

## EFFECT OF SEED EXTRACT OF *PICRALIMA NITIDA* ON HAEMATOLOGICAL PARAMETERS OF MALARIA-INFECTED ALBINO MICE AND ITS INTERFERENCE WITH THE SERUM ELECTROLYTE LEVELS

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### ABSTRACT

Low red blood cell (RBC) and white blood cell (WBC) counts, packed cell volume (PCV) and haemoglobin (Hb) concentration are indicative of parasitaemia in malaria patients. One of the adverse effects of drugs is their interference with the serum electrolytes balance thereby impairing certain movements such as muscular movement in the body. This research project was aimed at assessing the ameliorative effects of ethanol seed extract of *Picralima nitida* on the haematological parameters of malaria-infected mice and its interference with the serum electrolytes especially potassium ion ( $K^+$ ) and sodium ion ( $Na^+$ ). Pulverised dried seeds of *P. nitida* were extracted using ethanol. The haematological parameters and the serum electrolyte levels were assessed in mice. The result of the analyses showed that the RBC and WBC counts, PCV and Hb concentration of mice groups treated with 20, 40 and 80 mg/kg b.w. of the extract and 5 mg/kg b.w. of artesunate were significantly ( $p < 0.05$ ) higher compared to the ones inoculated with malaria parasite and treated with placebo (positive control) on days 3 and 5 post treatment. The serum  $K^+$  concentration of the treated groups was non-significantly ( $p > 0.05$ ) lower compared to the positive control on days 3 and 5 post treatment. However, the  $Na^+$  concentration of the treated groups was significantly ( $p < 0.05$ ) higher compared to the positive control on days 3 and 5 post treatment. The seed extract of *P. nitida* was found to increase the blood cells count, haemoglobin concentration and PCV; these parameters are normally adversely affected in malaria patient. However, treatment with this extract can interfere with the level of the serum electrolytes thereby causing electrolyte imbalance which could be life-threatening.

**Keywords:** Anti-malarial, haematological, serum electrolytes, *Picralima nitida*

### INTRODUCTION

Different parts of *Picralima nitida* plant such as the seeds, stem bark, fruit and leaves are used as preparations for the treatment of ailments (Erharuyi *et al.* 2014). The seeds are used as aphrodisiac, antipyretic, for the treatment of malaria, pneumonia and other respiratory tract diseases. The fruit is used for the treatment of gastrointestinal disorders, dysmenorrhoea and fever. The leaves are used as a vermifuge and the leafsap is applied to the ears for otitis. Preparation from the bark is used as laxatives/purgative, anthelmintic, febrifuges, treatment of venereal diseases and hernias. Its root extract is used as vermifuge, aphrodisiac, febrifuge, malaria, pneumonia and gastrointestinal disorders.

The cytoplasm of red blood cells is rich in haemoglobin- a complex metalloprotein containing heme groups whose iron atoms temporarily bind to  $O_2$  in the lungs or gills and release them throughout the body. Haemoglobin in

the erythrocytes also carries some of the waste product (especially carbon dioxide) back from the tissues (Vinay *et al.*, 2007). The white blood cells make up approximately 1% of the total blood volume in a healthy adult. They are found throughout the body including the lymphatic system (LaFleur-Brooks, 2008). Decrease in haematocrit may be associated with an increase in thrombotic events and cardiovascular mortality (Lowe, 1999). It is considered an integral part of a person's complete blood count results, along with haemoglobin concentration, white blood cell count, and platelet count.

Serum electrolytes such as potassium, sodium and calcium ions are very important in life process such as transmission of impulses, contraction and relaxation of muscles among others. The loss of much quantity of these electrolytes in the body leads to life-threatening conditions. An increase above the normal range of serum electrolytes is

sometimes associated with the decrease in another (Jigam *et al.*, 2011), and may be indicative of some respiratory or renal toxicity.

