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ANTI-MALARIA AND HEMATOLOGICAL ANALYSES OF ETHANOL LEAF EXTRACT OF MORINGA OLEIFERA ON MALARIA INFECTED MICE

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ABSTRACT

Percentage parasitaemia and haematological parameters of ethanol leaf extract of Moringa oleifera were evaluated in a twenty four mice consisting of six groups. Groups 1 (positive control) and 6 (negative control) were treated with 5mg/kg body weight of distilled water, group 5 (standard control) was treated with 5mg/kg body weight of artesunate while groups 2, 3 and 4 were treated with 45, 90 and 180 mg/kg body weight of Moringa oleifera ethanol leaf extract. The results showed that percentage parasitaemia of the mice treated with ethanol leaf extract of moringa oleifera increased significantly ($p<0.05$) in group 1 (positive control) when compared to group 6 (negative control) and other groups. Group 4 (180 mg/kg body weight of the extract), group 5 (5 mg/kg body weight of Artesunate) showed no significant difference ($p>0.05$) in percentage parasitaemia compared to group 6 (negative control). The haematological parameters of packed cell volume (PCV), haemoglobin concentration of the cell (Hb) and total red blood cell counts (TRBC) increased significantly ($p<0.05$) in groups 4 (180 mg/kg body weight of the extract), group 5 (5 mg/kg body weight of Artesunate) and group 6 (negative control) compared to group 1 (positive control) while the haematological parameter of total white blood cell (TWBC) increased significantly ($p<0.05$) in groups 3 (90 mg/kg body weight of the extract) and group 6 (negative control) compared to group 1 (positive control).

KEY WORDS

Moringa oleifera, percentage parasitaemia ,haematological parameters.

INTRODUCTION

Malaria is a mosquito-borne disease of humans caused by eukaryotic protists of the genus Plasmodium. It is transmitted from one human to another by a bite of an infected female anopheles mosquito. It is widespread in tropical and sub-tropical regions, including much of Sub-Sahara Africa, Asia and the Americas (Clark and Cowden, 2003). Plasmodium species are generally host specific and vector specific in that each species will only infect a limited range of hosts and vectors. Four species of plasmodium can infect and be transmitted by humans. They are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*. Malaria caused by *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae* is generally milder and rarely fatal. The fifth species, *Plasmodium knowlesi* is a *zoonosis* that causes malaria in *Macaques* but can also infect humans.Severe disease results largely from *Plasmodium falciparum*.

In humans, the parasites called sporozoites travel to the liver, where they mature and release another form, the merozoites. These enter the bloodstream and infect the red blood cells. The parasites multiply inside the red blood cells, which then ruptures after 48 to 78 hours, infecting more red blood cells (Trampuz *et al*., 2003). The first symptoms usually occur 10 days to 4 weeks after infection, though they can appear as early as 8 days or as long as a year after infection. The symptoms occur in cycles of 48 to 72 hours.

The majority of symptoms are caused by the massive release of merozoites into the bloodstream, the anaemia resulting from the destruction of the red blood cells and the problems caused by large amount of free hemoglobin released into circulation after red blood cells rupture. Malaria can also be transmitted from a mother to her unborn baby (congenitally) and through blood transmission (Clark and Cowden, 2003). Malaria is transmitted by mosquitoes in temperate climates, but the parasites disappear over the winter. The disease is a major health problem in most of the tropics and sub-tropics (Clark and Cowden, 2003). WHO (2005) estimates that there are three hundred to five hundred million cases of malaria each year and more than one million people die. It presents a major health hazard for travelers to warm climates. In some areas of the world, mosquitoes that transmit malaria have developed resistance to insecticides. In addition, the parasites have developed resistance to some antibiotics. This has led to difficulties in controlling both the rate of infection and the spread of the disease.

Symptoms of malaria include flu-like illness with fever, chills, muscle aches and headache. Some patients develop nausea, vomiting, cough and diarrhoea. Cycles of chills, fever and sweating that repeat every one, two or three days are typical. There can be sometimes vomiting, diarrhoea, coughing and yellowing (jaundice) of the skin and whitening of the eyes due to destruction of red blood and liver cells (Mueller *et al*., 2007). People with severe *Plasmodium falciparum* malaria can develop bleeding problems, shocks, liver and kidney failure, central nervous system problems and they can die from infection or its complications. Celebral malaria (coma, altered mental status or seizures) can occur with severe *Plasmodium falciparum* infection. It can be lethal if not treated quickly. Even with treatment, about 15 -20% die (Adams *et al*., 2002).

