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Haematological changes in malaria infested Wistar albino rats treated with the crude extract of Artemisia Annua and Artemisinin combination therapy

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Abstract
The haematological changes in malaria infested wistar albino rats treated with the crude extract of *Artemisia annua* and artemisinin combination therapy was investigated using 24 wistar albino rats weighing between 180 – 220g. The rats were divided into four groups of six rats each. Group 1 served as the normal control and received 0.2ml of bottled water, group 2 animals were malaria induced but untreated and received 0.2ml of bottled water, group 3 animals were malaria induced and received ACT (1.142mg/kg body weight), group 4 animals were malaria induced and received the crude extract from *Artemisia annua* (300mg/kg body weight). The administration was carried out twice a day for three consecutive days. The rats were given free access to food and water. At the end of administration, blood samples were taken through cardiac puncture and the haematological indices, PCV (%), Hb (g/dL), RBC, ($x10^9$/L), WBC, ($x10^9$/L) and platelets ($x10^9$/L) were assayed. From the result, the PCV, Hb and RBC of group 3 showed no significant difference when compared with the control, though the values were non-significantly higher ($p>0.05$) when compared to group 2. The WBC count showed a significant increase ($p<0.05$) when compared to group 2 and1 while the platelet count showed a significant difference when compared to group 2 and1. The WBC (13.38±0.55) and platelet (725.00±6.45) counts of group 4 showed a significant difference when compared to group 2 and1; there was a non significant increase in PCV, Hb and RBC count when compared to the control. The results show that ACT and the crude extract of *A. annua* have no negative effects on the haematological indices and hence the crude extract of *A. annua* may be recommended for those who cannot have access to the refined artemisinin combination therapy.

Keywords: plasmodium, Haematology, *Artemisia annua*, Artemisinin, phytochemical

Introduction
Malaria now a global burden is one of the most serious health challenges facing the world today. It is a mosquito-borne infectious disease of humans and other animals caused by *Plasmodia* and are also definitely the single most destructive and dangerous infectious agent in developing countries of the world (Olayinka and Ore, 2013) [7]. This disease results from the multiplication of *Plasmodium* parasites within red blood cells. Studies revealed that five species of *Plasmodium* can infect and be transmitted by humans (Sutherland *et al*., 2010) [12]. Malaria is largely caused by *Plasmodium falciparum* while the malaria caused by *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae* is generally a milder disease that is rarely fatal (Sutherland *et al.,* 2010) [12]. *Plasmodium knowlesi* is a zoonosis that causes malaria in macaques which can also infect humans (Sibley *et al.*, 2004) [11]. Malaria is transmitted through the bite of an infected female Anopheles mosquito, on biting a healthy individual; it injects its saliva which contains the sporozoites. The sporozoites enter the blood stream and migrate to the liver. They infect the liver cells, where they feed, grow and multiply at the expense of the liver cells into merozoites. The liver cells ruptures and the merozoites are released into the blood stream, some merozoites infects red blood cells where they feed, multiply and develop into the mature trophozoites and schizonts through a process called schizogony. While some merozoites re-enter new liver cells to form a second generation sporozoites, merozoites, trophozoites and schizonts, respectively. These organs/tissues (liver and blood) are prone to damage by toxins or metabolites which are released by the plasmodial parasites to the host organism's circulatory system during the process of metabolism. This tends to disrupt the blood components/cells or blood forming tissues. Blood is one of the most specialized body fluid responsible for the transportation of nutrients, oxygen, hormones and other metabolites to the body cells and in turn transports metabolic waste products away from these cells to sites of elimination. It is known to be the most important body fluid that regulates various vital functions of the body. In the mammalian circulatory system, the blood transports such specific chemical substances as nutrients, gases, minerals, metabolic products and hormones between different tissues/cells and organs (Baynes and Dominiczak, 2005) [2].
There were an estimated 225 million cases of malaria worldwide in 2010. An estimated 655,000 people died from malaria in 2010, a decrease from the 781,000 who died in 2009 (WHO, 2011) [11], accounting for 2.23% of deaths worldwide. However, a 2012 meta-study from the University of Washington and University of Queensland estimates that malaria deaths are significantly higher (Christopher *et al*., 2012) [3]. The study estimates that 1,238,000 people died from malaria in 2010. Ninety percent of malaria-related deaths occur in sub-Saharan Africa, with 60% of deaths being young children under the age of five (Christopher *et al*., 2012) [3]. Despite advances in scientific knowledge, malaria continues to cause significant morbidity and mortality worldwide.
In the light of this endemic disease that have defied numerous preventive or control measures and drugs, artemisinin combination therapies (ACTs) have proved to be

