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Effects of selected aqueous plant extracts on heamatological parameters of *Plasmodium berghei* infected mice

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Abstract

Cymbopogon citrates leaves, *Khaya senagalesis* stem bark, *Parquetina nigrescens* leaves, *Psidium guajava* leaves, *Terminalia glaucescens* stem bark and *Zingiber officinale* rhizome are used traditionally to treat malaria. This study evaluate the effects of the aqueous extracts of *C. citrates* leaves, *K. senagalesis* stem bark, *P. nigrescens* leaves, *P. guajava*, *T. glaucescens* stem bark and *Z. officinale* rhizome respectively on the heamatological status of NK 65 *Plasmodium berghei* (chloroquine sensitive parasite) infected mice. Fourty five (45) albino mice with average weight $25.5 \pm 1.45\text{g}$ were randomly grouped into nine (9). Mice in group A serve as control not infected. Groups B, C, D, E, F, G, H and I were inoculated intraperitoneally with *P. berghei*. Groups C, D, E, F, G, H and I were treated with 5 mg/kg body weight of chloroquine, 200mg/kg body weight of *C. citrates*, *K. senagalesis*, *P. nigrescens*, *P. guajava*, *T. glaucescens* and *Z. officinale* aqueous extracts respectively. On day 5, red blood cell count (RBC), white blood cell count (WBC), packed cell volume, haemoglobin count (Hb), lymphocyte and neutrophil counts were assessed. There were significant ($p < 0.05$) increase in the Hb count, RBC and PCV levels of the extract treated *P. berghei* infected mice in each group when compared with the untreated *P. berghei* infected mice. In the extracts treated infected mice, lymphocyte count was significantly ($p < 0.05$) increased with concomitant decrease in neutrophil count. Our Findings show that all the extracts used has anti-anaemic properties and anti-inflammatory potential with extracts of *P. nigrescens*, *P. guajava*, *T. glaucescens* and *Z. officinale* demonstrating higher potential.

Keywords: *Cymbopogon citrates*, *Khaya senagalesis*, *Parquetina nigrescens*, *Psidium guajava*, *Terminalia glaucescens*, *Zingiber officinale*, *Plasmodium berghei*, anti-anaemic properties.

Introduction

Malaria is a leading killer disease in the world, with about 3.2 billion people – almost half of the world's population at risk of malaria (WHO, 2013). In 2013, there were about 198 million malaria cases with an uncertainty range

of 124 million to 283 million and an estimated 584 000 malaria deaths with an uncertainty range of 367 000 to 755 000 (WHO, 2014). More than 150 million people were at risk of getting the disease in Nigeria (WHO 2013).

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Blood is the most easily accessible diagnostic tissue. Changes in haematological parameters are likely to be influenced by any disease condition including endemic diseases, such as malaria, that can affect health of mankind with various clinical presentations (Manas *et al.*, 2014). Malaria patients exhibit important changes in haematological parameter, including low platelet, white blood cells and lymphocyte counts (Manas *et al.*, 2014). Anemia has long been established in human and animal malaria (Carter and Diggs, 1977). Over half of deaths caused by malaria are attributed to severe anemia (Murphy and Breman, 2001). Malaria is clearly and extremely important causative factor of anemia (Rogerson, 2006), principally due to the destruction of the red blood cell by the invasion of the red blood cell by the plasmodium parasite (Jasen and Waters, 2006).

Diagnostic value of these haematological alterations may be easily used in diagnosis of malaria in endemic areas. The haematological abnormalities that have been reported include anaemia, thrombocytopenia, lymphocytosis and rarely, disseminated intravascular coagulation (Facer, 1994). Leucopenia, leucocytosis, neutropenia, neutrophilia, and eosinophilia, and monocytosis also have been reported (Murphy and Oldfield, 1996; Jandle, 1996). The present study aimed at evaluating the antianaemic potential of aqueous extract of *C. citrates*, *K. senagalesis*, *P. nigrescens*, *P. guajava*, *T. glaucescens* and *Z. officinale* using haematological indices in *P. berghei*-parasitized mice.

