that of Chloroquine diphosphate is shown in Table 1. While, the separate administration of propolis-extract and chloroquine diphosphate resulted in significant ($p<0.05$) decrease in level of parasitaemia in the mice, starting from day 2 through day 5 post-treatment, the reverse was the case in the untreated infected mice (i.e., negative control). However, the parasite-clearance effects of chloroquine diphosphate were significantly more pronounced than those of the tested extract. Also while the former treatment was most active within 24 h of administration, the latter had its

Table 1: Mean±SE daily parasitaemia levels in *Plasmodium berghei* infected mice, treated with methanolic extract of honey-bee propolis

	Days post-administration					
Treatment (kg^{-1} b.wt.)	1	2	3	4	5	Mean
Normal saline (0.20 mL) (negative control)	36.00±0.24^{a}	38.00±1.92^{a}	35.00±1.20^{a}	34.00±2.11^{a}	36.00±2.81^{a}	35.80±1.65^{a}
Propolis extract (600 mg)	35.00±1.20^{a}	33.00±2.55^{b}	28.00±2.77^{b}	25.00±0.56^{b}	13.00±1.33^{b}	26.80±1.68^{b}
Chloroquine diphosphate (5 mg) (positive control)	32.00±2.88^{a}	18.22±0.87^{c}	11.32±1.23^{c}	5.000±0.99^{c}	1.200±0.34^{c}	13.55±1.26^{c}

*Value followed by same superscript alphabet are not significantly different at $p<0.05$. **Values followed by same superscript alphabet in a column, are not significantly different at $p<0.05$. Each value is a mean of data from 5 mice

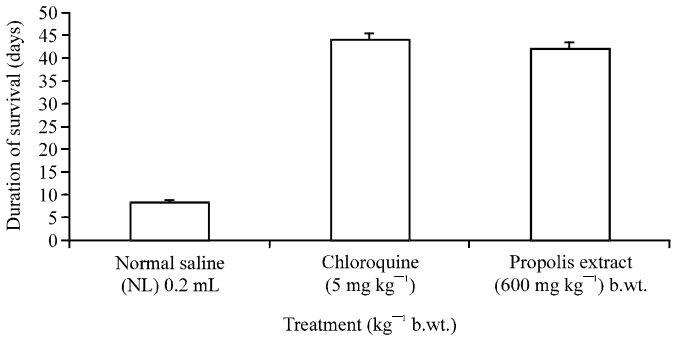

Fig. 1: Mean duration of survival (days) of *Plasmodium berghei*-infected mice, treated with methanolic extract of honey-bee propolis. Values are means of data from five mice

Table 2: Mean±SE (%) of packed cell volume (PCV) in relation to parasite infection and treatment with methanolic extract of honey-bee Propolis, in *Plasmodium berghei* mice

Treatment (kg^{-1} b.wt.)	Pre-infection (Day 0)	Post-infection (Day 4)	Post-treatment (Day 7)
Normal saline (0.20 mL) (negative control)	55.00±1.00^{b}	56.00±2.23^{a}	50.00±3.50^{a}
Propolis Extract (600 mg)	44.00±1.50^{a}	57.00±4.22^{a}	47.00±3.12^{a}
Chloroquine diphosphate (5 mg)	46.00±3.00^{a}	50.00±1.89^{a}	57.00±1.49^{b}

Values are means of data from five mice. *Value followed by same superscript alphabet are not significantly different at p = 0.05. **Values followed by same superscript alphabet in a column, are not significantly different at p = 0.05. Each value is a mean of data from 5 mice

equivalent efficacy between the 4th and 5th days. At the expiration of the monitoring of parasitaemia level (i.e., day-5), total clearance was almost achieved in the group of mice treated with chloroquine diphosphate while those treated with the extract had a mean plasmodial density of 13.00±1.33.