a potent cure or prevention of malaria. Artemisinin is the active ingredient of *Artemisia annua. Artemisia annua* also known as qinghao or sweet wormwood is a medicinal plant native to China and well known for its anti-plasmodial, anti-rheumatic and anti-cancer properties (Ogbole *et al.*, 2014) [6]. Phytochemical extracts of the *A. annua* leaf powder contains, alkaloids, coumarins, flavonoids, sterols and triterpenes, tannins, volatile oils, higher fatty acids, saponins, glycosides, and reducing compounds (Ajah and Eteng, 2010) [1].

Since the discovery of *A. Annua* in early 1970s, hundreds of papers have focused on the anti-parasitic effects of artemisinin and its semi-synthetic analogs, artemether, dihydroartemisinin, arteether, and artesunate. However, the haematological changes and anomalies that follow its administration have not been critically or carefully assessed and analyzed. Therefore, there is paucity of information in the literatures on the haematological changes following malarial treatment. Because of the danger malaria has posed to mankind and not knowing how the anti-malarial drugs affect man's blood biochemistry, and since haematological indices provide crucial information to assessing the well-being of an organism, it therefore became interesting to investigate the haematological changes in malaria infested wistar albino rats treated with the crude extract of *Artemisia annua* and an artemisinin combination therapy.

Materials and methods

Collection and preparation of materials

ACTs, Dextrose and Distil Water: The anti malarial drug zymal® (Artemether Tablet 80mg + Lumenfantrine Tablets 480mg) manufactured by Innova CapTab, Pharmaceutical Co Ltd, 81-B EPIP, Phase-I, Jharmajri, Baddi (H.P) India. Manufacturing Lic. No.: MNB/06/394, Distilled water and 5% dextrose water was bought from Turtle Bay pharmacy in Calabar.

Plant material: The leaves of *Artemisia annua* was collected from the biotechnology farm operated by Prof. Ebiamadon Andi Brisibe, of the Department of Genetics and Biotechnology, Faculty of Science, University of Calabar. It was taken to the Botany Department of the University for Identification and specimen was deposited at the department's herbarium.

Parasites: The strain of *Plasmodium falciparum* that was used for this study was obtained from the Calabar office of Roll Back Malaria.

Animals: A total of 24 Inbreed adult male and female wistar albino rats weighing between 180 - 220g were used for this study, they were purchased from the animal house unit of the Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, and were housed in a well ventilated wooden cages in the animal house, and were fed with rat pellets (growers' marsh manufactured by Vital feeds Ltd, Lagos) and tap water *ad libitum.* The animals were acclimatized for three weeks and their body weights noted before and after the commencement of the experiment. The animals were divided into four groups, based on their weights as shown in table 1.

Innoculation: The infection of the recipient rats was initiated by injection of the parasites preparation gotten from the Calabar office of Roll Back Malaria to healthy test rats via intramuscular route as described by David *et al* (2004) [4] and Peter and Anatoli (1998) [9]. 2ml of *Plasmodium falciparum* sample with a parasite load of 161.5 was diluted with 5% dextrose water using a dilution factor of 1:4 (Shakya *et al.*, 2012) [10]. 0.5ml per kilogram of body weight of the diluted plasmodium base solution was subsequently injected into the animals in group 2, 3 and 4 via intramuscular method (David *et al.*, 2004) [4].

Determination of Degree of Parasitaemia

The CareStart™ Malaria HRP2 Pf (Cat #: G0141) test kit, manufactured by Access Bio, Inc. 65 Clyde Road, Somerset, NJ, 08873, USA, was used to investigate the level of infection in the groups of rats that were inoculated with the malaria parasites. 48 hours after inoculation, a drop of blood was collect from the tails of the infected rats and tested for the presence of *plasmodium* according to the method describe by the manufacturer.

Administration of Drug: The antimalarial drug, zymal® (Artemether 80mg + Lumenfantrine 480mg) tablet was used as the artemisinin combination therapy. It was powdered in a mortar, mixed with 50ml distilled water and administered as aqueous suspension by oral gavage at a dose of 1.143mg/kg body weight twice a day for three consecutive days.

