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Hematological Responses and Percentage Parastaemia in Malaria-Infected Mice Treated with Ethanol Extract of *Zapoteca portoricensis* Roots

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Abstract

The increasing use of *Zapoteca portoricensis* roots in the treatment of malaria has necessitated the need to assay for the haematological responses and parasitaemia levels in malaria-infected mice treated with ethanol extract of *Zapoteca portoricensis* roots. This was done by determining the percentage parasitaemia and some haematological parameters such as packed cell volume (PCV), haemoglobin concentration (Hb), total white blood cell count (TWBC) and red blood cell count (RBC). The *Zapoteca portoricensis* roots were randomly collected from Umabor Ehalumona, Nsukka, Enugu State, Nigeria. Treatment of infected mice with the ethanol extract of *Zapoteca portoricensis* roots caused mean percentage parasitaemia to reduce significantly ($p < 0.05$) in groups 4, 5 and 6 administered 100, 200 and 300 mg/kg b.w. of the extract respectively when compared to the group 2 mice (malaria untreated). Haematological parameters such as PCV, Hb concentration and RBC count significantly ($p < 0.05$) increased in groups 4, 5 and 6 administered 100, 200 and 300 mg/kg b.w. of the extract respectively when compared to group 2 (malaria untreated) but TWBC count did not change significantly ($p > 0.05$) in the controls, group 1 (normal control), groups 2 (malaria untreated) and 3 administered 28 mg/kg b.w. of artemether and lumefantrine and test groups 4 and 6 administered 100 and 300 mg/kg b.w. of the extract respectively. The investigation validated the use of the root extract of *Zapoteca portoricensis* in the treatment of malaria in traditional medicine.

Keywords: *Zapoteca portoricensis*; Percentage parasitaemia; Haematological parameters

Introduction

Malaria remains one of the most widespread infectious diseases of our time. The latest estimates reveal that approximately (~) 250 million people are infected with malaria across the globe, of which ~800,000 die every year [1], the vast majority being young children. Most available anti-malarials were designed to target the pathogenic blood stages in humans and to address the constant threat of drug resistance [2]. Five species of *Plasmodium* can infect and be transmitted by humans. The vast majority of deaths are caused by *P. falciparum* while *P. vivax*, *P. ovale*, and *P. malariae* cause a generally milder form of malaria that is rarely fatal. Malaria is prevalent in tropical and subtropical regions because of rainfall, warm temperatures, and stagnant waters provide habitats ideal for mosquito larvae [3].

The signs and symptoms of malaria typically begin 8-25 days following infection [4]. However, symptoms may occur later in those who have taken antimalarial medications as prevention. According to Nadjm and Behrens [5], initial manifestations of the disease common to all malaria species are similar to flu-like symptoms [6] and can resemble other conditions such as septicemia, gastroenteritis, and viral diseases. The classic symptom of malaria is paroxysm which is a cyclical occurrence of sudden coldness followed by rigour and then fever and sweating, occurring every two days (tertian fever) in *P. vivax* and *P. ovale* infections, and every three days (quartan fever) for *P. malariae*, *P. falciparum* infection can cause recurrent fever every 36-48 hours or a less pronounced and almost continuous fever [7].

Traditional methods of treatment and control of malaria could be a promising source of potential anti-malaria drugs [8-11]. More than 80% of the world's population relies on traditional medicine for their primary healthcare needs [12]. In developing countries, low income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infectious diseases. These plants are ingested as decoctions, teas or juice preparations [13].

Zapoteca portoricensis belonging to the family *Fabaceae* are traditionally used as anti diarrhoel, anti canvulsant, antispasmodic and in the treatment of tonsillitis. Terpenoids and steroids obtained from the column fractions of the root extracts are proved to be responsible for the production of significant anti-inflammatory activity [14]. This study was designed to investigate the antimalarial properties of the ethanol extract of *Zapoteca portoricensis* root.

Severe malaria is usually caused by *P. falciparum* (often referred to as *falciparum malariae*). Symptoms of falciparium malaria arise 9-30 days after infection [6]. Splenomegaly, severe headache, hepatomegaly (enlarged liver), hypoglycemia, and haemoglobinuria with renal failure may occur. Renal failure is a feature of blackwater fever, where haemoglobin from lysed red blood cells leaks into the urine [6].

Materials and Methods

Plant material

The roots of *Zapoteca portoricensis* (Elugelu) were collected from Umabor-Ehalumona in Nsukka Local Government Area of Enugu State, Nigeria. The plant was identified by Mr. A. Ozioko of Bioresources Development and Conservation Program (BDCP) Research Centre Nsukka.

