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Antimalarial activity and heamatological parameters of *Plasmodium berthgei*- infected mice treated with combination of aqueous extract of *Nigella sativa* and Honey

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Abstract

Malaria is one of the most deadly parasitic diseases in endemic areas including Nigeria. The aim of the study was to investigate antimalarial potential of the aqueous extract of *Nigella sativa* in combination with honey in ratio 1: 1 (w/v) and its effect on heamatological parameters of *P. berthgei* infected mice. Dose levels of the combined extract and honey at 50, 100, and 200 mg/kg body weight were evaluated for the curative effects against *Plasmodium berthgei* (NK 65 chloroquine sensitive) infected mice. Chloroquine (20 mg/kg body weight) was used as standard drug. The results showed significant ($p < 0.05$) decrease in the parasitaemia levels in treated mice when compared to those of the untreated group. Chemosuppressive inhibition (91.37%) of the parasite in *P. berthgei* treated mice at 100 mg/kg body weight of the combination was relatively higher when compared to those treated with the standard drug (81.06%). Also, there was significant ($p < 0.05$) increase in white blood cell, red blood cell, heamoglobin, packed cell volume and platelets counts of the treated *P. berthgei* mice when compared with the untreated *P. berthgei* mice. The findings suggested that the combination of aqueous extract of *N. sativa* and honey has antimalarial property and restored the deranged hematological indices caused by the *P. berthgei* parasite.

Keywords: Antimalarial, *Plasmodium berthgei*, *Nigella sativa*, Honey, Hematological parameters

Introduction

Malaria is a major global health problem and a predisposition risk to 97% of Nigeria's population where it accounts for more clinical episodes (100 million/ year) and deaths (300,000/ year) than in any other country in the world (WHO, 2015). This devastating parasitic infectious disease competes with death rate (215,000/ year) from HIV/AIDS in the country. Children and pregnant women are the most susceptible groups to the disease resulting in an estimated 11% of maternal mortality rate (WHO, 2015). Recent report from World Health Organization reveals that nearly half of the world's population is at risk of malaria, with about 216 million malaria cases and about 445,000 cases of malaria-related deaths (WHO, 2016).

Most common complications in malaria infection are changes or alteration in the hematological parameters of the infected individuals. These changes involve the major cell lines such as red blood cells, leucocytes and thrombocytes. As such they play a major role in malaria pathology (Maina *et al.*, 2010). Some degree of anaemia is linked to most malaria infections (Lamikanra *et al.*, 2007). Severe injury and death may arise from malaria anaemia particularly in children and pregnant women (Menendez *et al.*, 2000).

Plant materials have been a major source of natural therapeutic remedies for treatment of various infectious diseases in many countries (Wendy *et al.*, 2017). Among various medicinal plants, *Nigella sativa* (*N. sativa*) (Family Ranunculaceae) is emerging as a miracle herb with a rich historical and religious background (Al-Buhari, 1976), since many researches revealed its wide spectrum of pharmacological potential (Khare, 2004). *N. sativa* is commonly known as black seed. Pharmacological properties include: antioxidant activity (Kruk *et al.*, 2000), analgesic effect and anti-inflammatory activity (Al-Ghamdi, 2001; Ali and Blunden, 2003; Hajhashemi *et al.*, 2004), hepato-protective activity (Sultan, *et al.*, 2012; Yesmin *et al.*, 2013; Rahmani and Aly, 2015). Antimalarial activity (Abdulelah *et al.*, 2007; Hadi *et al.*, 2016). They stimulate the body's energy and help recovery from fatigue (Tembhurne *et al.*, 2014; Krishnaveni and Saranya, 2016; Botnick *et al.*, 2012). Also they can be administered internally in intermittent fever (Tembhurne *et al.*, 2014).