Based on the claimed ethnomedicinal uses, scientists have investigated a number of pharmacological parameters such as antimalarial, anti-inflammatory, analgesic, antimicrobial, antioxidant, antiulcer and cytotoxic properties of extracts from *P. nitida* (Erharuyi *et al.* 2014). An *in vitro* antimalarial study of *P. nitida* extracts by Iwu and Klayman (2002), showed an inhibitory activity against drug resistant clones of *Plasmodium falciparum*. The *in vivo* antiplasmodial activity study of the ethanol seed extract of *P. nitida* was conducted in chloroquine-sensitive *Plasmodium berghei* infected mice, the result of which proved the extract to possess the activity in both early (4-Day chemo-suppressive test) and established infections (Curative test) (Okokon *et al.* 2007).

The methanol fruit extract of *P. nitida* showed potent and dose-dependent anti-inflammatory activity. The extract when administered intraperitoneally inhibited carrageenan-induced rat paw oedema with  $IC_{50}$  value of 102 mg/kg. The highest dose tested was 300 mg/kg of the extract which produced 72.2% inhibition. The antipyretic activity showed that the methanol fruit extract at a dose of 50 mg/kg produced a mean percentage antipyrexia of 38.7% on lipopolysaccharide-induced pyrexia in rabbits, which was comparable to aspirin (29.0% at 200 mg/kg). The ethanol seed extract of *P. nitida* evaluated for analgesic effect showed that it increased the mean pain threshold of rats in a dose-dependent manner and also significantly suppressed bradykinin-induced hyperalgesia in rats (Ezeamuzie *et al.* 1994).

The basic fraction of the methanol extract of the stem bark of *P. nitida* was shown to exhibit significant antimicrobial activity against a wide range of Gram-positive bacteria and fungi, but limited activity against Gram-negative bacteria (Fakeye *et al.* 2000). The antioxidant capacity of ethanol, ether, ethyl acetate, butanol and aqueous extracts of *P. nitida* seeds were determined using free radical induced hemolysis in red blood cells. The result of the study showed that *P. nitida* seed extract have good antioxidant capacity with the butanol extract exhibiting the highest activity

(Shittu *et al.* 2010). The antiulcer activity of the methanol extract, chloroform and methanol fractions of *P. nitida* seeds were evaluated using the aspirin-pylorus-ligation method in rats. The study revealed that the extract and fractions of *P. nitida* seeds produced significant reduction of ulcer index, total acidity, pepsin activity and increase in mucoprotective parameter such as phenol red content (Mabeku *et al.* 2008).

The antiproliferative and apoptotic effects of the crude methanol extract and fractions of *P. nitida* root bark were investigated *in vitro* using human breast cancer cell line (MCF-7). The result indicated a marked reduction in cell proliferation and increase apoptosis in MCF-7 cells after extract treatment (Osayemwenre *et al.* 2011). The effects of *P. nitida* seed extract on haematological parameters and serum electrolytes levels have rarely been investigated. This research was aimed at assessing ameliorative effect of ethanol seed extract of *Picralima nitida* on the haematological parameters in malaria-infected mice and its effect on the serum electrolytes balance.

## MATERIALS

### Animals

The experimental animals used for this study were 3-4 months old albino mice of either sex weighing 20-34 g. They were obtained from the Animal Unit of Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were acclimatized for two weeks before usage.

### Collection of *Picralima nitida* Seeds

Seeds of *Picralima nitida* were collected from Isuofia, Aguata Local Government Area of Anambra State and were authenticated by Mr. Ozioko A. of the Bioresource Development and Conservation Programme (BDGP) Research Centre, Nsukka.

### Chemicals and reagents

All the chemicals and reagents used in this study were of analytical grade and products of May and Baker, England; BDH, England and Merck, Darmstadt, Germany.

## METHODS

### Extraction

The seeds of *Picralima nitida* plant were harvested

and dried under room temperature for three weeks, after which they were pulverized with a Crestor high speed milling machine. A certain amount (1 kg) was macerated in 5 volume (w/v) absolute ethanol and left to stand for 48 hours. Afterwards, the extract was filtered using muslin cloth on a plug of glass wool in a 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 throughout the period of the experiment.