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Animals

Thirty six (36) Wistar albino mice weighing 20-40 g were used for the study. The mice were obtained from the Faculty of Biological Sciences, University of Nigeria, Nsukka. The animals were acclimatized for one week, under a standard condition with 12 hr light and dark conditions with free access to food and water before the commencement of the experiments.

Extraction procedure

The roots of *Zapoteca portoricensis* were collected, washed with distilled water and dried under room temperature for 4 weeks. The dried roots were pulverized into powdered form with a high speed milling machine. The powdered sample (500 g) was macerated in 2.5 L absolute ethanol for 24 hr. After that, the resulting extract was filtered using Wattman No 1 filter paper. The resulting filtrate was concentrated to dryness using rotary evaporator at temperature of 60°C. The concentrated extract was stored in the refrigerator and used for the study.

Experimental design

Thirty six Wistar albino mice weighing 20-40 g were housed in separate cages, acclimatized for one week and then divided randomly into six groups of six mice each. The animals were allowed free access to water and feed. The route of administration (treatment) was oral with the aid of an oral intubations tube. The animals were grouped, inoculated and treated as follows:

Group 1: Normal control which was not inoculated with malaria parasite and was treated with 5 ml/kg body weight of distilled water.

Group 2: The positive control inoculated with malaria parasite (mp^+) and was treated with 5 ml/kg body weight of distilled water.

Group 3: Inoculated with malaria parasite (standard control) and was treated with 28 mg/kg body weight of artemeter and lumenfantrine.

Group 4: Inoculated with malaria parasite and treated with 100 mg/kg body weight of the ethanol extract of *Zapoteca portoricensis* roots.

Group 5: Inoculated with malaria parasite and treated with 200 mg/kg body weight of the ethanol extract of *Zapoteca portoricensis* roots.

Group 6: Inoculated with malaria parasite and treated with 300 mg/kg body weight of the ethanol extract of *Zapoteca portoricensis* roots.

The animals in group 2-6 were inoculated with malaria parasite as shown above and confirmed positive on the 7^{th} day before treatment commenced. The experiment lasted for 28 days during which blood samples were collected through ocular puncture on days 0, 7, 14 and 28 in EDTA bottles and non-heparinized tubes for the analysis.

Determination of percentage yield of the extract

The percentage yield of the extract was determined by weighing the pulverized dry roots and the concentrated extract obtained after extraction and then calculated using the formula:

$$\text{Percentage yield} = \frac{\text{Weight (g) of the extract}}{\text{Weight (g) of pulverized roots}} \text{ X } 100\%$$

Determination of percentage parasitaemia

The determination of malaria parasitaemia (Mp+) was carried out according to the method of Dacie and Lewis [15].

Determination of hematological parameters

The determination of hematological parameters, hemoglobin concentration, packed cell volume; total white blood cell count and red blood cell count were carried out according to the method of Dacie and Lewis [15].

Result

From the result in Table 1, Percentage yield of the ethanol extract of *Zapoteca portoricensis* roots was 3.18%. As shown in Figure 1, Day 0 result showed significant ($p < 0.05$) decrease in the mean percentage parasitaemia in groups 3, 4 and 5 malaria-passaged mice administered standard drug (28 mg/kg b.w of artemether and lumenfantrine), 100 and 200 mg/kg b.w. of the extract respectively compared to the percentage parasitaemia in group 2 mice (malaria untreated) group. The mean percentage parasitaemia showed significant ($p < 0.05$) decrease in groups 3 treated with 28 mg/kg b.w. of artemeter and lumenfantrine, 4, 5 and 6 administered 100, 200 and 300 mg/kg b.w. of the extract respectively when compared to the percentage parasitaemia in group 2 (malaria untreated) on days 7, 14 and 28.

Figure 2 shows significant elevation ($p < 0.05$) on Day 0, in the mean packed cell volume (PCV) of the normal control mice in group 1 compared to the mean PCV of group 2 mice which represented malaria-passaged mice untreated. Dose-dependent increase ($p < 0.05$) was observed from group 3 to group 6 representing malaria-passaged mice treated with 28 mg/kg b.w. of artemether and lumenfantrine, 100, 200 and 300 mg/kg b.w. of the extract respectively on Days 7, 14 and 28.

Figure 3 shows significant ($p < 0.05$) reduction on day 0, in the hemoglobin concentration in all the groups when compared to group 1 mice (normal control). Dose dependent ($p < 0.05$) increase was observed from group 3 to group 6 representing malaria-passaged mice administered 28 mg/kg b.w. of artemether and lumenfantrine, 100, 200 and 300 mg/kg b.w. of the extract respectively on Days 7, 14 and 28.