Honey is a natural substance produced, when the nectar and sweet deposits from plants are gathered, modified and stored in the honeycombs by honeybees of the genera *Apis* and *Meliponini* (Namias, 2003; Al-jabri, 2005). Honey forms part of traditional medicine in many cultures (Viuda-Martos *et al.*, 2008), although it is most widely used as sweetener. There is currently resurgence in interest in the use of honey and honey products by the public. This alternative branch of medicine is called apitherapy (Manyi-Loh *et al.*, 2011; Mamo, 2018). It is composed of at least 181 components and is basically a solution supersaturated in sugars (Wei *et al.*, 2009). The actual composition of honey varies; depending on many factors such as the pollen source, climate, environmental conditions, and the processing it undergoes (Bertoncelj *et al.*, 2007).

Pharmacological properties include: anti-oxidant activity (Jaganathan *et al.*, 2010, Manyi-Loh *et al.*, 2011, Khan *et al.*, 2018), anti-inflammatory activity (Kassim *et al.*, 2010, Candiracci *et al.*, 2012, Vallianou *et al.*, 2014), anti-malarial activities (Jirattikarn *et al.*, 2012, Olayemi, 2014) and boosting of the immune system (Jones *et al.*, 2000, Molan, 2001, Tonks *et al.*, 2007). The objective of the present work was to assess the antimalarial potential of the combination of aqueous extract of *N. sativa* seed and honey in ratio 1:1 and its effects on the hematological parameter on the *P. berghei* infected mice.

Materials and methods

Plant materials and authentication

Nigella sativa seeds and honey were obtained from Alhamed shop in Taiwo Road Ilorin, Kwara State, Nigeria. The seeds were authenticated at herbarium unit of the Department of Plant Biology, University of Ilorin, Nigeria with voucher number UIH039.

Plant Preparation

100g of dry black seed was ground using a mortar and pestle, the powdered seed was dissolved in 2L of distilled water in a plastic container and shaken intermittently for 48 hours. The resulting mixture was filtered and the filtrate was freeze dried.

Laboratory Animals and Ethical protocol

Adult albino mice, with average weight of $21.5 \pm 1.4\text{g}$, obtained from Small Animal Holding unit, Department of Biochemistry, University of Ilorin, were used for the experiment. The mice were placed on standard pellet feed and allowed to drink water freely. They were acclimatized for 2 weeks before the commencement of the study. The guide line on animal handling and treatment were duly followed (CCAC, 1993).

Malaria parasites

NK65 strain of *P. berghei* was obtained from the Institute for Advance Malaria Research and Training (IMRAT), College of Medicine, University of Ibadan, Ibadan Nigeria, and sustained in the laboratory by continuous passage of parasitized blood into mice.

Antimalarial assay

Curative antimalarial test used was a modified method of Ryley and Peters (1970).

Preparation of smear

A drop of blood was collected from the tail end of each mouse and was placed on slides, smear, and stain to determine its parasitemia using a method described by Adebayo *et al.* (2013).

Blood preparation

The mice were sacrificed by anaesthetizing them in a jar containing cotton wool soaked with diethyl ether. Blood was collected in well labeled EDTA sample bottles for the hematological analysis.

Results

There was a significant ($p < 0.05$) decrease in percentage parasitemia level of treated parasitized mice when compared with untreated parasitized mice as shown in figure 1. All the treated group shown a significant ($p < 0.05$) reduction in parasitemia level from day 4 to day 8 with the treatment at 100 mg/kg bw of the combination demonstrating more reduction in parasite level when compared with the chloroquine treated (fig. 1).

There was a significant ($p<0.05$) increase in percentage chemo suppression of parasite inhibition in all the treated groups with 100 mg/kg bw of the combination (91.37%) demonstrated a significant parasite inhibition when compared with chloroquine treated (87.73%) as shown in figure 2.