The classic symptoms of malaria is cyclical occurrence of sudden coldness followed by rigour, then fever and sweating lasting four to six hours, occurring every two days in *Plasmodium vivax* and *Plasmodium ovale* infections, while every three days for *Plasmodium malariae***.** Malaria due to *Plasmodium falciparum* can give recurrent fever every 36 – 48 hours or a less pronounced and almost continous fever. For reasons that are poorly understood, but that may be related to high intracranial pressure, children with malaria frequently exhibit abnormal posturing, a sign indicating severe brain damage (Idro *et al*., 2005). Malaria has been also found to cause cognitive impairments, especially in children. It causes widespread anaemia during a period of rapid brain development and also direct brain damage. The neurologic damage results from celebral malaria in which children are more vulnerable (Trampuz *et al*., 2003). Celebral Malaria is associated with retinal whitening, which may be a useful clinical sign in distinguishing between malaria and other causes of fever (Trampuz, *et al*., 2003). Severe malaria is almost exclusively caused by *Plasmodium* falciparum infection and usually arises 6 – 14 days after infection.

Consequences of severe malaria include coma and death if untreated. Young children and pregnant women are more vulnerable. Splenomegaly (enlarged spleen), severe headache, celebral ischemia, hepatomegally (enlarged liver), hypoglycemia and hemoglobinuria with renal failure may occur. Renal failure is a feature of blackwater fever, where hemoglobin from lysed red blood cells leak into the urine. Severe malaria can progress extremely rapidly and cause death within hours or days (Makintosh *et al*., 2004). In most severe cases of the disease, fatality rate can exceed 20% even with intensive care and treatment (Makintosh *et al*., 2004). In endemic areas, treatment is often less satisfactory and the overall fatality rate for all cases of malaria can be as high as one in ten (Trampuz *et al*., 2003).The long term developmental impairments have been documented in children who have suffered episodes of severe malaria.

Malaria has been and is still the cause of major human morbidity and mortality (Clark and Cowden, 2003). It is the most important parasitic disease worldwide with an incidence of almost three hundred million clinical cases and over one million deaths yearly (WHO, 2000). Malaria is directly responsible for one in five childhood deaths in Africa and indirectly contributes to illnesses and deaths from other diseases (WHO, 1999). Pregnant women and children under five years of age are the most vulnerable. In the absence of an effective vaccine, the fight against malaria depends on chemotherapy, the reduction and prevention of anopheles mosquito contacts with human (Winstainley, 2000). The loss in effectiveness of chemotherapy due to the emergence of resistant strains constitutes the greatest threat to the control of malaria. Therefore, to overcome malaria, new knowledge, products, and tools especially new drugs are urgently needed (Omulokoli *et al*., 1997). Traditional methods of treatment and control of malaria could be a promising source of potential anti-malaria drugs. (Wright and Phillipson, 1990; Venkat et al., 2012; and Sumalata and Sreedevi, 2012) *Moringa oleifera* was massively grown and promoted by the local media in Uganda in the 1980s as a plant which is capable of curing a number of diseases ,including malaria, and relieving some symptoms of HIV/AIDS. *Moringa oleifera* is referred to as a MIRACLE TREE (Fuglie, 2001). This is due to its socio-economic, nutritional, pharmacological and industrial benefits (Makkar and Becker, 2007). As a result of the impact of malaria on the human race and claimed effectiveness of *Moringa oleifera* in curing diseases such as diabetes,typhoid and high blood pressure, it was considered necessary to investigate the anti-malarial effect of *Moringa oleifera*.

This study was designed to determine the percentage parasitaemia and haematological parameters of ethanol leaf extract of *Moringa oleifera.*

MATERIALS AND METHODS

Plant material

Fresh leaves of *Moringa oleifera* were obtained from Ovoko, Igbo-Eze South L.G.A of Enugu State, Nigeria.The leaves were identified by Mr. O. Chijioke of the *Hebarium* unit of the Department of Botany, University of Nigeria, Nsukka.

Animals

The experimental animals used for this study were white albino mice of either sex weighing 20-34g.The mice were between 3-4 months old and were obtained from the animal unit of Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

Chemicals/Reagents

All chemicals used in this study were of analytical grade and products of May and Baker, England; BDH, England and Merck, Darmstand, Germany. Reagents used for the assays were products of Radox commercial kits.

Extraction Procedure

The fresh leaves of *Moringa oleifera* plant were plucked and dried under room temperature at (29°C-35°C) for three weeks, after which the leaves were pulverized into coarse form with a crestor high speed milling machine. The coarse form (130g) was then macerated in absolute ethanol. This was left to stand for 48 hours. After that the extract was filtered through muslin cloth on a plug of glass wool in a glass column.The resulting ethanol extract was concentrated and evaporated to dryness using rotary evaporator at an optimum temperature of between 40 and 45°C to avoid denaturation of the active ingredients. The concentrated extract was stored in the refrigerator.

EXPERIMENTAL DESIGN

Twenty-four white albino mice of either sex weighing 20 – 34kg were housed in separate cages, acclimatized for one week and then divided into six groups of four mice each. The route of administration (treatment) was via oral with the aid of an oral intubation tube.

Group 1 was the (positive control) inoculated with malaria parasite (Mp^+) and treated with 5mg/kg body weight of distilled water.

Group II was inoculated with malaria parasite and treated with 45mg/kg body weight of *Moringa oleifera* ethanol leaf extract.