### Experimental Design

A total of 180 albino mice of either sex weighing 20-34 kg were housed in separate cages, acclimatized for one week and then divided into six groups of thirty mice each as follows:

**Group 1:** Mice not inoculated with malaria parasite; treated with 3% tween 80 (Normal control)

**Group 2:** Mice inoculated with malaria parasite; treated with 3% tween 80 (Positive control)

**Group 3:** Mice inoculated with malaria parasite; treated with 20 mg/kg body weight of the extract

**Group 4:** Mice inoculated with malaria parasite; treated with 40 mg/kg body weight of the extract

**Group 5:** Mice inoculated with malaria parasite; treated with 80 mg/kg body weight of the extract

**Group 6:** Mice inoculated with malaria parasite; treated with 5 mg/kg Artesunate (Standard control)

The route of administration (treatment) was via oral route with the aid of an oral intubation tube.

After three days of malaria inoculation, analyses were performed to determine the baseline parameters of the mice. Treatment lasted for 5 days, during which blood samples were collected on days 3 and 5 within the period of treatment for analyses.

### Determination of haematological parameters

The RBC and WBC counts, packed cell volume and haemoglobin concentration were carried out according to the method of Dacie and Lewis (2000).

### Determination of serum electrolyte concentrations

The serum electrolyte concentrations were

determined using flame emission photometric method.

### Statistical analysis

The data obtained were subjected to ANOVA with repeated measures and t-test. Significant difference was accepted at  $p < 0.05$ . Results are expressed as mean  $\pm$  standard error of mean (S.E.M.). This analysis was carried out using IBM SPSS Statistics version 18.

### RESULTS

As presented in figures 1, 2, 3, 4 and table 1, the RBC and WBC counts, PCV and Hb concentration of normal control (mice not inoculated with malaria parasite but treated with 3% tween 80) were  $10.45 \pm 0.33$  ( $\times 10^6/\mu\text{l}$ ),  $14700.00 \pm 517.687$  ( $10^9/\text{L}$ ),  $40.00 \pm 0.71\%$  and  $14.16 \pm 0.31$  g/dl respectively. For the positive control (mice inoculated malaria parasite and not treated), the values for these parameter were  $6.71 \pm 0.37 \times 10^6/\mu\text{l}$  (RBC count),  $6940.00 \pm 347.275$   $10^9/\text{L}$  (WBC count),  $21.00 \pm 1.00\%$  (PCV) and  $8.54 \pm 0.20$  g/dl (Hb concentration). On day 3 post treatment, the mice treated with 20 mg/kg b.w. of the extract showed the RBC and WBC, PCV and Hb concentration values of  $8.01 \pm 0.23$  ( $\times 10^6/\mu\text{l}$ ),  $7760.00 \pm 312.410$  ( $10^9/\text{L}$ ),  $26.40 \pm 1.03\%$  and  $11.24 \pm 0.55$  g/dl respectively. These values increased further to  $8.52 \pm 0.44$  ( $\times 10^6/\mu\text{l}$ ),  $9320.00 \pm 289.583$  ( $10^9/\text{L}$ ),  $11.60 \pm 0.59$  g/dl and  $29.40 \pm 0.75\%$  on day 5 post treatment. The same trend of increment in the values of these parameters between day 3 and day 5 post treatments followed in mice groups treated with 40 and 80 mg/kg b.w. of the extract. The RBC count of mice treated with 5 mg/kg b.w. of artesunate (standard control) was  $10.02 \pm 0.17$  ( $\times 10^6/\text{ml}$ ) on day 3 post treatment, which increased to  $10.31 \pm 0.20$  ( $\times 10^6/\mu\text{l}$ ) on day 5 post treatment. The WBC count of the standard control was  $12160 \pm 317.175$  ( $10^9/\text{L}$ ) on day 3 post treatment and increased to  $13190.00 \pm 263.818$  ( $10^9/\text{L}$ ) on day 5 post treatment. The Hb concentration was  $11.20 \pm 0.41$  g/dl on day 3 post treatment and  $11.60 \pm 0.44$  g/dl on day 5 post treatment and the PCV increased by 5.00% between day 3 post treatment and day 5 post treatment.