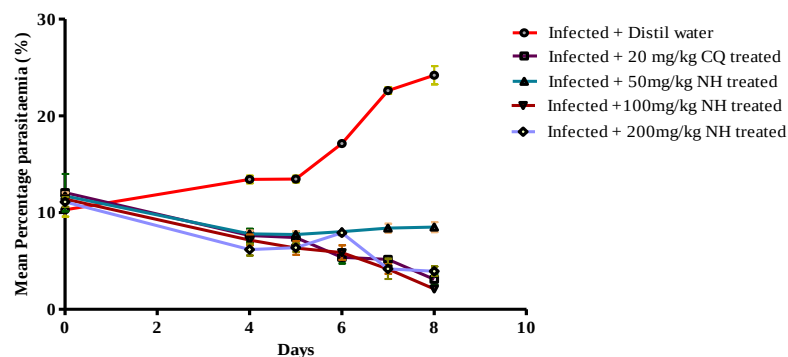


Figure 1: Mean percentage parasitaemia of *P. berghei* infected mice treated with combination of aqueous extracts of *N. sativa* seed and honey

lines are mean $\pm$ SEM; $n = 5$, the alphabets show the statistical different with $P<0.05$. NH: *Nigella sativa* aqueous extract and honey

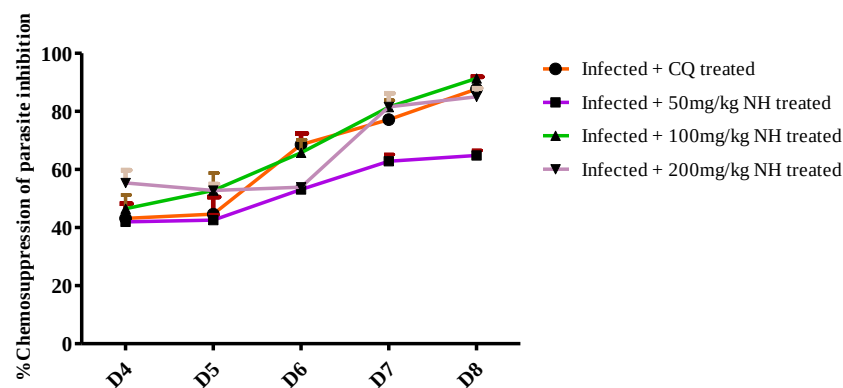


Figure 2: Percentage chemosuppression of parasite inhibition of *P. berghei* infected mice treated combination of aqueous extracts of *N. sativa* seed and honey

line are mean $\pm$ SEM; $n = 5$, the alphabets show the statistical different with $P < 0.05$. NH: *Nigella sativa* aqueous extract and honey

There was a significant ($p < 0.05$) decrease in the red blood cell (RBC) count, hemoglobin (Hb) and packed cell volume (PCV) of untreated infected *P. berghei* mice when compared with the treated parasitized mice as shown in figure 3,4 and 5. The rate of increase in RBC, Hb, and PCV of treated mice at 100 mg/kg bw of the combinations and chloroquine (standard drug) was compare favorably with the normal control (uninfected mice). Also, there was a significant ($p < 0.05$) decrease in the white blood cell (WBC) count and platelet count of the untreated parasitized mice when compared with treated parasitized mice as presented in figure 6 and 7. However, the treatment at 100 mg/kg bw of the combination and chloroquine treated shown appreciable increase in the level of WBC count and platelet count when compared with the normal control (figure 6 and 7).

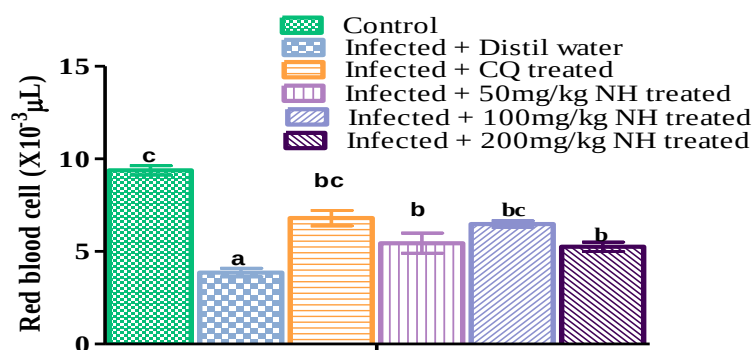


Figure 3: Red blood cell of *P. berghei* infected mice treated with combination of aqueous extracts of *N. sativa* seed and honey