Prior to infection with plasmodial parasites (i.e., day-0), erythrocyte Packed Cell Volume (PCV) was significantly higher (55.00±1.00%) in the group of mice that were eventually injected with normal saline solution (i.e., negative Control) than the other two groups namely, propolis-extract and chloroquine diphosphate treated-mice (i.e., 44.00±1.50 and 46.00±3.00%, respectively) (Table 2). After 72 h parasite-infection (i.e., day 4) but before commencement of anti-plasmodial treatments, a general increase in PCV was recorded in all three mice groups (range = 50.00±1.89 to 57.00±4.22%). However, 4 days after the commencement of anti-plasmodial treatments (i.e., day 7) while PCV increased significantly ($p<0.05$) in the group of mice treated with chloroquine diphosphate (57.00±1.49%), significant reductions were recorded in those treated with the extract (47.00±3.12%) as well as normal saline solution (50.00±3.50%).

The mean survivorship of the three groups of mice is presented in Fig. 1. Survival period of the mice ranged significantly ($p<0.50$) from 8.30±0.50 days in the negative Control group to 44.00±1.38 days in the group treated with Chloroquine diphosphate. However, mice survivorship was not significantly different ($p>0.05$) between Chloroquine diphosphate and propolis-extract groups.

DISCUSSION

The results of this study indicate that methanolic extract of the Nigerian honey-bee propolis possesses significant anti-plasmodial activity. Though, the plasmodial parasite clearance efficacy of the positive Control (i.e., mice treated with Chloroquine diphosphate), was significantly higher

than that of the extract, a cursory look at the result reveals that the latter may be as potent as the former in malaria treatment. For example while Chloroquine diphosphate was most active within 24 h post-administration, peak activity of the extract was recorded towards the end of the monitoring of level of parasitaemia in the mice (i.e., between days 4 and 5). Also, if the results for the extract in Fig. 1 were extrapolated, the same level of parasite-clearance achieved by Chloroquine diphosphate on day 5 would probably had been attained by the extract a day or two later (i.e., day 6 or 7). These two classical examples, therefore, suggest that propolis extract possesses equally high anti-plasmodial efficacy though, probably with a different mode of action. Usually, the receptor-targets and mechanism of action of drug compounds are largely determined by their chemical composition and organismal origin. Therefore, quinine (the major component of Chloroquine diphosphate) and extract of honey-bee propolis, being of wholly phytochemical and partly insect metabolite origins, respectively, are likely to employ different mechanisms against equally varied receptor sites in plasmodial parasites. This possibility greatly serves the objective of this study; identifying anti-plasmodial lead-agents, of sources other than botanicals, that will be less vulnerable to parasite-resistance.

The anti-plasmodial activities demonstrated by Propolis in this study may be due to inherent composition of bio-active compounds including, Flavonoid, Cinnamic acid, Ketones, triterpenes, steroids, etc. (Marcucci, 1995; Komericki and Kranker, 2009). These physiologically-active substances have been largely responsible for the pathogenicidal activities of Propolis, especially, those of anti-bacterial (Sforcin *et al*., 2000; Farnesi *et al*., 2009), anti-fungal (Pena, 2008), antiviral (Freitas *et al*., 2006) and anti-protozoan (Koru *et al*., 2007). This finding is consistent with those of an earlier report (Syamsudin *et al*., 2008) who reported immune-modulatory activities of the extracts of propolis against plasmodial parasites. The anti parasite-resistance potential of Propolis may be further enhanced by the fact that as much as 40% of its constituents are wax or oil-based (Burdock, 1998; Silici and Kutlaca, 2005); insecticidal oils are the only compounds to which insects are yet to develop resistance (Pfadt, 1985).

Erythrocyte Packed Cell Volume (PCV) varied significantly among the groups of mice even before infection with Plasmodium parasites-a traditional red blood cell-lysing agent (Muturi *et al*., 2008). Such differential PCV in mammalian populations, not under erythrocytic-lysing effects of pathogenic or nutritional-deficiency disease conditions, have been attributed to individual differences in sex, age, genetic vigour, etc. (Lawler *et al*., 2005). However, there was a general rise in PCV in the three groups of mice, even till 72 h post-parasite infection. At the early stage, it may be possible for PCV to rise in *Plasmodium*-infected mice, as it takes more than three days after parasite infection, before the erythrocytes are invaded and eventually destroyed. According to Miller *et al*. (2013), the pre-erythrocytic stage of *Plasmodium* parasites takes up to 10 days. Four days after the commencement of anti-plasmodial treatments while there was significant improvement in the level of PCV among the mice treated with Chloroquine disulphate, those of Propolis extract and normal saline solution dropped significantly. The results obtained for Chloroquine diphosphate and normal saline solution are expected, as while treatment with the formal arrest the progression of plasmodial development in the erythrocytes thereby preserving them for increased PCV; destruction of red blood cells continued unabated in the latter (i.e., normal saline solution). Then, one wonders why erythrocyte PCV dropped in the group of mice administered with Propolis extract despite the significant reduction in the level of parasitaemia induced by the extract. The explanation for these observations may be in the plasmodial developmental life-stage affected by the two treatments, i.e., Chloroquine diphosphate and Propolis