Group III was also inoculated with malaria parasite and treated with 90mg/kg body weight of *Moringa oleifera* ethanol leaf extract.

Group IV was inoculated with malaria parasite and treated with 180mg/kg body weight of *Moringa oleifera* ethanol leaf extract.

Group V which was also inoculated with malaria parasite (standard control) and was treated with 5mg/kg body weight of artesunate (standard drug).

Group VI was the negative control which was not inoculated with malaria parasite and was finally treated with 5mg/kg body weight of distilled water.

Before the treatments, the mice in Groups I – V were inoculated with malaria parasite and 3 days after that analyses were carried out to determine the baseline parameter in all the groups, then, two days later, treatment began. The treatment lasted for 5 days during which analyses were done on day 3, day 5 of treatment and 28 days post treatment

Determination of percentage yield of extract

The percentage yield of the extract was determined by weighing the coarse *Moringa oleifera* leaf before extraction and the *Moringa oleifera* ethanol leaf extract after concentration and then calculated using the formula.

$$\text{Percentage (\%) yield} = \frac{\text{Weight (g) of concentrated extract}}{\text{Weight (g) of ground Moringa leaves}} \times 100$$

Determination of percentage parasiteamia

The determination of malaria parasitemia (Mp^+) was carried out according to the Method of Dacie and Lewis (2000).

Determination of haematological parameters

The determination of haematological parameters of total red blood cell count, total white blood cell count, packed cell volume and haemoglobin

concentration were carried out according to the method of Dacie and Lewis (2000).

RESULTS

Percentage yield of the extract

Table 1: The percentage yield of the ethanol leaf extract of *Moringa oleifera*

Initial weight of ground extract (g)	Final weight of Extract (g)	Percentage (%) yield of extract
130	23.20	17.85

From the result in Table 1 the (%) yield of the ethanol leaf extract of *Moringa oleifera* was found to be 17.85%.

Effect of Ethanol Leaf Extract of *Moringa oleifera* on Percentage Parasitaemia

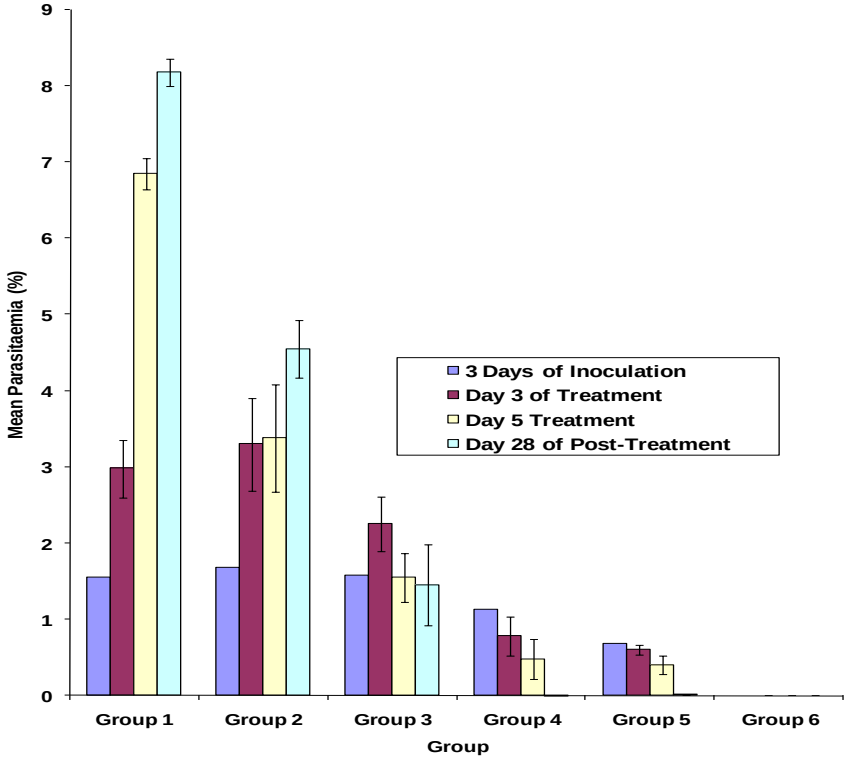

Fig. 1: Effect of ethanol leaf extract *Moringa oleifera* on percentage parasitaemia in mice

Group 1= Positive Control
Group 2= 45mg/kg b.w of *Moringa oleifera*
Group 3=90mg/kg b.w of *Moringa oleifera*
Group 4=180mg/kg b.w of *Moringa oleifera*
Group 5=5mg/kg b.w of Artesunate
Group 6=Negative Control

Fig 1:shows that 3 days of inoculation mean values for Percentage Parasitaemia of mice in groups 4 and 5 significantly decreased ($p<0.05$) compared to the values of mice in groups 1 (positive control), 2 and 3. On day 3 of treatment the mean values for Percentage Parasitaemia in all the groups significantly decreased ($p<0.05$) compared to the mean percentage parasitaemia of mice in groups 1 (positive control) and 2. Also, on day 5 of treatment the mean percentage parasitaemia in all the groups significantly decreased ($p<0.05$) compared to the values for the percentage parasitaemia of mice in group 1 (positive control). Hence,on day 28 of post treatment the mean values of the percentage parasitaemia significantly decreased in all the groups compared to the mean percentage parasitaemia of group 1 (positive control). Finally on day 28 of post treatment also showed significant ($p<0.05$) clearance of the parasitaemia in groups 4 and 5 compared to the mean values of the percentage parasitaemia in groups 1 (positive control), 2 and 3 animals.