Bars are mean $\pm$ SEM, $n = 5$, the alphabets show the statistical different with $P < 0.05$

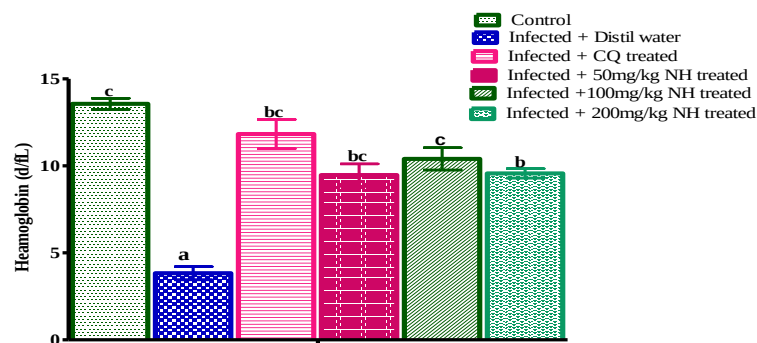


Figure 4: Hemoglobin of *P. berghei* infected mice treated with combination of aqueous extracts of *N. sativa* seed and honey

Bars are mean $\pm$ SEM, $n = 5$, the alphabets show the statistical different with $P < 0.05$. NH: *Nigella sativa* aqueous extract and honey

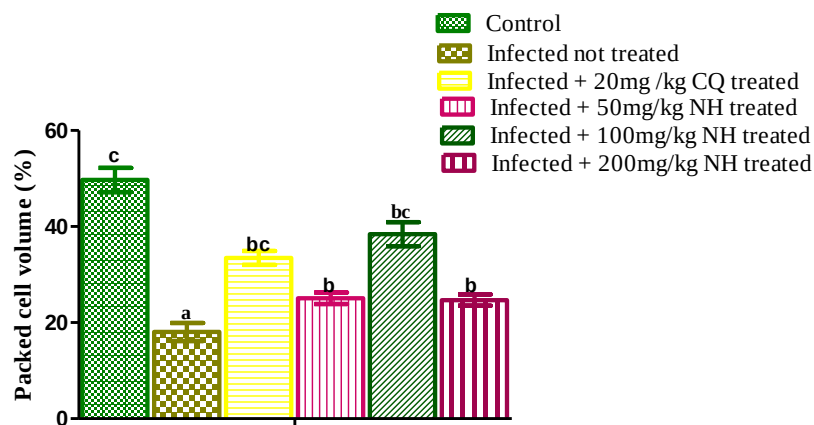


Figure 5: Pack cell volume of *P. berghei* infected mice treated with combination of aqueous extracts of *N. sativa* seed extract and honey

Bars are mean $\pm$ SEM, $n = 5$, the alphabets show the statistical different with $P < 0.05$ NH: *Nigella sativa* aqueous extract and honey

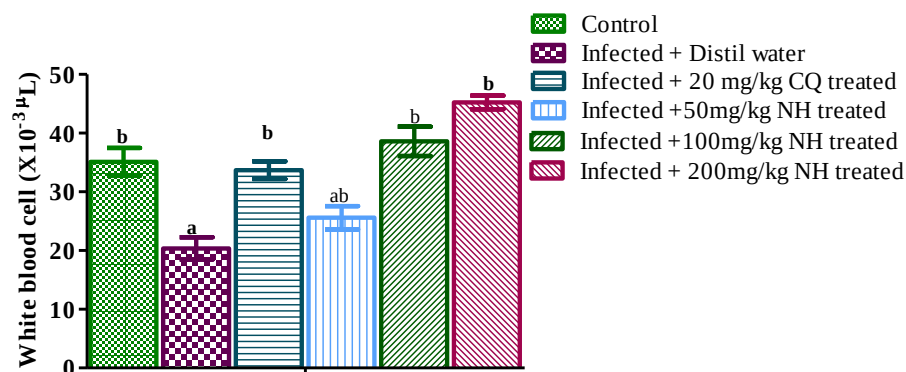


Figure 6: White blood cell of *P. berghei* infected mice treated with combination of aqueous extracts of *N. sativa* seed and honey