extract. Generally, there are two categories of antimalarial drugs namely, blood and tissue Schizonticides (Ekpeyong and Eyo, 2006). While, the former destroys parasites in the erythrocytic stage, the latter attacks exo-erythrocytic plasmodial stages. Therefore, Chloroquine diphosphate being a blood schizonticide (Obaldia *et al*., 1997), prevents the lysing of infected erythrocytes thus enhancing PCV. It seems, therefore, that Propolis extract is a tissue Schizonticide hence, its significant anti-plasmodial activity but may not be able to stop lyses of red blood cells already infected with parasites. This finding further suggests that the extract of Propolis adopts a different anti-plasmodial mechanism of action or target a different receptor site, from those of Chloroquine diphosphate.

CONCLUSION

The Nigerian honey bee Propolis is a viable potential source of cost-effective anti-malarial lead-agent, judging by the significant plasmodial parasite-clearance and very low mammalian toxicity demonstrated by the extract in this study. However, its mechanism of anti-plasmodial activity and/or target receptor site appears to be different from those of the parasite-resistance vulnerable Chloroquine. Therefore, further screening of the bio-active compounds of Propolis may yield a classical anti-plasmodial bio-active compound, for strengthening the sustainability of the gains of chemotherapeutic control of malaria.

REFERENCES

Adebayo, J.O., M.T. Yakubu, E.C. Egwin, V.B. Owoyele and B.U. Enaibe, 2003. Effect of ethanolic extract of *Khaya senegalensis* on some biochemical parameters of rat kidney. J. Ethnopharmacol., 88: 69-72.

Bankova, V., R. Christov, S. Popov, M.C. Marcucci, I. Tsvetkova and A. Kujumgiev, 1999. Antibacterial activity of essential oils from Brazilian propolis. Fitoterapia, 70: 190-193.

Bankova, V., 2005. Recent trends and important developments in propolis research. Evid. Based Complement Alternat. Med., 2: 29-32.

Burdock, G.A., 1998. Review of the biological properties and toxicity of bee propolis (propolis). Food Chem. Toxicol., 36: 347-363.

Chakravorty, J., S. Ghosh and V.B. Meyer-Rochow, 2011. Practices of entomophagy and entomotherapy by members of the Nyishi and Galo tribes, two ethnic groups of the state of Arunachal Pradesh (North-East India). J. Ethnobiol. Entomed., Vol. 7. 10.1186/1746-4269-7-5

Costa-Neto, E.M., 2005. Entomotherapy, or the medicinal use of insects. J. Ethnobiol., 25: 93-114.

Dacie, J.V. and M.S. Lewis, 1991. Practical Haematology. 7th Edn., ELBS Churchill Living Stone, England.

Ekpeyong, E.A. and J.E. Eyo, 2006. Infection in man: A review. Ann. Res. Int., 3: 573-580.

Farnesi, A.P., R. Aquino-Ferreira, D. de Jong, J.K. Bastos and A.E. Soares, 2009. Effects of stingless bee and honey bee propolis on four species of bacteria. Genet. Mol. Res., 8: 635-640.

Freitas, S.F., L. Shinohara, J.M. Sforcin and S.Guimaraes, 2006. *In vitro* effects of propolis on *Giardia duodenalis* trophozoites. Phytomedicine, 13: 170-175.

Jigam, A.A., H.O. Akanya, E.O. Ogbadoyi, B.E.N. Dauda and E.C. Evans, 2009. *In vivo* antiplasmodial, analgesic and anti-inflammatory activities of the leaf extract of *Lippia multiflora* mold. J. Med. Plants Res., 3: 148-154.