Materials and methods

Parasites

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

Laboratory Animals

A total of 45 adult albino mice with average weight range of 25.5 ± 1.45 g, obtained from small animal holding unit, Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were placed on standard pellet feed and were allowed free access to water. The animals were acclimatized for 2 weeks before the commencement of the experiment.

Plant Preparation

C. citrates leaves, *K. senagalesis* stem bark, *P. nigrescens* leaves, *P. guajava* leaves, *T. glaucescens* stem bark and *Z. officinale* rhizome were bought from Oja Tuntun, Ilorin, Kwara State, Nigeria and identified in the Department of Plant Biology, University of Ilorin, Nigeria. Specimens with voucher numbers UIH 800, UIH 852, UIH 876, UIH 973, UIH 1039 and UIH 1083 respectively were deposited in the herbarium. The plant materials were rinsed and air dried to constant weight and then pulverized into powder with blender (Marlex excella, Model no: L 31454). Powder sample (50g) was suspended in distilled water for 48 hours, then filtered through whatman no 1 filtered paper and the filtrate was freeze dried.

Experimental Design

The forty five (45) mice were distributed into nine groups (A-I) of five mice per group. B-I were inoculated intra-peritoneally with 0.2ml of 1×10^7 infected erythrocytes and were left for three days for parasitaemia establishment. Groups A and B were treated with 0.2 ml distilled water while Group C was administered 5 mg/kg body weight chloroquine. Other groups (D-I) were treated with 200 mg/kg weight of *C. citrates*, *K. senagalesis*, *P. nigrescens*, *P. guajava*, *T. glaucescens*, *Z. officinale* respectively. All treatment were given orally for four days.

Blood Collection and Haematological Assays

On the 5th day, the mice were sacrificed by stunning and blood was collected through jugular vein puncture. Blood was collected in EDTA bottles. The following haematological parameters were evaluated using automated haematologic analyzer, sysmex, KX- 21 (Japan): red blood cell (RBC) counts, white blood cell (WBC) count; platelet count, packed cell volume (PCV), haemoglobin (Hb), neutrophil and lymphocyte counts. The analysis were carried out based on standard methods (Dacie and Lewis, 1975; Alexander and Griffiths, 1993)

Statistical Analysis

All data were presented as mean of five determination $\pm$ standard error of mean (SEM). The significance of difference among groups was determined by the one-way Analysis of Variance (ANOVA), the Duncan's Test was used for the Post Hoc analyses and $p < 0.05$ was accepted as significant (Mahajan, 1997). The graph pad prism 5 was used for the analysis.

Results

At the end of the 4 days treatment some heamatological parameter on the blood of extracts and chloroquine treated infected mice were analyzed. There was a significant ($p > 0.05$) decrease in WBC of untreated *P. berghei* infected mice when compared with extracts and chloroquine treated mice (Fig. 1). There was no significant ($p < 0.05$) difference in WBC count of extracts treated infected mice when compared with the standard drug (chloroquine) treated mice. However, the WBC count of *T. glaucescens* and *Z. officinale* extracts treated compared favourably with the control.

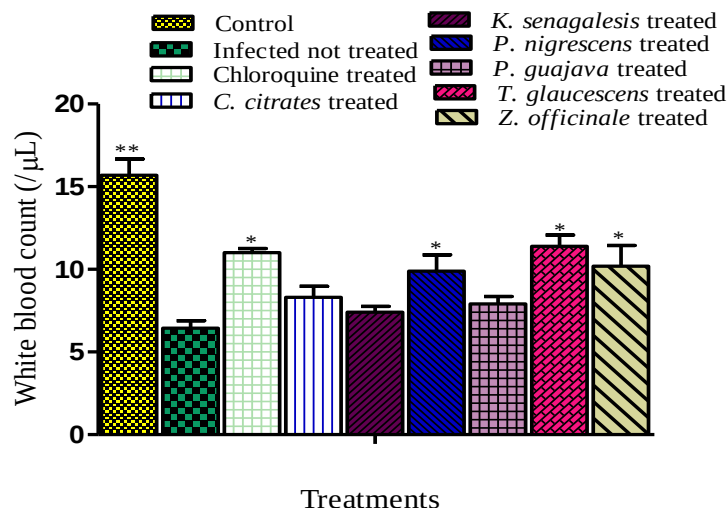


Figure 1: White blood cell of *P. berghei* parasitized mice treated with aqueous extracts (200 mg/kg bwt) of some medicinal plant and 5mg/kg bwt of chloroquine. The bars are mean $\pm$ SEM of determinations, the * show the statistical different at $P < 0.05$ and ** $p < 0.01$