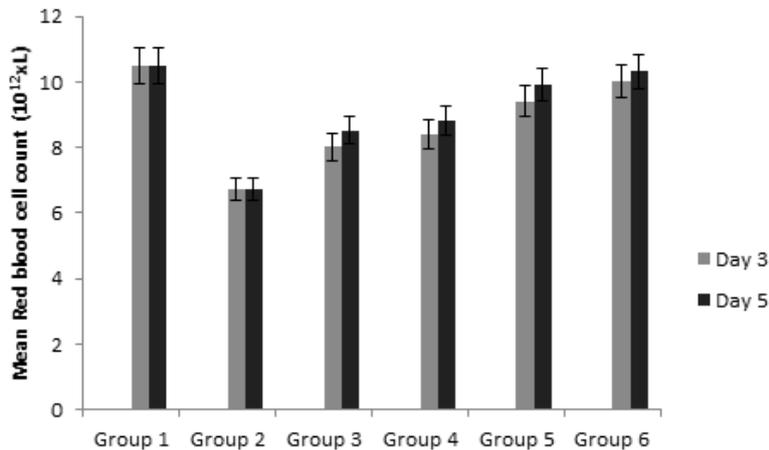


Figure 1: Effect of treatment with ethanol seed extract on red blood cell count of malaria-infected mice

Group 1: Mice not inoculated with malaria parasite; treated with 3% tween 80 (Normal control)  
 Group 2: Mice inoculated with malaria parasite; treated with 3% tween 80 (Positive control)  
 Group 3: Mice inoculated with malaria parasite; treated with 20 mg/kg body weight of the extract  
 Group 4: Mice inoculated with malaria parasite; treated with 40 mg/kg body weight of the extract  
 Group 5: Mice inoculated with malaria parasite; treated with 80 mg/kg body weight of the extract  
 Group 6: Mice inoculated with malaria parasite; treated with 5 mg/kg Artesunate (Standard control)

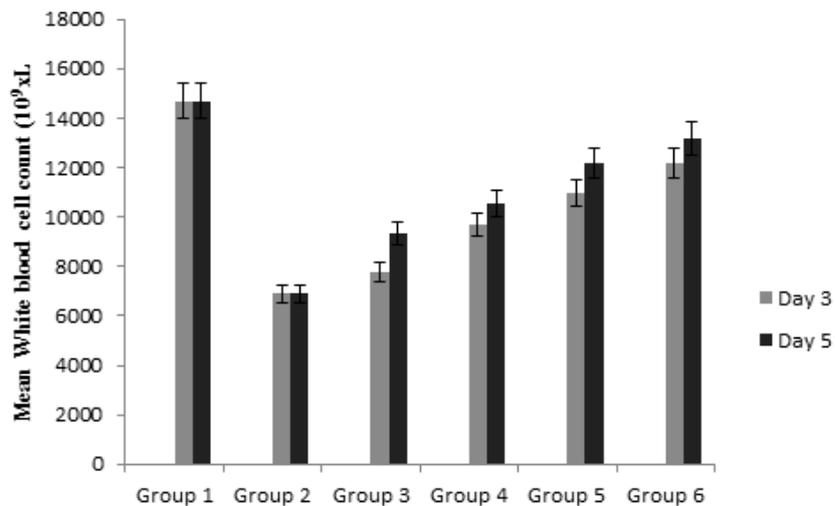


Figure 2: Effect of treatment with ethanol seed extract of *P. nitida* on white blood cell count of malaria-infected mice

Group 1: Mice not inoculated with malaria parasite; treated with 3% tween 80 (Normal control)  
 Group 2: Mice inoculated with malaria parasite; treated with 3% tween 80 (Positive control)  
 Group 3: Mice inoculated with malaria parasite; treated with 20 mg/kg body weight of the extract  
 Group 4: Mice inoculated with malaria parasite; treated with 40 mg/kg body weight of the extract  
 Group 5: Mice inoculated with malaria parasite; treated with 80 mg/kg body weight of the extract  
 Group 6: Mice inoculated with malaria parasite; treated with 5 mg/kg Artesunate (Standard control)