Effect of Ethanol Leaf Extract of *Moringa oleifera* on Haemoglobin Concentration

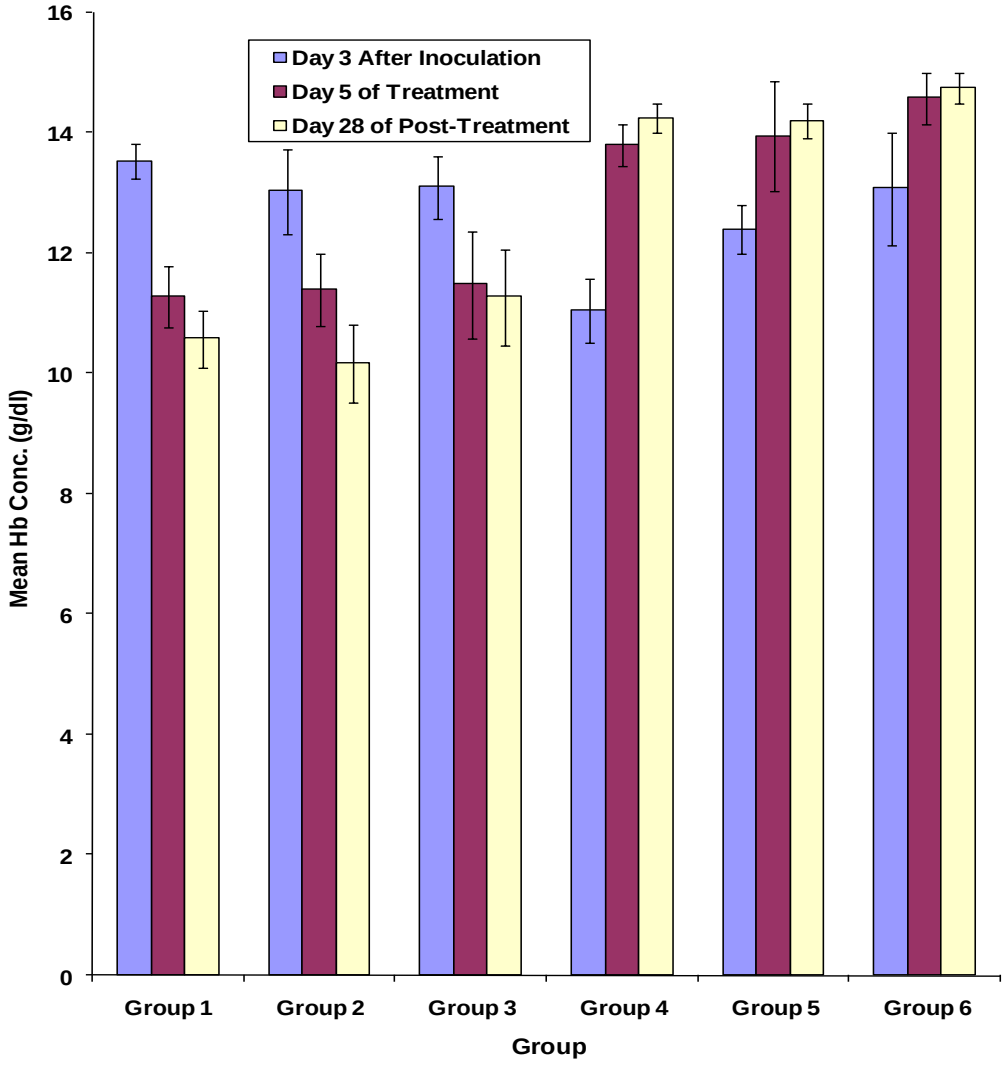

Fig. 2: Effect of ethanol leaf extract of *Moringa oleifera* on haemoglobin concentration concentration in mice

Group 1= Positive Control
Group 2= 45mg/kg b.w of *Moringa oleifera*
Group 3=90mg/kg b.w of *Moringa oleifera*
Group 4=180mg/kg b.w of *Moringa oleifera*
Group 5=5mg/kg b.w of Artesunate
Group 6=Negative Control

Fig. 2: shows that 3 days after inoculation the mean values for heamoglobin in all the groups were essentially similar, while the value obtained for group 4 was significantly ($p<0.05$) lower than for mice in group 1 (positive control) . On day 5 of treatment the mean values for heamoglobin in groups 4, 5 and 6 significantly increased ($p<0.05$) compared to group 1 (positive control). Finally, on day 28 of post treatment the mean values for heamoglobin in groups 4, 5 and 6 (negative control) significantly increased ($p<0.05$) compared to group 1 (positive control).