Weight of pulverized root	Weight of the concentrated extract	Percentage Yield (%)
500 g	15.9 g	3.18%

Table 1: Percentage yield of the ethanol extract of *Zapoteca portoricensis* roots.

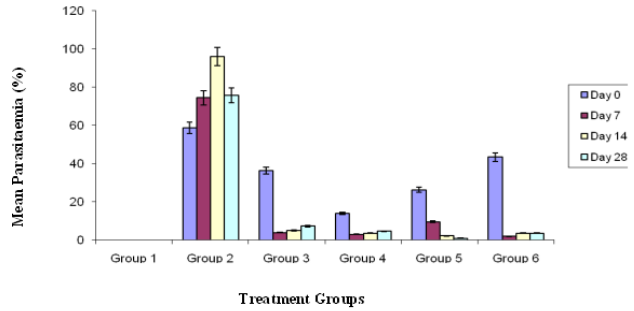

Figure 1: Effect of ethanol extract of *Zapoteca portoricensis* root on percentage parasitaemia in malaria-passaged mice.

Group 1 = Normal/Negative control.
Group 2 = Positive Control (malaria-passaged).
Group 3 = Standard Control (malaria-passaged + 28 mg/kg of artemether and lumefetrine).
Group 4 = Malaria-passaged + 100 mg/kg b.w. of ethanol extract of *Zapoteca portoricensis* root.
Group 5 = Malaria-passaged + 200 mg/kg b.w. of ethanol extract of *Zapoteca portoricensis* root.
Group 6 = Malaria-passaged + 300 mg/kg b.w. of ethanol extract of *Zapoteca portoricensis* root.

Figure 4 shows non-significant ($p > 0.05$) difference in the mean values of total white blood cell count (TWBC) in all the groups on day 0. On day 7 significant ($p < 0.05$) increase was observed in the mean value for TWBC count in group 4 treated with 100 mg/kg b.w. of the extract when compared to group 1 mice (normal control). Also on day 14 non-significant ($p > 0.05$) difference was observed in the mean values for TWBC count in all the groups. Significant ($p < 0.05$) increase was observed in the mean values of TWBC count for group 5 treated with 200 mg/kg b.w. of the extract compared to group 2 (malaria untreated) mice on Day 28.

As shown in Figure 5, Day 0 result showed significant ($p < 0.05$) decrease in the mean red blood cell (RBC) count in all the groups when compared to group 1 (normal control). Significant increase was observed in groups 3, 4, 5 and 6 administered standard drug (28 mg/kg b.w. of artemether and lumenfantrine), 100 and 200 and 300 mg/kg b.w. of the extract respectively compared to group 2 (malaria untreated) on Days 7, 14 and 28 respectively.

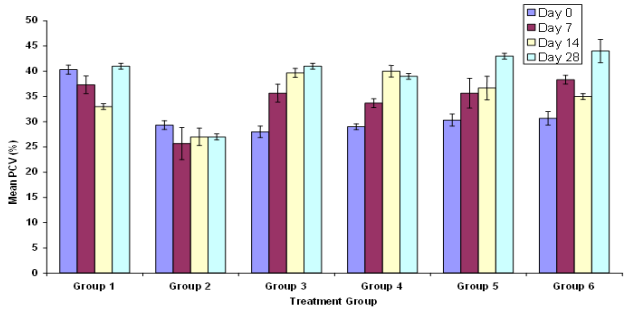

Figure 2: Effect of ethanol extract of *Zapoteca portoricensis* root on packed cell volume in malaria-passaged mice.

Group 1 = Normal/Negative control.
Group 2 = Positive Control (malaria-passaged).
Group 3 = Standard Control (malaria-passaged + 28 mg/kg of artemether and lumefetrine).
Group 4 = Malaria-passaged + 100 mg/kg b.w. of ethanol extract of *Zapoteca portoricensis* root.
Group 5 = Malaria-passaged + 200 mg/kg b.w. of ethanol extract of *Zapoteca portoricensis* root.
Group 6 = Malaria-passaged + 300 mg/kg b.w. of ethanol extract of *Zapoteca portoricensis* root.

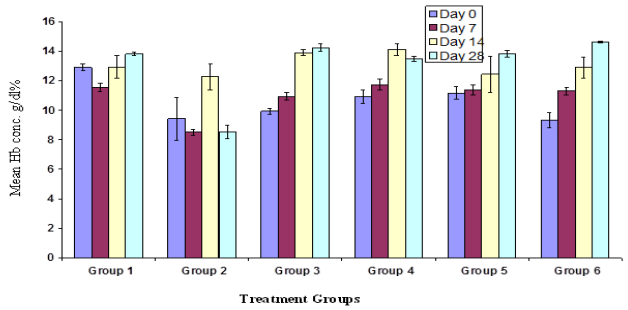

Figure 3: Effect of ethanol extract of *Zapoteca portoricensis* root on hemoglobin concentration in malaria-passaged mice.