There was a significant ($p < 0.05$) decrease in RBC, Hb and PCV of untreated *P. berghei* parasitized mice when compared with extracts and standard drug (chloroquine) treated mice as presented in figure 2a, 2b and 2c respectively. The increase in RBC, Hb and PCV count of extracts treated was

not significant when compared with standard drug (chloroquine). However, the increase in RBC (fig. 2a), Hb (fig. 2b) and PCV (fig. 2c) count was more pronounced in *P. nigrescens*, *T. glaucescens* and *Z. officinale* treated mice when compared with other extracts used.

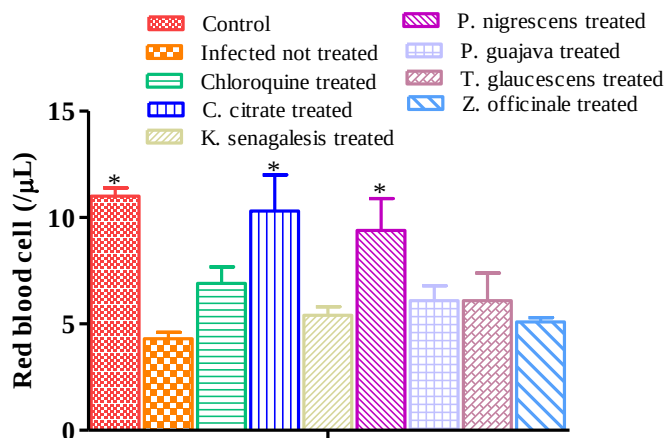


Figure 2a: Red blood cell of *P. berghei* parasitized mice treated with aqueous extracts (200mg/kg bwt.) of some medicinal plant and 5mg/kg bwt chloroquine. The bars are mean $\pm$ SEM of determinations, the * show the statistical different at $P < 0.05$

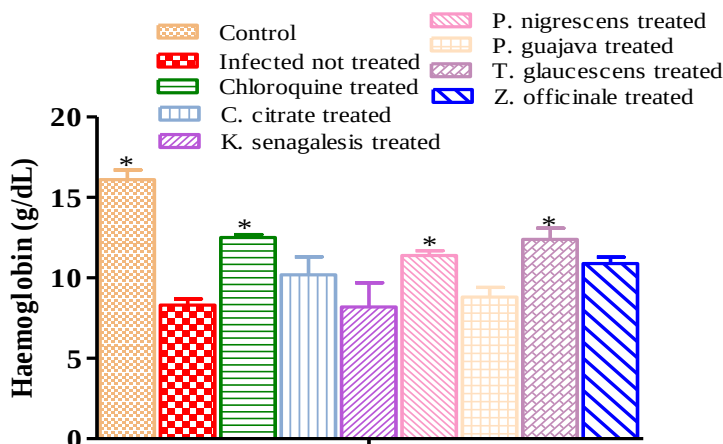


Figure 2b: Heamoglobin count of *P. berghei* parasitized mice treated with aqueous extracts (200mg/kg bwt.) of some medicinal plant and 5mg/kg bwt chloroquine. The bars are mean $\pm$ SEM of determinations, the * show the statistical different at $P < 0.05$

