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Original Research

Effects of *Citrus grandis*, *Azadirachta indica* and *Vernonia amygdalina* extracts on Haematological Parameters of *Plasmodium berghei* Infected Mice

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Abstract

The decoction of *Citrus grandis* leaves, *Azadirachta indica* stem bark, and *Vernonia amygdalina* leaves are used traditionally to treat malaria and other forms of fever in Ilorin metropolis. This study evaluated the effects of aqueous extracts of *Citrus grandis*, *Azadirachta indica* and *Vernonia amygdalina* on the haematological status of *Plasmodium berghei* infected mice. Twenty albino mice were inoculated intraperitoneally with *P. berghei* while five (5) mice were left uninfected to serve as control (Group A). Group B, was infected but not treated and received distilled water. Group C was treated with chloroquine at 5 mg/kg body weight while *C. grandis*, *A. indica*, and *V. amygdalina* extracts were orally administered at 200 mg/kg body weight for 4 days to Groups D, E and F respectively. On the fifth day of treatment, haematological parameters; red blood cell (RBC), white blood cell (WBC) and platelet counts; packed cell volume (PCV), haemoglobin (Hb) concentrations, leukocyte and neutrophils counts were determined. *C. grandis*, *A. indica*, and *V. amygdalina* at 200 mg/kg body weight increased the haemoglobin, RBC and PCV levels of treated *P. berghei* infected mice compared to the untreated mice. Lymphocyte levels of these same groups significantly ($p < 0.05$) increased while neutrophil level reduced. Our findings show that *C. grandis*, *A. indica*, and *V. amygdalina* possess anti-anaemic properties.

Keywords

Haematology; *Citrus grandis*; *Azadirachta indica*; *Vernonia amygdalina*; anaemia; *Plasmodium berghei*

History

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Introduction

Blood is the most easily accessible diagnostic tissue. Variations in haematological parameters are influenced by disease condition, which affects the haemopoietic physiology. This is likely to happen with an endemic disease such as malaria that affects homeostasis of the host (Maina, *et al.*, 2010). The target of malaria parasite is red blood cells.

Haematological changes associated with malaria infection are well recognized, but specific changes may differ from the level of malaria endemicity, background haemoglobinopathy, nutritional status, demographic factors and immunity to malaria (Price *et al.*, 2001). Hence, haematological changes are the most common and important complications encountered and are considered a hallmark of malaria; playing major role in fatality. Prediction of the haematological changes

Prediction of the haematological changes enables the clinician to establish an effective and early therapeutic intervention in order to prevent the occurrence of major complications (Taha *et al.*, 2014). The haematological abnormalities that have been reported include anaemia, thrombocytopenia, lymphocytosis and rarely disseminated intravascular coagulation (Facer, 1994), leucopenia, leucocytosis, neutropenia, neutrophilia, eosinophilia, and monocytosis also have been reported (Murphy and Oldfield, 1996; Jandle, 1996).

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Medicinal plants are important in ethno medical practices with malaria ranking as the most important disease treated with herbal remedies. *Citrus grandis* (grape fruit) is a medium-sized fruit tree in the *Rutaceae* (citrus family) of recent hybrid origin (Akinola *et al.*, 2007). The grape fruit tree typically grows 4.5 to 6 m (15 to 20 ft) tall, leaves are typically ovate, 7.5 to 17.5 cm (3 to 7 in) long, and 4.5 to 7.5 cm wide (William, 2007). *Citrus grandis* were found to have anxiolytic, antioxidant, antidepressant, hepatoprotective, antidiabetic, apoptotic and antimalaria activities (Hata *et al.*, 2003; Adeneye, 2008; Gupta *et al.*, 2009; Jun *et al.*, 2009).

Azadirachta indica, also known as Neem *Azadirachta* is a tree in the mahogany family *Meliaceae*; they are fast growing evergreen trees ranging in height from 12 – 24 m. Neem extract has been reported to have antidiabetic, antibacterial and antiviral activities (Kirtikar and Basu, 1987). The phytochemical present in *A. Indica* are alkaloids, flavonoids, tannins, terpenoids, phenol and saponins (Harry-Asobara and Eno-Obong, 2014).