Komericki, P. and B. Kranke, 2009. Maculopapular exanthem from propolis: Case report and review of systemic cutaneous and non-cutaneous reactions. Contact Dermatitis, 61: 353-355.

Koru, O., F. Toksoy, C.H. Acikel, Y.M. Tunca and M. Baysallar *et al*., 2007. *In vitro* antimicrobial activity of propolis samples from different geographical origins against certain oral pathogens. Anaerobe, 13: 140-145.

Lawler, K.A., J.W. Younger, R.L. Piferi, R.L. Jobe, K.A. Edmondson and W.H. Jones, 2005. The unique effect of forgiveness on health: An exploration of pathways. J. Behv. Med., 28: 157-167.

Mahajan, B.K., 1997. Significance of Difference in Means. In: Methods in Biostatistics for Medical and Research Workers, Mahajan, B.K. (Ed.). 6th Edn., JAYPEE Brothers Medical Publishers, New Delhi, pp: 130-155.

Marcucci, M.C., 1995. Propolis: Chemical composition, biological properties and therapeutic activity. Apidologie, 26: 83-99.

Matuschewski, K., 2006. Vaccine development against malaria. Curr. Opin. Immunol., 18: 449-457.

Miller, J.L., S. Murray, A.M. Vaughan, A. Harupa and B. Sack *et al*., 2013. Quantitative bioluminescent imaging of pre-erythrocytic malaria parasite infection using Luciferase-Expressing *Plasmodium yoelii*. PloS ONE, Vol. 8. 10.1371/journal.pone.0060820

Muturi, E.J., P. Burgess and R.J. Navak, 2008. Malaria vector management: Where have we come from and where are we headed. Am. J. Trop. Med. Hyg., 78: 536-537.

Najafi, M.F., F. Vehedy, M. Seyyidin, H.B. Jomehzadeh and K. Bozary, 2007. Effect of the water extracts of Propolis on stimulation and inhibition of different cells. Cytotechnology, 54: 49-56.

Obaldia, N., R.N. Rossan, R.D. Cooper, D.E. Kyle, E.O. Nuzum, K.H. Rieckmann and G.D. Shanks, 1997. WR 238605, chloroquine and their combinations as blood schizonticides against a chloroquine-resistant strain of Plasmodium vivax in Aotus monkeys. Am. J. Trop. Med. Hyg., 56: 508-510.

Pemberton, R.W., 1999. Insects and other arthropods used as drugs in Korean traditional medicine. J. Ethnopharmacol., 65: 207-216.

Pena, R.C., 2008. Propolis standardization: A chemical and biological review. Ciencia Invest. Agraria, 35: 17-26.

Pfadt, R.E., 1985. Fundamentals of Applied Entomology. 4th Edn., Macmillan Publishing Company, New York, USA., Pages: 742.

Roll-Back-Malaria, 2002. A Global partnership. http://www.rbm.who.int/

Schwartlander, B., 1997. Global burden of disease. Lancet, 350: 141-142.

Sforcin, J.M., J.A. Fernandes, C.A. Lopes, V. Bankova and S.R. Funari, 2000. Seasonal effect on Brazilian propolis antibacterial activity. J. Ethnopharmacol., 73: 243-249.

Sherman, R.A., M.J.R. Hall and S. Thomas, 2000. Medicinal maggots: An ancient remedy for some contemporary afflictions. Ann. Rev. Entomol., 25: 55-81.

Shittu, O.K., I.K. Olayemi, I.C.J. Omalu and A.K. Adeniyi, 2013. Anti-plasmodial properties of methanolic extract of *Musca domestica* maggot on *P. berghei*-infected mice. Int. J. Biol. Pharm. Allied Sci., 2: 1064-1070.

Silici, S. and S. Kutluca, 2005. Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. J. Ethnopharmacol., 99: 69-73.

Syamsudin, R.M. Dewi and Kusmardi, 2008. Immunomodulatory and *in vivo* antiplasmodial activities of propolis extracts. Global J. Phamacol., 2: 37-40.

USAID., 2005. Health: USAID?s malaria program. U.S. Agency for International Development, USA.

WHO, 2010. World malaria report summary. World Health Organisation, Geneva. http://www.who.int/malaria/world_malaria_report_2010/world_malaria_report_2010.pdf