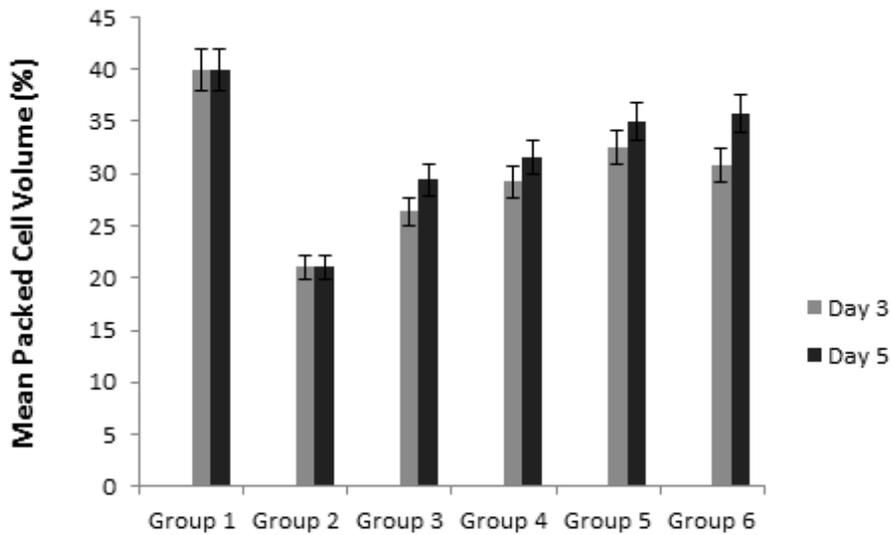


Figure 3: Effect of treatment with ethanol seed extract of *P. nitida* on packed cell volume (PCV) of malaria-infected mice

- Group 1: Mice not inoculated with malaria parasite; treated with 3 % tween 80 (Normal control)
- Group 2: Mice inoculated with malaria parasite; treated with 3 % tween 80 (Positive control)
- Group 3: Mice inoculated with malaria parasite; treated with 20 mg/kg body weight of the extract
- Group 4: Mice inoculated with malaria parasite; treated with 40 mg/kg body weight of the extract
- Group 5: Mice inoculated with malaria parasite; treated with 80 mg/kg body weight of the extract
- Group 6: Mice inoculated with malaria parasite; treated with 5 mg/kg Artesunate (Standard control)

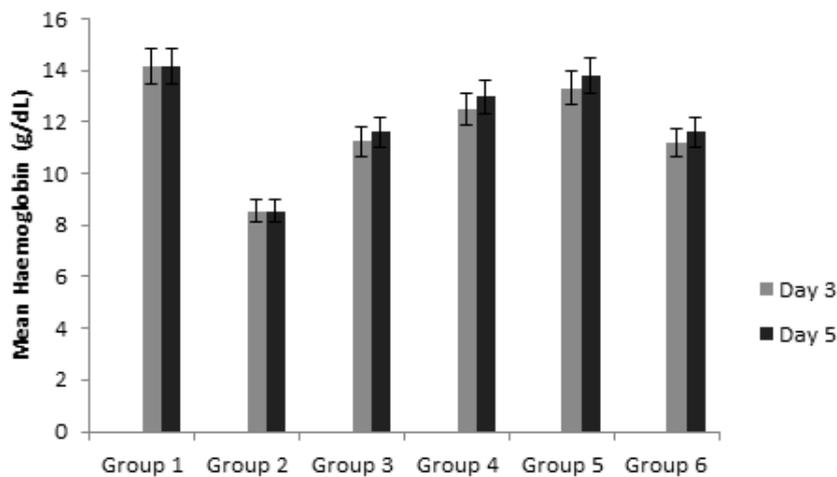


Figure 4: Effect of treatment with ethanol seed extract of *P. nitida* on haemoglobin concentration of mice

- Group 1: Mice not inoculated with malaria parasite; treated with 3 % tween 80 (Normal control)
- Group 2: Mice inoculated with malaria parasite; treated with 3 % tween 80 (Positive control)
- Group 3: Mice inoculated with malaria parasite; treated with 20 mg/kg body weight of the extract
- Group 4: Mice inoculated with malaria parasite; treated with 40 mg/kg body weight of the extract
- Group 5: Mice inoculated with malaria parasite; treated with 80 mg/kg body weight of the extract
- Group 6: Mice inoculated with malaria parasite; treated with 5 mg/kg Artesunate (Standard control)