Effect of Ethanol Leaf Extract of *Moringa oleifera* on Total White Blood Cell Count in Mice

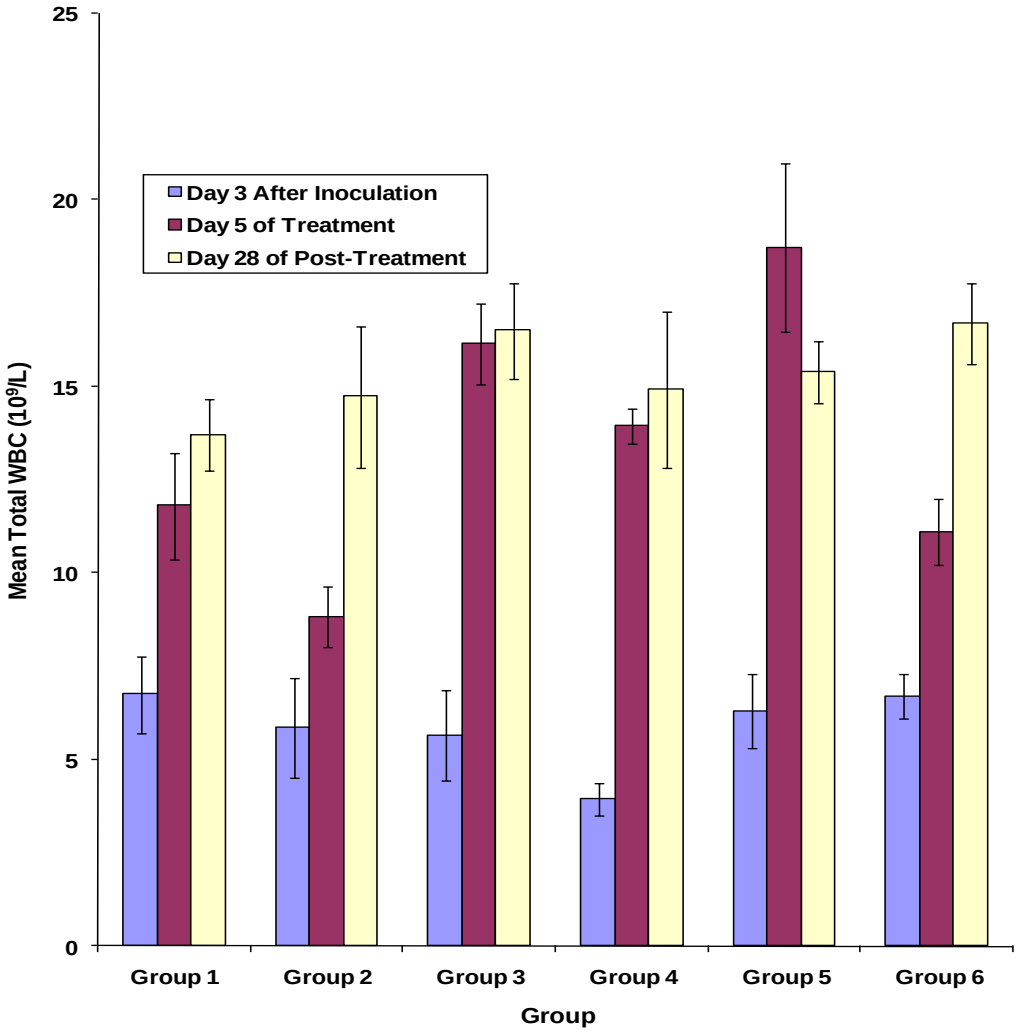

Fig. 3: Effect of ethanol leaf extract of *Moringa oleifera* on total white blood cell count in mice

Group 1= Positive Control
Group 2= 45mg/kg b.w of *Moringa oleifera*
Group 3=90mg/kg b.w of *Moringa oleifera*
Group 4=180mg/kg b.w of *Moringa oleifera*
Group 5=5mg/kg b.w of Artesunate
Group 6=Negative Control

Fig.3: Shows the effect of ethanol leaf extract of *Moringa oleifera* on total white blood cell count. The TWBC (baseline) count obtained 3 days after inoculation for mice in groups 1, 2 ,3, 5 and 6 were essentially similar ,while the value obtained for mice in group 4 was significantly lower than that for mice in group 1. On day 5 after commencement of treatment, mean value for group 2 mice was significantly ($p<0.05$) lower than that of group 1 mice,while mean values for mice in groups 3 and 5 were significantly ($p<0.05$) higher than that of group 1 mice.There was no significant difference ($p>0.05$) between the mean values for mice in groups 4 and 6 when compared with that for mice in group 1. TWBC count on day 28 of treatment in group 3 mice was essentially similar to the value of TWBC in group 6 (negative control) mice,while the values obtained for mice in groups 2,3,4,5 and 6 were significantly ($p<0.05$) higher than that for group 1 (positive control) mice.