Bars are mean ± SEM, n = 5, the alphabets show the statistical different with P<0.05. NH: Nigella sativa aqueous extract and honey

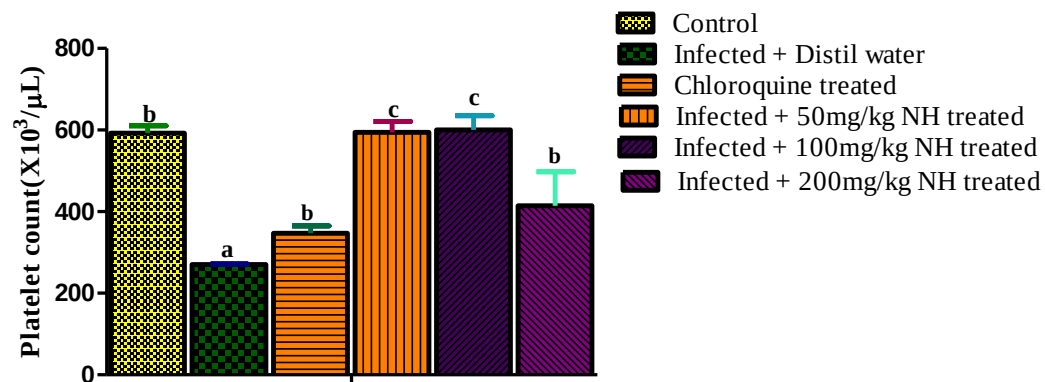


Figure 7: Platelet count of *P. berghei* infected mice treated with combination of aqueous extracts of *N. sativa* seed and honey

Bars are mean ± SEM, n = 5, the alphabets show the statistical different with P<0.05. NH: Nigella sativa aqueous extract and honey

Discussion

The results show that the combination of aqueous extract *N. sativa* seed and honey at 100 mg/kg bw was able to reduce the level of the parasite in the *P. berghei* infected mice. The treatment at various dosages of the combination and chloroquine also exhibit growth inhibition of parasite above 50%. This is in line with the work of Isha *et al.* (2003) who reported that an extract is considered active if it demonstrates 50% growth inhibition. In addition, the growth inhibition of the combination at 100 mg/kg bw (91.37%) was significantly higher than that of the chloroquine (81.06%) treated. Therefore, the treatment at 100 mg/kg bw of the combination was considered more effective for the treatment of malaria.

Hematological parameter plays a major role in malaria pathology (Maina *et al.*, 2010). The hematological assay revealed that RBC, Hb and PCV level significantly decrease ($p < 0.05$) after infection with *P. berghei* parasite. This is an indication of anemia due to hemolysis. Anemia is a consistent finding in blood protozoa parasite infection (Ajagbonna *et al.*, 2002). The increase in RBC, Hb and PCV counts after the treatment with the combination at 100 mg/kg bw that was brought to near normal was an indication that the combination at 100 mg/kg bw can revert the anaemic condition of the infected parasitized mice to near normal. The WBC and platelets counts of the treated *P.berghei* mice also increased significantly ($p < 0.05$) following the treatment at 100 mg/kg bw of the combination. This could also be an indication that the immune system of the *P. berghei* infected mice could have been boosted after the treatment.

Conclusion

The combination of *N. sativa* aqueous extract and honey at 100 mg/kg bw has antimalarial potential which was higher than that of standard drug (chloroquine) and demonstrated the ability to restore the deranged hematological indices caused by *P. berghei* infected mice.

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