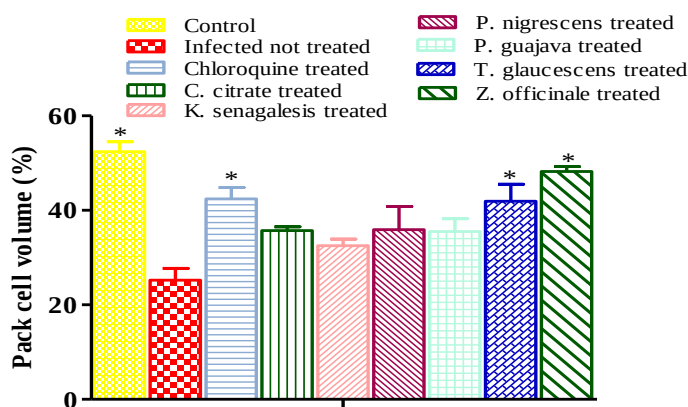


Figure 2c: Pack cell volume of *P. berghei* parasitized mice treated with aqueous extracts (200mg/kg bwt.) of some medicinal plant and 5mg/kg bwt chloroquine. The bars are mean $\pm$ SEM of determinations, the * show the statistical different at $P < 0.05$

There was a significant ($p > 0.05$) decrease in the platelet count of untreated *P. berghei* parasitized mice when compared with extracts and standard drug treated parasitized mice as shown on figure

3. The increase in platelet count in extracts treated was not significant ($p < 0.05$) when compared with chloroquine treated mice.

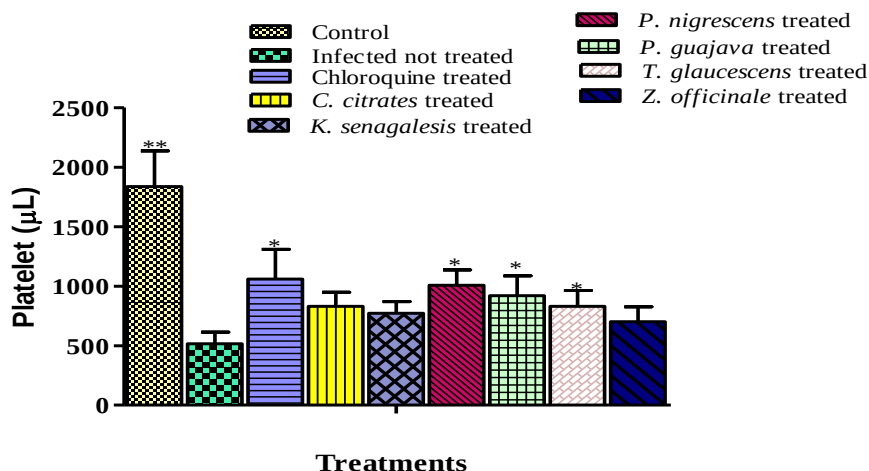


Figure 3: Platelet count of *P. berghei* parasitized mice with treated aqueous extracts (200 mg/kg bwt) of some medicinal plant and 5mg/kg bwt of chloroquine. The bars are mean $\pm$ SEM of determinations, the * show the statistical different at $P < 0.05$ and ** $p < 0.01$

There was a significant increase ($p > 0.05$) in the neutrophil count and decrease in lymphocyte count of untreated *P. berghei* parasitized mice when compared with extracts and chloroquine treated parasitized mice as shown in figure 4a and 4b respectively. The significant ($p > 0.05$) increase in lymphocyte count of extracts treated mice was compared favourably with the control.

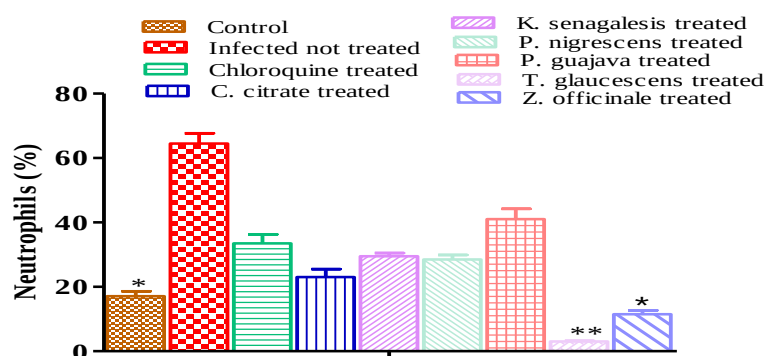


Figure 4a: Neutrophil counts of *P. berghei* parasitized mice with treated aqueous extracts (200 mg/kg bwt) of some medicinal plant and 5mg/kg bwt of chloroquine. The bars are mean $\pm$ SEM of determinations, the * show the statistical different at $P < 0.05$