Effect of Ethanol Leaf Extract of *Moringa oleifera* on Packed Cell Volume in Mice

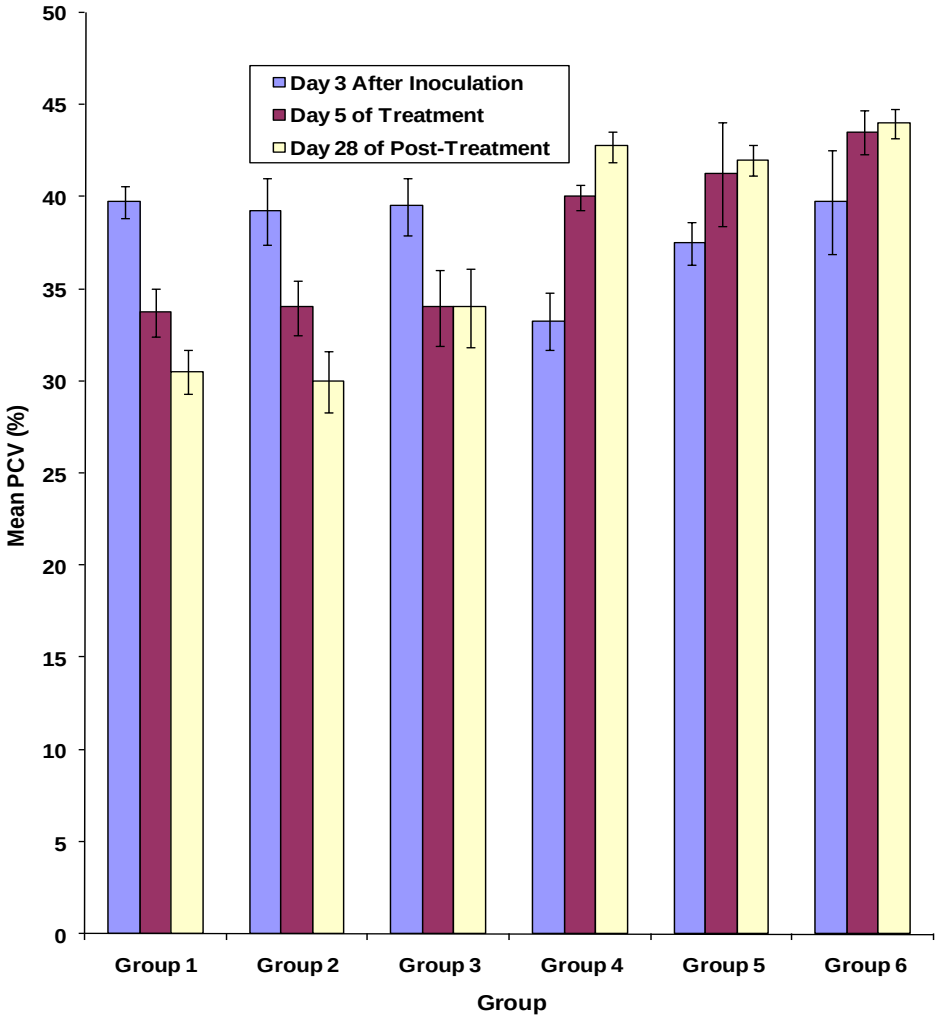

Fig. 4: Effect of ethanol leaf extract of *Moringa oleifera* on packed cell volume in mice

Group 1= Positive Control
Group 2= 45mg/kg b.w of *Moringa oleifera*
Group 3=90mg/kg b.w of *Moringa oleifera*
Group 4=180mg/kg b.w of *Moringa oleifera*
Group 5=5mg/kg b.w of Artesunate
Group 6=Negative Control

Fig.4: Shows that 3 days after inoculation mean values for PCV of mice in groups 2 ,3,5, and 6 were not significantly ($p>0.05$) different from the value obtained for mice in group 1 (positive control) ; but the value obtained for mice in group 4 was significantly ($p<0.05$) lower than that for mice in group 1 (positive control).On day 5 of treatment , mean values for PCV for groups 2 and 3 were essentially similar to that of animals in group 1. On the other hand,values obtained for mice in groups 4, 5 and 6 showed significant ($p<0.05$) increases above the value for animals in the group 1 (positive control). For day 28 post treatment,whereas the mean PCV values for groups 4 , 5 and 6 animals were significantly ($p<0.05$) higher than that of group 1 mice ,mean values for those in groups 2 and 3 showed no significant ($p>0.05$) difference when compared with the value for group 1 mice.Also , mean PCV values for mice in groups 4 ,5 and 6 were similar.