Vernonia amygdalina, commonly known as bitter leaf, is a shrub or small tree belonging to the family *Asteraceae*. The leaf extract have been reported to possess hypoglycemic, antineoplastic, antibacterial, antioxidant properties and antiparasitic properties (Akah and Okafor, 1992; Taiwo *et al.*, 1999; Masaba, 2000; Abosi and Raseroka, 2003; Iwalewa *et al.*, 2003; Izevbogie *et al.*, 2004; Tona *et al.*, 2004). Phytochemical constituents of the plant include oxalate, phytate, tannins, saponins, flavonoids, cyanogenic glycosides, alkaloids, anthraquinones, sesquiterpenes lactones, vernoniosides, steroids, phenolics (Igile *et al.*, 1995; Cimanga *et al.*, 2004). This study evaluated the effects of aqueous extracts of *C. grandis* leaves, *A. Indica* stem bark, and *V. amygdalina* leaves on the haematological status of mice infected with *P. berghei*.

Materials and methods

Plant materials, authentication and preparation

Citrus grandis leaves, *Azadirachta indica* bark and *Vernonia amygdalina* leaves were bought from Oja Tuntun, Ilorin, Kwara State, Nigeria and identified in the Department of Plant Biology, University of Ilorin, Nigeria. Herbarium specimens with their voucher numbers UIH1081, UIH979, UIH972 respectively were deposited at the University of Ilorin Herbarium, Nigeria.

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 passage of parasitized blood into mice.

Experimental animals

Swiss albino mice (25) with mean weight of 21.5 ± 2.3 g were obtained from small animal holding unit, Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were placed on standard pellet feed and allowed to drink water *ad libitum*. They were allowed to acclimatize for 2 weeks before the commencement of the study.

Preparation of plant extracts

Plant samples were washed, air-dried to constant weights and pulverized into powder. The powdered samples, 150 g each, were extracted for two days using distilled water. The resulting mixture was filtered with Whatmann filter paper, concentrated using freeze dryer

serial dilutions made with distilled water to get the appropriate concentrations for oral administration to the mice.

Blood Collection and Haematological Assays

On the 5th day, the mice were sacrificed by stunning and blood was collected by heart puncture. Blood for haematological parameters was collected in EDTA bottles. The following haematological parameters were evaluated using standard methods: red blood cell (RBC) counts, white blood cell (WBC) count; platelet count, packed cell volume (PCV), haemoglobin (Hb) and differential leukocyte count, using automated haematologic analyzer, Sysmex, KX- 21 (Japan).

Statistical Analysis

The significance of difference among groups was determined by the one-way analysis of variance (ANOVA), the Duncan's Test was used for Post Hoc analyses and $P < 0.05$ was accepted as significant at $p < 0.05$. Graphpad prism 6 and SPSS 16.0, SPSS Inc., Chicago, Illinois, USA, were used for analyzing the data obtained from this study.

Results

The packed cell volume (PCV) was significantly ($p < 0.05$) lower in the group that untreated group when compared with the uninfected group and other treated groups (Fig. 1). The haemoglobin (Hb) levels were the same in all the treated animals, while the level was insignificantly ($p > 0.05$) lowered in the group that was not treated as compared to extract treated groups (Fig. 2). Figure 3 shows that the red blood cell (RBC) level was significantly ($p < 0.05$) higher in the group treated with 5 mg/kg body weight of chloroquine than all other groups treated with extracts and it was lower in the group that was not treated when compared with the group that was treated with extract.

Platelet count for uninfected mice was significantly ($p < 0.05$) higher compared to the untreated group. However, mice treated with extracts had significant increase ($p < 0.05$) in platelet count when compared to untreated group. Although, the extracts did not compare with the control, there was no significant ($p < 0.05$) between the treatment groups and reference drug, chloroquine treated groups (Fig. 4).

Similarly, white blood cell of control mice was significantly ($p < 0.05$) higher than untreated mice. While extracts (except *C. grandis*) treatment compared significantly with chloroquine treated group, *A. indica* and *V. amygdalina* extracts compared well with chloroquine treated group (Fig. 5).

There was significant increase ($p < 0.05$) in the neutrophil count of mice infected but not treat when compared to the control mice. This increase was significantly lowered by the extracts, which compared with both control and chloroquine treated groups (Fig. 6). Conversely, the lowered lymphocyte in untreated group was significantly annulled by raised by the extracts and compared significantly with control (Fig. 7).