Administration of Extract: 40g of the powdered *Artemisia annua* leaves was socked in ethanol for 12 hours and filtered thereafter. The filtrate was further filtered using a watman filter paper and then concentrated by evaporation using a water bath at 40ºC. The 40g of powdered *A. annua* leaves yielded 2.9g of extract. The crude extract was administered to group 4 animals at a dose of 300mg/kg body weight twice a day for three consecutive days.

Collection and preparation of tissue for analysis: After 3 days of treatment, the rats were weighed and fasted overnight. Blood samples were collected from the untreated, treated and control groups for haematological investigation. The animals were anaesthetized with trichloromethane (chloroform). They were then dissected and blood samples were collected through cardiac puncture using sterile syringes into screw cap test tubes containing EDTA for haematological analysis.

Haematological methods: The Red Blood Cell (RBC) Count, Haemoglobin (Hb) concentration, The Packed cell volume (PCV), White blood cell (WBC) count and Platelet (PLT) counts were determined using Sysmex KX-21N automated haematology analyzer (Sysmex America Inc. USA).

Statistical analysis: Data was expressed as mean ± standard error of mean. The data obtained were analyzed statistically using one-way analysis of variance (ANOVA) at a 95% (0.05) probability level.

Results: Table 2 shows results of the haematological parameters, packed cell volume (PCV) (%), haemoglobin (Hb) (g/dL), red blood cell (RBC) count ($x10^9$/L), white blood cell (WBC) count ($x10^9$/L) and platelets (PLT) count ($x10^9$/L).

Packed cell volume (PCV) (%): From Table 2, there was a non-significant decrease ($p>0.05$) in the packed cell volume count of the untreated group (42.00±2.10) when compared with the control group (43.17±2.48). However, there was a

non significant increase (p>0.05) in the group treated with ACT (44.33±2.25) when compared with the control group, and also shows a non significant increase (p>0.05) when compared with the untreated group. Also, there was a non significant increase (p>0.05) in the PCV of the group treated with the *A. annua* extract (45.00±3.29) compared to the control group, untreated group and the ACT treated group.

Haemoglobin concentration (g/dL): Table 4.2 shows that, the malaria induced but untreated group (13.50±0.80) showed a non-significant decrease (p>0.05) when compared to the control group (14.20±0.66). The group treated with ACT (14.03±1.02) showed no significant difference (p>0.05) when compared with the control group, but showed a non significant increase (p>0.05) when compared with the untreated group. However, the group treated with *Artemisia annua* extract (14.62±1.02) showed a significant increase (p<0.05) when compared to the untreated group, but showed no significant difference (p>0.05) when compared with the control group and the ACT treated group.

Red Blood Cell (RBC) counts (x10^9/L): Table 2 shows that, there was a non significant decrease (p>0.05) in the red blood cell counts of the untreated group (848.17±5.01) when compared to the control group (861.83±6.99). The RBC counts of the ACT treated group (857.83±6.01) also showed a non significant decrease (p>0.05) when compared with the control group but gives a non significant increase (p>0.05) when compared with the untreated group. However, the group treated with crude extract of *A. annua* (865.33±6.36) showed a non significant increase (p>0.05) compared to the control group, untreated and ACT treated group.

White Blood Cell (WBC) Counts (x10^9/L):
Table 2 shows no significant difference (p>0.05) between the WBC counts of the malaria infested but untreated group (7.67±0.44) and the control group (7.88±0.58). However, there was a significant increase (p<0.05) in the group treated with ACT (12.58±1.76) when compared with the untreated and control group, but non-significant decrease (p>0.05) when compared with the group treated with the crude extracts of *Artemisia annua* (13.38±0.55). The group treated with the *A. annua* extract showed a significant increase (p<0.05) in WBC counts compared to the untreated and control groups but showed a non significant increase (p>0.05) when compared with the ACT treated group.

Platelets (PLT) Count (x10^9/L): In table 2, the platelets count of the rats in the malaria induced but untreated group (631.00±9.22) was significantly lower (p<0.05) when compared to the control group (747.00±7.42). The platelet count of the ACT treated group (709.33±7.94) showed a significant decrease (p<0.05) when compared with the control group, and its value is significantly higher (p<0.05) than the untreated group but shows a non significant decrease when compared with the group administered with the crude extract of *A. Annua* (725.00±6.45). However, the group treated with the extract of *A. annua* had a significantly lower (p<0.05) platelet count compared to the control group but significantly higher (p<0.05) than that of the malaria induced but untreated group.