**Table 1:** Table Showing the Values for WBC and WBC Counts, Hb Concentration and PCV of Mice Groups

Parameters/Post Treatment Days	Mice Groups					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
<b>RBC (<math>10^{12}/L</math>)</b>						
Day 3	10.45 ± 0.33	6.71 ± 0.37	8.01 ± 0.23	8.40 ± 0.20	9.40 ± 0.32	10.02 ± 0.17
Day 5	N.D.	N.D.	8.52 ± 0.44	8.81 ± 0.24	9.91 ± 0.35	10.31 ± 0.20
<b>WBC (<math>10^9/L</math>)</b>						
Day 3	14700.00 ± 517.687	6940.00 ± 347.275	7760.00 ± 312.410	9700.00 ± 852.056	10960.00 ± 177.764	12160.00 ± 317.175
Day 5	N.D.	N.D.	9320.00 ± 239.583	10560.00 ± 777.560	12180.00 ± 363.868	13190.00 ± 263.818
<b>Hb (g/dl)</b>						
Day 3	14.16 ± 0.31	8.54 ± 0.20	11.24 ± 0.55	12.50 ± 0.32	13.32 ± 0.34	11.20 ± 0.41
Day 5	N.D.	N.D.	11.60 ± 0.59	12.98 ± 0.32	13.78 ± 0.37	11.60 ± 0.44
<b>PCV (%)</b>						
Day 3	40.00 ± 0.71	21.00 ± 1.00	26.40 ± 1.03	29.20 ± 0.53	32.60 ± 1.54	30.80 ± 1.99
Day 5	N.D.	N.D.	29.40 ± 0.75	31.60 ± 0.75	35.00 ± 1.73	35.80 ± 1.96

The results are expressed as mean ± standard error of mean (S.E.M)

**N.D. = Not determined**

Group 1: Mice not inoculated with malaria parasite; treated with 3% tween 80 (Normal control)

Group 2: Mice inoculated with malaria parasite; treated with 3% tween 80 (Positive control)

Group 3: Mice inoculated with malaria parasite; treated with 20 mg/kg body weight of the extract

Group 4: Mice inoculated with malaria parasite; treated with 40 mg/kg body weight of the extract

Group 5: Mice inoculated with malaria parasite; treated with 80 mg/kg body weight of the extract

Group 6: Mice inoculated with malaria parasite; treated with 5 mg/kg Artesunate (Standard control)

The increases in RBC counts of mice treated with 20 (group 3), 40 (group 4) and 80 (group 5) mg/kg b.w. of the extract between day 3 and 5 post treatment were significantly ( $p < 0.05$ ) higher when compared to the positive control. The RBC counts of groups 4 and 5 were significantly ( $p < 0.05$ ) higher compared to the standard control. The WBC counts of groups 3, 4 and 5 on days 3 and 5 post treatment were significantly ( $p < 0.05$ ) higher compared to the positive control. In comparison with the standard control, the WBC count of group 5 was significantly ( $p < 0.05$ ) lower and was found to have decreased by 190.00 ( $10^9/L$ ) between day 3 and day 5 post treatment. Compared to the positive control, the Hb concentrations of groups 3, 4 and 5 on days 3 and 5 post treatment were significantly ( $p < 0.05$ ) higher on days 3 and 5 post treatment. Even in comparison with the standard control, the Hb concentrations of groups 4 and 5 were significantly ( $p < 0.05$ ) higher. The PCVs of groups 3, 4 and 5 on days 3 and 5 post treatment were significantly ( $p < 0.05$ ) higher compared to the positive control. When compared with the standard control, the PCVs of groups 3 and 4 were significantly ( $p < 0.05$ ) higher. The effects of the extract on these haematological parameters

were dose-dependent.