Discussion

Evaluation of complete blood count provides useful information on the haematological status in disease conditions (Lathia and Joshi, 2004). In this study, the untreated *P. berghei* infected mice had the lowest values of PCV, Hb, RBC. This is consistent with the anaemia seen in malaria. An unusually low haemoglobin concentration recorded is implicative of anaemia. This may result from a reduction in erythrocyte number or a reduction in the concentration of haemoglobin in each erythrocyte (Lathia and Joshi, 2004). Treatment with *C. grandis*, *A. indica* and *V. amygdalina* extract however increased the values of PCV, Hb and RBC when compared with the untreated group; this

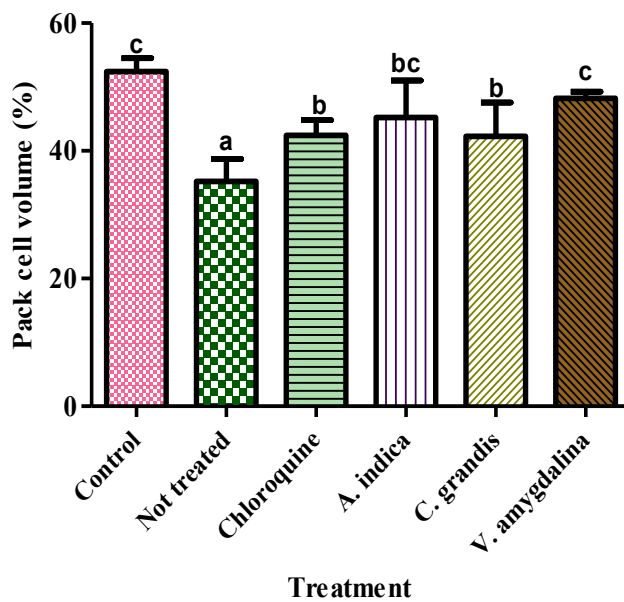


Figure 1: Effects of *C. grandis*, *A. indica* and *V. amygdalina* extracts at 200 mg/kg body weight on packed cell volume (PCV) of *P. berghei*-Infected Mice. Data are presented as means of 5 determinations $\pm$ SEM. Different alphabet notations are significant different at $p < 0.05$

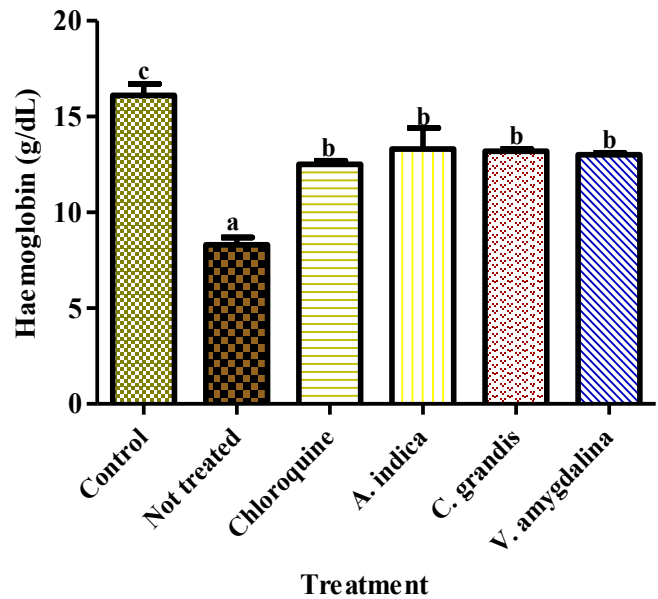


Figure 2: Effects of *C. grandis*, *A. indica* and *V. amygdalina* extracts at 200 mg/kg body weight on haemoglobin (Hb) concentration of *P. berghei*-Infected Mice. Data are presented as means of 5 determinations $\pm$ SEM. Different alphabet notations are significant different at $p < 0.05$

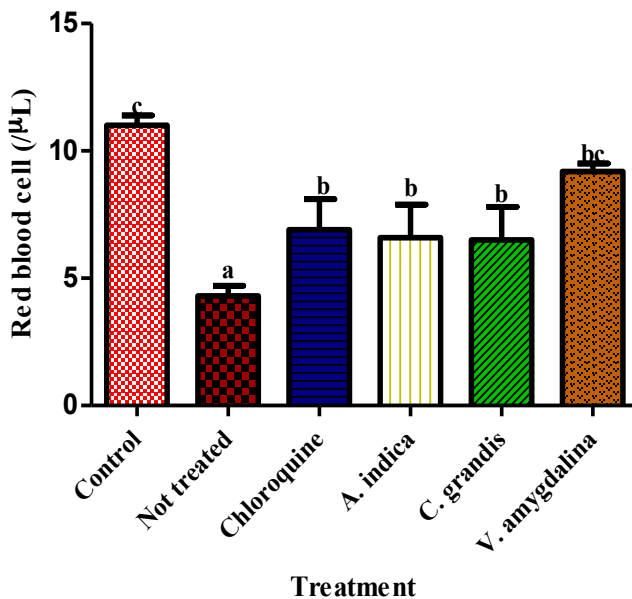


Figure 3: Effects of *C. grandis*, *A. indica* and *V. amygdalina* extracts at 200 mg/kg body weight on red blood cell (RBC) count of *P. berghei*-Infected Mice. Data are presented as means of 5 determinations $\pm$ SEM. Different alphabet notations are significant different at $p < 0.05$