Group 1 = Normal/Negative control.
Group 2 = Positive Control (malaria-passaged).
Group 3 = Standard Control (malaria-passaged + 28 mg/kg of artemether and lumefetrine).
Group 4 = Malaria-passaged + 100 mg/kg b.w. of ethanol extract of *Zapoteca portoricensis* root.
Group 5 = Malaria-passaged + 200 mg/kg b.w. of ethanol extract of *Zapoteca portoricensis* root.
Group 6 = Malaria-passaged + 300 mg/kg b.w. of ethanol extract of *Zapoteca portoricensis* root.

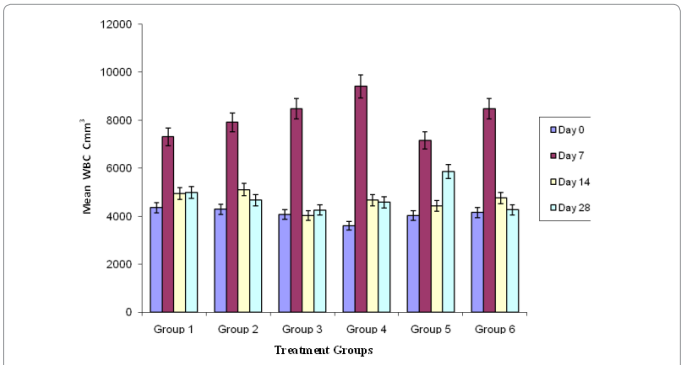

Figure 4: Effect of ethanol extract of *Zapoteca portoricensis* root on total white blood cell count in malaria-passaged mice.

Group 1 = Normal/Negative control.
Group 2 = Positive Control (malaria-Passaged).
Group 3 = Standard Control (malaria-Passaged + 28 mg/kg of Artemether and Lumefetrine).
Group 4 = Malaria-Passaged + 100 mg/kg b.w. of ethanol extract *Zapoteca portoricensis* root.
Group 5 = Malaria- + 200 mg/kg b.w. of ethanol extract *Zapoteca portoricensis* root.
Group 6 = Malaria-Passaged + 300 mg/kg b.w. of ethanol extract *Zapoteca portoricensis* root.

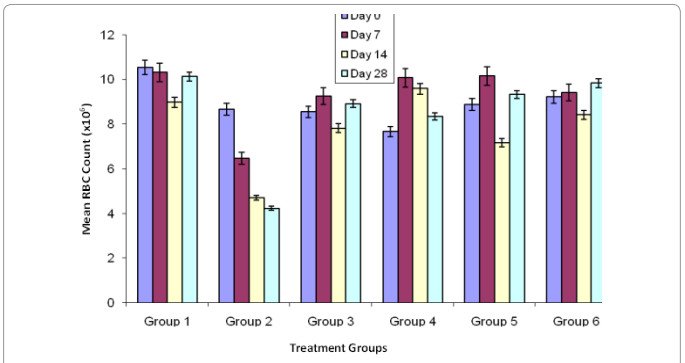

Figure 5: Effect of ethanol extract of *Zapoteca portoricensis* root on red blood cell count in malaria-passaged mice.

Group 1 = Normal/Negative control.
Group 2 = Positive Control (malaria-Passaged).
Group 3 = Standard Control (malaria-Passaged + 28 mg/kg of Artemether and Lumefetrine).
Group 4 = Malaria-Passaged + 100 mg/kg b.w. of ethanol extract of *Zapoteca portoricensis* root.
Group 5 = Malaria-Passaged + 200 mg/kg b.w. of ethanol extract of *Zapoteca portoricensis* root.
Group 6 = Malaria-Passaged + 300 mg/kg b.w. of ethanol extract of *Zapoteca portoricensis* root.

Discussion

Malaria is a mosquito-borne infectious disease of humans and other animals caused by protists (a type of microorganism) of the genus *Plasmodium* [4]. It begins with a bite from an infected female mosquito (Anopheles mosquito), which introduces the protists via its saliva into the circulatory system, and ultimately to the liver where they mature and reproduce. The disease causes symptoms that typically include fever and headache, which in severe cases can progress to coma or death [3].