As shown in figures 5, 6 and table 2, the serum electrolytes ( $K^+$  and  $Na^+$ ) concentrations of normal control were found to be  $16.28 \pm 1.03$  and  $90.40 \pm 5.12$  mmol/l respectively. The positive control had serum  $K^+$  concentration of  $16.30 \pm 0.35$  mmol/l and  $Na^+$  concentration of  $91.40 \pm 5.12$  mmol/l. On day 3 post treatment, the potassium ion concentrations of groups 3, 4 and 5 were  $16.00 \pm 0.35$ ,  $15.88 \pm 0.34$  and  $15.70 \pm 0.34$  mmol/l respectively. These extract-treated groups showed  $Na^+$  concentrations of  $93.00 \pm 5.44$ ,  $93.40 \pm 5.60$  and  $95.80 \pm 5.42$  mmol/l respectively. The  $K^+$  concentration of the extract-treated groups decreased non-significantly to  $15.96 \pm 0.36$ ,  $15.84 \pm 0.34$  and  $15.60 \pm 0.34$  mmol/l respectively on day 5 post treatment. However, on day 5 post treatment, the  $Na^+$  concentration of these groups increased to  $93.40 \pm 5.48$ ,  $94.40 \pm 5.60$  and  $96.80 \pm 5.42$  mmol/l. The standard control had  $K^+$  concentrations of  $16.02 \pm 1.12$  mmol/l on day 3 post treatment and  $16.02 \pm 1.09$  mmol/l on day 5. The standard control had  $Na^+$  concentrations of  $92.40 \pm 4.91$  mmol/l on day 3 post treatment which increased to  $92.80 \pm 5.09$  mmol/l on day 5 post treatment.

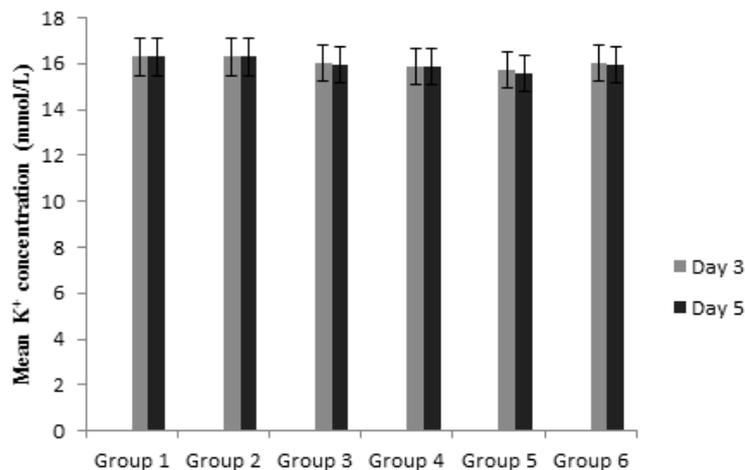


Figure 5: Effect of treatment with ethanol seed extract of *P. nitida* on serum K<sup>+</sup> concentration of malaria-infected mice

- Group 1: Mice not inoculated with malaria parasite; treated with 3 % tween 80 (Normal control)
- Group 2: Mice inoculated with malaria parasite; treated with 3 % tween 80 (Positive control)
- Group 3: Mice inoculated with malaria parasite; treated with 20 mg/kg body weight of the extract
- Group 4: Mice inoculated with malaria parasite; treated with 40 mg/kg body weight of the extract
- Group 5: Mice inoculated with malaria parasite; treated with 80 mg/kg body weight of the extract
- Group 6: Mice inoculated with malaria parasite; treated with 5 mg/kg Artesunate (Standard control)

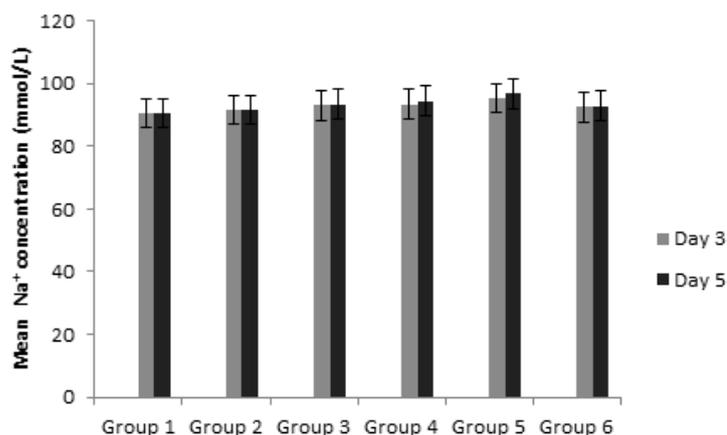


Figure 6: Effect of treatment with ethanol seed extract of *P. nitida* on serum Na<sup>+</sup> electrolyte of mice