Lathia and Joshi (2004) reported that reduced haemoglobin and platelet count increase the probability of malaria by factors of 1.95 and 5.04 respectively. The slight increase in platelet count seen in *P. berghei* infected mice and treated with chloroquine and all the extract in this study may be as a result of the short period of infection; it is possible that prolonged or repeated infections may lead to thrombocytopenia.

White blood cells (WBCs), as well as other cells, are involved in the body's immune system and help to fight diseases. They are increased in normal situations such as exertion and pregnancy, and abnormally in situations such as loss of blood, cancer, and most infections. Though decrease in WBCs have been demonstrated to be linked to severe malaria (Modiano *et al.*, 2001), during acute malaria WBC counts are generally observed to be low or normal (White and Breman, 2001). Our results show that WBC counts for all groups were not significantly ($p < 0.05$); however, Chloroquine, *C. grandis*, *A. indica* and *V. amygdalina* in separate dosages slightly increase WBC count (Fig. 5).

A high neutrophil count usually implies an infection while a reduced lymphocyte count may indicate a compromised immune system or if the case of increased neutrophils, imply an infection (Jaeger and Hedegaard, 2002). *P. berghei* infection significantly ($p < 0.05$) altered neutrophil and lymphocyte counts, whereas treatment with *C. grandis*, *A. indica* and *V. amygdalina* at 200 mg/kg body weight increase the levels of neutrophil and lymphocyte counts when compared with the value of uninfected group (Figs. 6 and 7).

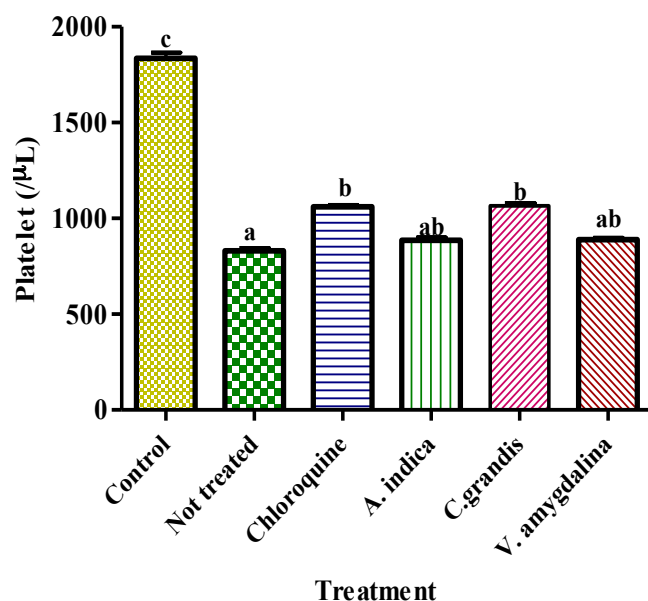


Figure 4: Effects of *C. grandis*, *A. indica* and *V. amygdalina* extracts at 200 mg/kg body weight on platelet count of *P. berghei*-Infected Mice. Data are presented as means of 5 determinations $\pm$ SEM. Different alphabet notations are significant different at $p < 0.05$