Discussion
Haematological parameters are good indicators to assess the physiological state of animals and the alterations of any of the haematological indices could be used to assess the response of the animals to various physiological situations. The various haematological indices investigated in this study are useful in evaluating the haematological changes associated with the administration of drugs and plant extracts.
From the results obtained in this study, significant (P<0.05) and non-significant (p>0.05) differences were observed in all the treated groups and the untreated group when compared with the control group. The results showed that, the packed cell volume (PCV), and the red blood cell (RBC) count decreased non-significantly in the untreated group when compared to the control group, which may be attributed to erythrocytes lyses caused by the malaria parasite infestation, a tendency that can result to anaemia. Ovuakporage (2011) [8] reported that malaria parasite reduces Red blood cell count, Packed Cell Volume (PCV) and Haemoglobin (Hb) concentration. The PCV, RBC count and Hb concentration of group 3 animals that were treated with ACT seems to return to normal, while in the *Artemisia annua* treated groups, there was a non significant increase in these haematological parameters when compared to other groups, indicating that the extracts may not have induced negative effect on these parameters. Previous studies have reported that dehydroartemisinin, a derivative of artemisinin significantly elevated the packed cell volume (Utoh-Nedosa *et al.*, 2009) [13], while artesunate and dehydroartemisinin has been reported to cause no significant effect on RBC and Hb, (Obianime and Aprioku, 2009) [5].
However, at the end of experiments, the groups treated with ACT and the extract from *A. annua* showed no significant difference on the haemoglobin concentration (Hb) when compared with the control group. Although, the group treated with the extract from *A. annua* showed a significant increase in haemoglobin concentration when compared with the untreated group, while the ACT treated group showed no significant difference when compared with the untreated group. The elevated levels of haemoglobin in the *A. annua* treated group could be as a result of increase in number or sizes of the RBCs. This observation is also in line with previous study by Obianime and Aprioku (2009) [5].
White blood cell (WBC) (x10^9/L) count showed a non-significant decrease in the untreated group when compared to the control group. This non significant decrease in WBC count (leucopenia) could be as a result of the infection. However, the groups treated with ACT and the *Artemisia annua* extract showed a significant increase in WBC count when compared to the control and untreated groups. The ability of the ACT and the crude extract of *Artemisia annua* to increase WBC could also leads to immune boosting tendency. There was a significant decrease in the platelets counts of the malaria induced but untreated group when compared to the control group. This condition of decreased (low) platelet count called thrombocytopenia, could only be as a result of the malaria infection. The *A. annua* extract treated group showed a non significant increase in the platelet count on comparison with the ACT treated group. Though, both ACT and *A. annua* treated groups showed a significant decrease when compared with the control group, but there was a significant increase in platelet counts of the

ACT and extract treated groups on comparison with the untreated group. This implies that treatment with ACT and *A. annua* are both effective in normalizing platelet counts during malaria infestation.

Table 1: Experimental protocol Experimental group distribution of wistar albino rats during treatment with anti malarial drug (ACT) and extract from *Artemisia annua* leaves.

Groups	Number of rats	Treatment
1	6	Normal control
2	6	Malaria infected and Untreated
3	6	Malaria infected and ACT treated
4	6	Malaria infected and *A. Annua* treated

Table 2: Haematological baseline values Haematological changes during the administration of ACT and *A. annua* extract in malaria infested wistar albino rats.

Groups	PCV (%)	Hb(g/dL)	RBC ($x10^9$/L)	WBC ($x10^9$/L)	Platelets ($x10^9$/L)
1 Normal control	43.17±2.48	14.20±0.66	861.83±6.99	07.88±0.58	747.00±7.42
2 (malaria) Untreated	42.00±2.10	13.50±0.80	848.17±5.01	07.67±0.44	631.00±9.22*
3 ACT treated	44.33±2.25	14.03±1.02	857.83±6.01	12.58±1.76*[a]	709.33±7.94*,[a]
4 *A. annua* treated	45.00±3.29	14.62±1.02[a]	865.33±6.36	13.38±0.55*,[a]	725.00±6.45*,[a]

Values are expressed as mean ± SEM, n=6.
*=significantly different from normal control at (P<0.05)
a=significantly different from untreated at (P<0.05)

Conclusion

The haematological changes in malaria infested wistar albino rats administered with the crude extract of *Artemisia annua* and artemisinin was investigated in this research. The results of this study indicate that the treatment of malaria using ACT results in improved haematological indices. However, using the crude extract of *Artemisia annua* appears to be more potent and should be encouraged.

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