This study on *Zapoteca portoricensis* root has become necessary both to meet the challenges of malaria eradication and to circumvent resistance to most antimalarial drugs. Several medicinal plants have also been used locally to treat malaria infection. Some of such plants

are *Enantia chloranta, Nauclea natifolia, Salacia nitida* and *Moringa oleifera*. Their use shows that they ameliorate the effects of malaria parasite as shown by Ugwu, et al. [8] and Ogbonna et al. [16].

The result of the effect of ethanol extract of *Zapoteca portoricensis* roots on the mean parasitaemia in mice showed significant ($p < 0.05$) reduction in all the groups when compared with group 2 (malaria untreated). The reduction could lead to the destruction of the essential organs such as liver, kidney, blood cells and other organs in the mice. It could also be as a result of the infection of the liver by sporozoites and increase number of meroziotes, this is in consistent with the findings of Ugwu et al. [8] and Trampuz et al. [17] Significant ($p < 0.05$) reduction was observed in the parasitaemia of groups 5 and 6 malaria-passaged mice treated with 200 and 300 mg/kg b.w. of the extract respectively compared to the parasitaemia of group 2 (malaria untreated) mice. This showed that the extract might be effective against malaria parasitaemia due to the reduction of the percentage parasitaemia level in the treatment groups.

Packed cell volume (PCV) is used to assess anaemia. A decrease in packed cell volume indicates anaemia. The effect of ethanol extract of *Zapoteca portoricensis* root on packed cell volume showed significant ($p < 0.05$) decrease in group 2 (malaria untreated) when compared with group 1 (normal control). Non-significant variation ($p > 0.05$) was observed in the PCV of groups 3 (treated with 28 mg/kg b.w. of the arthemeter and lumenfantrine), 4, 5, and 6 treated with 100, 200 and 300 mg/kg b.w. of the extract respectively compared to group 2 (malaria untreated). This indicated that *Zapoteca portoricensis* root and artemeter + lumefantrine ameliorated the effect of malaria parasitaemia on the PCV and *Zapoteca portoricensis* root boosted PCV levels in mice. This agrees with the work of George and Ewelike-Ezeani [18] who reported significant reduction of PCV in patient with malaria parasitaemia when compared with PCV levels of non-malaria parasitaemia.

A low haemoglobin concentration is used to assess symptoms such as weakness, fatigue, anaemia and other disoders. The result of the effect of ethanol extract of *Zapoteca portoricensis* root on haemoglobin concentration in mice showed significant ($p < 0.05$) increase in haemoglobin concentration of groups 1 (normal control), 3 administered 28 mg/kg b.w. of the arthemeter and lumenfantrine, 4 and 5 (treated with 100 and 200 mg/kg b.w. of the extract) compared to group 2 (malaria untreated) but non-significant ($p > 0.05$) different was observed in groups 4 and 5 treated with 100 and 200 mg/kg b.w. of the extract respectively compared to group 2 (malaria untreated). This shows that *Zapoteca portoricensis* root extract increased hemoglobin concentration in the treated mice. This corroborates the work of Dacies and Lewis [15] that significant reduction occurs in the hemoglobin concentration of a patient with malaria parasitaemia compared to non-malaria parasitaemia patients.

White blood cells (WBC) play a vital role in the body's immune defense against disease. The effect of ethanol extract of *Zapoteca portoricensis* root on white blood cell count shows non-significant ($p > 0.05$) difference in the WBC count in groups 2 (malaria untreatedl), 4, 5 and 6 treated with 100, 200 and 300 mg/kg b.w. of the extract respectively compared to group 1 (normal control). This is consistent with the studies of Bashawri et al. [19] and Chiwakata et al. [20] who reported non-significant difference ($p > 0.05$) in WBC count between the malaria-infected and non-malaria-infected groups. In contrast, other studies have demonstrated leucopoenia [21-24].

The effect of ethanol extract of *Zapoteca portoricensis* root on red blood cell count showed non-significant difference ($p > 0.05$) in groups 3 treated with 28 mg/kg b.w. of the artemether and lumenfantrine, 4, 5 and 6 administered 100, 200 and 300 mg/kg b.w. of the extract respectively compared to group 2 (malaria untreated) with significant decrease ($P < 0.05$) observed in group 2 (malaria untreated) mice compared to all the groups. This showed that malaria parasites destroy red blood cells with a consistent report of George and Ewelike-Ezeani [18] who reported that malaria parasitaemia patients suffer low red blood cell count.

Conclusion

The findings from this study indicate that the ethanol extract of *Zapoteca portoricensis* root has antimalarial properties through the reduction of percentage parasitaemia, and the improvement of the levels of some hematological indices such as PCV, Hb and RBC.

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