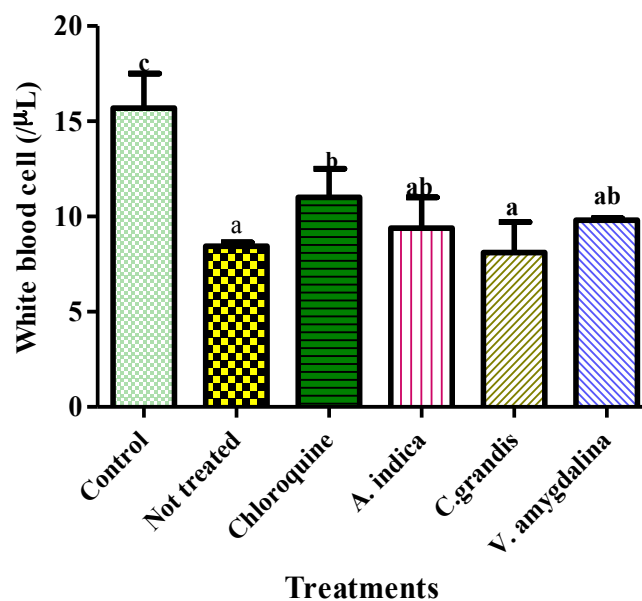


Figure 5: Effects of *C. grandis*, *A. indica* and *V. amygdalina* extracts at 200 mg/kg body weight on white blood cell (WBC) count of *P. berghei*-Infected Mice. Data are presented as means of 5 determinations $\pm$ SEM. Different alphabet notations are significant different at $p < 0.05$

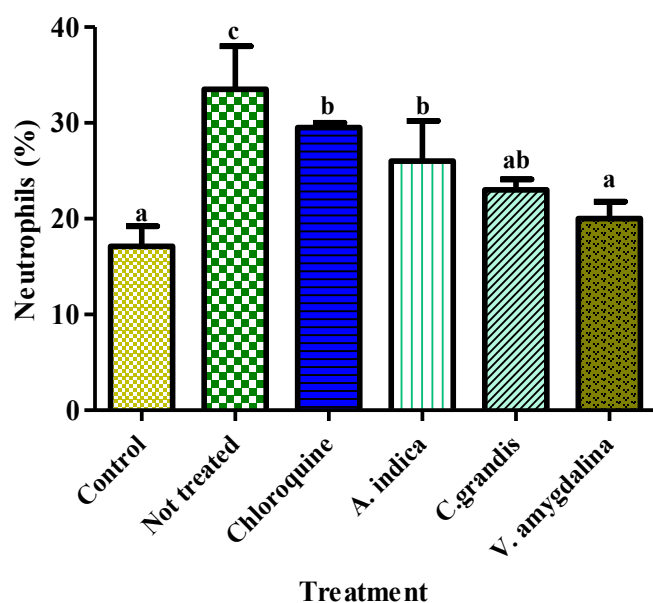


Figure 6: Effects of *C. grandis*, *A. indica* and *V. amygdalina* extracts at 200 mg/kg body weight on neutrophil count of *P. berghei*-Infected Mice. Data are presented as means of 5 determinations $\pm$ SEM. Different alphabet notations are significant different at $p < 0.05$

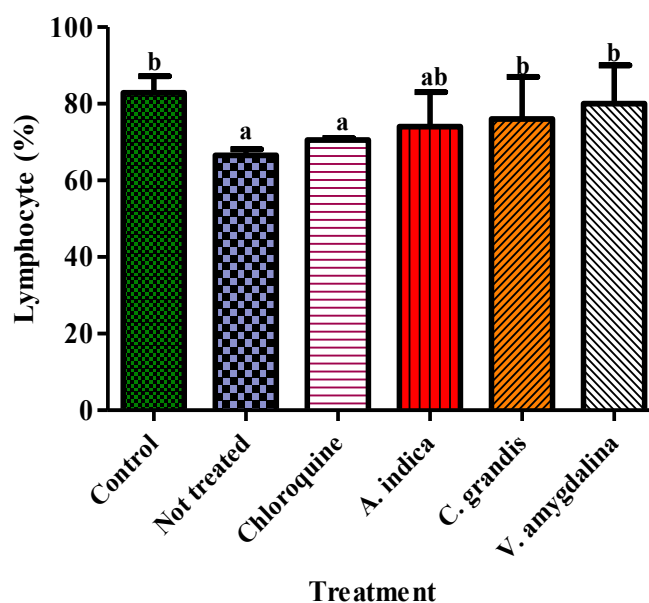


Figure 7: Effects of *C. grandis*, *A. indica* and *V. amygdalina* extracts at 200 mg/kg body weight on lymphocyte count of *P. berghei*-Infected Mice. Data are presented as means of 5 determinations $\pm$ SEM. Different alphabet notations are significant different at $p < 0.05$

Conclusion

The findings of this study showed that aqueous extract of *Citrus grandis* leaf, *Azadirachta indica* bark, and *Vernonia amygdalina* leaf possess antianaemic activity as evident from the modulation of plasmodium-mediated alterations in hematological parameters. compares closely with the chloroquine and the effects on These findings therefore support the ethnomedicinal use of the aqueous extract of *Citrus grandis* leave, *Azadirachta indica* stem bark, and *V. amygdalina* leave for treating malaria related anaemia.

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