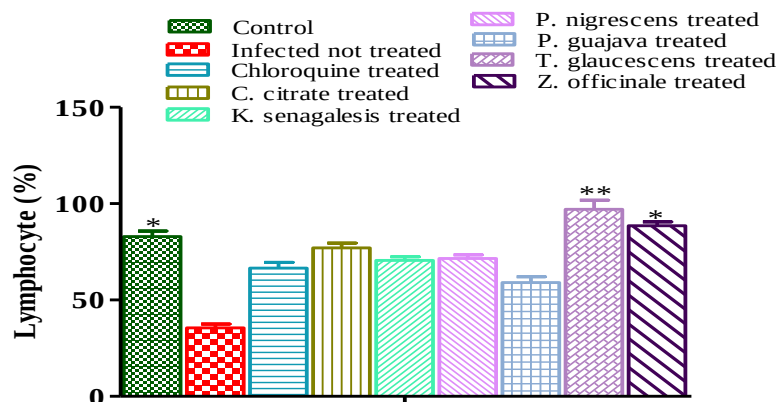


Figure 4b: Lymphocyte counts of *P. berghei* parasitized mice with treated aqueous extracts (200 mg/kg bwt) of some medicinal plant and 5mg/kg bwt of chloroquine. The bars are mean $\pm$ SEM of determinations, the * show the statistical different at $P < 0.05$ and ** $p < 0.01$

Discussion

Haematological changes are some of the most common complications in malaria and they play a major role in malaria pathology (Maina *et al.*, 2010). Reduction in haemoglobin concentration may be accompanied by a fall in the red blood cell count (RBC) and packed cell volume (PCV) (Moss, 1999). The major function of red cells is to contain haemoglobin, which in turn carries oxygen from the lungs to the tissue and carbondioxide from the tissues to the lungs (Waugh and Granti, 2001). In this study there was a significant reduction in RBC, PCV and heamoglobin of untreated *P. berghei* parsitized mice which could have predisposed the animals to anaemic condition (Muhammed and Oloyede, 2009). Lathia and Joshi (2004) reported that reduced heamoglobin and platelet count are associated with malaria. Significant increase in RBC, PCV and heamoglobin of the extract treated parsitized mice in this study implied that the extracts demonstrated an anti anemic property.

High neutrophils count usually implies infection while reduced lymphocyte count indicate a compromised immune system (Jaeger and Hedegaard, 2002). Increased level of neutrophils may also indicate inflammatory response due to toxic effect of the product of the parasite on the tissue, neutrophils are recruited to the site of injury within minutes following trauma and are the hall mark of acute inflammation (Cohen *et al.*, 2002). Increased neutrophil count and reduced lymphocyte count of the untreated *P. berghei* parasitized mice in this study was an indication of inflammation in the system. Significant reduction in neutrophil count and increase in lymphocyte count of the extract - treated parasitized mice were brought to near normal and showed that the extracts demonstrated anti inflammatory property.

White blood cells count are generally observed to be lower during malaria infection (White and Breman, 2001). Decreased in WBC count in the untreated *P. berghei* parsitized mice in this

study was an indication of malaria infection. Whereas there were increase in WBC count in extract-treated mice close to normal indicating that the extract demonstrated antiinflammatory property.

Conclusion

The aqueous extracts of *C. citrates* leaves, *K. senagalesis* stem bark, *P. nigrescens* leaves, *P. guajava* leaves, *T. glaucescens* stem bark and *Z. officinale* rhizome compares favorably with the standard drug (chloroquine) in their effects on the haematological parameters associated with the presence of *P. bergei* infection. Hence these extracts have been established in this work to be anti anaemic. However, extracts of *P. nigrescens*, *T. glaucescens* and *Z. officinale* demonstrate higher anti-anaemic properties than the other extract and chloroquine (standard drug). These findings therefore support the ethno-medicinal use of the aqueous extracts of the plants for treating malaria related anaemia.

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