Effect of Ethanol Leaf Extract of *Moringa oleifera* on Red Blood Cell Count in Mice

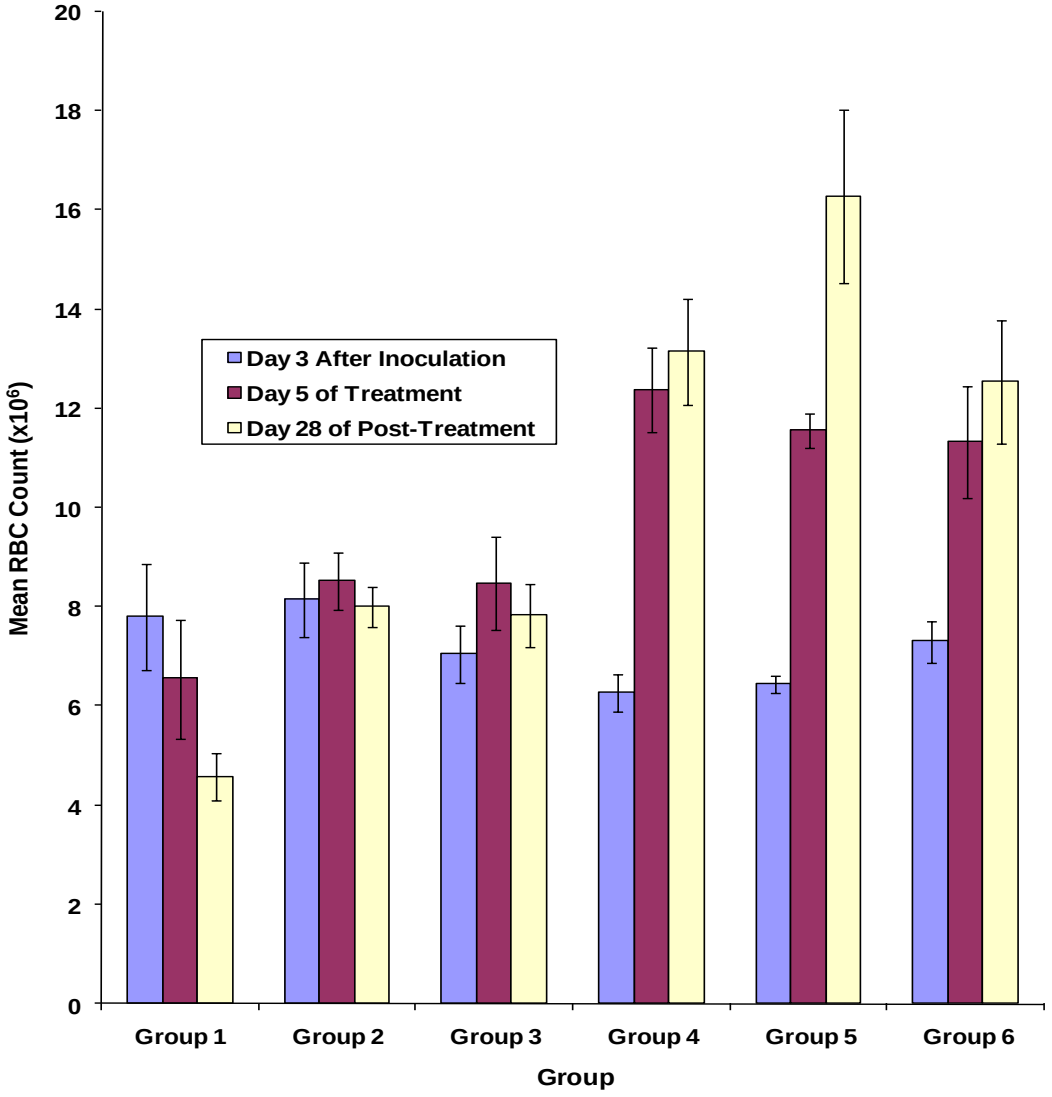

Fig. 5: Effect of ethanol leaf extract of *Moringa oleifera* on red blood cell count in mice

Group 1= Positive Control
Group 2= 45mg/kg b.w of *Moringa oleifera*
Group 3=90mg/kg b.w of *Moringa oleifera*
Group 4=180mg/kg b.w of *Moringa oleifera*
Group 5=5mg/kg b.w of Artesunate
Group 6=Negative Control

Fig. 5: Shows that mean RBC baseline obtained 3 days after inoculation for mice in groups 2 , 3 ,4, 5 and 6 (negative control) mice were not significantly ($p>0.05$) different compared to the value in group 1 (positive control) mice. On day 5 of treatment showed significant increase ($p<0.05$) in RBC count of groups 2, 3 ,4, 5 and 6 (negative control) mice compared to the mean value for RBC count of mice in group 1 (positive control). Also day 28 of post treatment, showed significant increase ($p<0.05$) in the mean RBC count of mice in groups 2,3,4,5 and 6 (negative control) mice when compared to the mean RBC count of group 1 (positive control) mice .But group 4 mice was essentially similar to group 6 (negative control) mice.

DISCUSSION

Malaria is a major public health problem and cause of much suffering and premature death in the poorer areas of the Tropical Africa, Asia and Latin America. Human beings are exposed to malaria through the bite of an infected female anopheles mosquito, blood transfusion and congenitally from mother to her child (Bruce, 1981). In many endemic areas, it is becoming difficult to control, because of the parasite resistance to antimalarial drugs and the failure of vector control measures. Due to resistance to some of the conventional drugs used for the treatment of malaria and the impact of malaria to world health, it is therefore necessary to search for new, cheap and easily available drug that will be used for the treatment of malaria (Dondorp, 2007). The medicinal uses of many plants like *Moringa oleifera* cannot be over-emphasised. The choice of this plant for the research work was based on its numerous ethnomedicinal properties.

The observation on the effect of ethanol leaf extract of *Moringa oleifera* on percentage parasitaemia in mice showing a significant ($p<0.05$) clearance of parasitaemia in group 4 (180mg/kg body weight of the extract) and group *5* (5mg/kg body weight of artesunate) when compared to group 1 (positive control) is consistent with the findings of Monzon (1995) in Phillipines, who administered *Moringa oleifera* leaf extract in mice that were infected with malaria and other parasitic diseases. The result showed that the extract might be effective against the parasites. The result also showed a significant increase ($p<0.05$) in parasitaemia in group 1 (positive control) treated with 5mg/kg distilled water which could lead to the destruction of the liver, blood cells, kidney and other vital organs in the mice (Trampuz *et al*., 2003). This could be as a result of the infection of the liver by the sporozoites and the resultant multiplication of the merozoites in the blood cells.

The result of the effect of ethanol leaf extract of *Moringa oleifera* on the haematological parameter of packed cell volume showed a non significant difference in packed cell volume ($p>0.05$) in group 4 (180 mg/kg body weight of the extract) and group 5 (5 mg/kg body weight of the artesunate) compared to group 6 (negative control). But, a significant reduction ($p<0.05$) in packed cell volume was observed in group 1 (positive control) when compared to group 6 (negative control). This showed that *Moringa oleifera* ethanol leaf extract has ameliorated the effect of malaria parasitaemia on the packed cell volume. This agrees with the work of Ambi *et al*., (2006) who showed that *Moringa oleifera* leaf extract boosted heamatological parameters of packed cell volume in rats. Packed cell volume is used to asses anaemia, erythrocytosis, haemodilution and haemoconcentration.A decrease in packed cell volume indicates anaemia (Dacie and Lewis, 2000).

The result of the effect of ethanol leaf extract of *Moringa oleifera* on red blood cell count showed a non significant difference ($p>0.05$) in group 4 (180 mg/kg body weight of the extract) compared to group 6 (negative control).This, also corroborates with the work of Ambi *et al*. ,(2006) showing that *Moringa oleifera* leaf extract boost red blood cell counts in rats.. There was a significant increase ($p<0.05$) in red blood cell count in group 4 (180mg/kg body weight of the extract) when compared to group 1 (positive control) . A decrease in red blood cell could be as a result of anaemia (Dacie and Lewis,2000). *Moringa oliefera* ethanol leaf extract has probably repaired the damages caused by merozoites to the red blood cell in mice that were infected with malaria.

The effect of ethanol leaf extract of *Moringa oleifera* on haemoglobin concentration in mice

showed a significant increase ($p<0.05$) in haemoglobin in group 4 (180 mg/kg body weight of the extract), group 5 (5mg/kg body weight of the artesunate) and group 6 (negative control) when compared to group 1 (positive control). But, group 4 (180mg/kg body weight of the extract) and group 5 (5 mg/kg body weight of the artesunate) showed no significant difference ($p>0.05$) in haemoglobin concentration when compared to group 6 (negative control). This corroborated with the work of Ambi *et al*., (2006) who showed that *Moringa oleifera* leaf extract boosted haemoglobin concentration in rat. A complete blood count is used to asses symptoms such as weakness, fatigue, anaemia, infection and other disorders. Haemoglobin molecule fills up the red blood cells. It transports oxygen and gives the blood cell its red colour. The higher the haemoglobin concentration, the higher its ability to transport oxygen throughout the body.

The effect of ethanol leaf extract of *Moringa oleifera* on total white blood cell count showed a significant increase ($p<0.05$) in total white blood cell count in other groups when compared to group 1 (positive control) .The *Moringa oleifera* ethanol leaf extract increased the total white blood cell in group 2 (45 mg/kg body weight of the extract), group 3 (90 mg/kg body weight of the extract) and group 4 (180 mg/kg body weight of the extract) when compared to group 1 (positive control). This is also consistent with the work of Ambi *et al*., (2006) that showed the potency of *Moringa oleifera* leaf extract in increasing white blood cell counts in rat. This could be the reason for reduced parasitaemia in groups 2 (45mg/kg body weight of the extract) and group 3 (90 mg/kg body weight of the extract) and total clearance of the parasitaemia in group 4 (180 mg/kg body weight of the extract) and group 5 (5 mg/kg body weight of the artesunate).

CONCLUSION

The results above have shown why ethanol leaf extract of Moringa oleifera have been used in numerous ethnomedicinal practises to combat malaria.

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