- Group 1: Mice not inoculated with malaria parasite; treated with 3 % tween 80 (Normal control)
- Group 2: Mice inoculated with malaria parasite; treated with 3 % tween 80 (Positive control)
- Group 3: Mice inoculated with malaria parasite; treated with 20 mg/kg body weight of the extract
- Group 4: Mice inoculated with malaria parasite; treated with 40 mg/kg body weight of the extract
- Group 5: Mice inoculated with malaria parasite; treated with 80 mg/kg body weight of the extract
- Group 6: Mice inoculated with malaria parasite; treated with 5 mg/kg Artesunate (Standard control)

**Table 2:** Table Showing the Values of Serum Electrolytes (K<sup>+</sup> and Na<sup>+</sup> ions) of Mice Groups

Parameters/Post Treatment Days	Mice Groups					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
<b>K<sup>+</sup> (mmol/l)</b>						
Day 3	16.28 ± 1.03	16.30 ± 0.35	16.00 ± 0.35	15.88 ± 0.34	15.70 ± 0.34	16.02 ± 1.12
Day 5	N.D.	N.D.	15.96 ± 0.36	15.84 ± 0.34	15.60 ± 0.34	16.02 ± 1.09
<b>Na<sup>+</sup> (mmol/l)</b>						
Day 3	90.40 ± 5.12	91.40 ± 5.12	93.00 ± 5.44	93.40 ± 5.60	95.80 ± 5.42	92.40 ± 4.91
Day 5	N.D.	N.D.	93.40 ± 5.48	94.40 ± 5.60	96.80 ± 5.42	92.80 ± 5.09

The results are expressed as mean ± standard error of mean (S.E.M)

**N.D. = Not determined**

Group 1: Mice not inoculated with malaria parasite; treated with 3 % tween 80 (Normal control)

Group 2: Mice inoculated with malaria parasite; treated with 3 % tween 80 (Positive control)

Group 3: Mice inoculated with malaria parasite; treated with 20 mg/kg body weight of the extract

Group 4: Mice inoculated with malaria parasite; treated with 40 mg/kg body weight of the extract

Group 5: Mice inoculated with malaria parasite; treated with 80 mg/kg body weight of the extract

Group 6: Mice inoculated with malaria parasite; treated with 5 mg/kg Artesunate (Standard control)

Compared to positive control, the  $K^+$  concentration of groups 3, 4 and 5 on days 3 and 5 post treatment were non-significantly ( $p > 0.05$ ) lower. In comparison with the standard control, the  $K^+$  concentrations of groups 3, 4 and 5 were non-significantly ( $p > 0.05$ ) lower and were found to have decreased by 0.04, 0.04 and 0.10 mmol/l respectively between day 3 and day 5 post treatment. On days 3 and 5 post treatment, the  $Na^+$  concentrations of groups 3, 4 and 5 were non-significantly ( $p > 0.05$ ) higher compared to the positive control. Compared to the standard control, the  $Na^+$  concentrations of groups 3, 4 and 5 were non-significantly ( $p > 0.05$ ) lower. The effects of the extract on serum electrolytes were dose-dependent.

**DISCUSSION**

Red blood cell (RBC) count tells us the amount of red blood cells contained in one's systemic circulation (Bunn, 2011). The result of the effect of the ethanol seed extract of *Picralima nitida* on RBC count of mice showed that group 5 produced the highest mean RBC count while group 3 produced the least among the extract-treated groups on days 3 and 5 of post treatment. The standard control produced more promising mean RBC count than the extract-treated groups. This evidence showing that the infected mice treated with graded doses of extract exhibited increase in RBC count proved that the extract had a protective effect on the integrity of the red blood cells. White blood cell (WBC) count measures the number of white blood cells in ones blood (Krucik). WBC count was analyzed on days 3 and 5 of post treatment. Group 5 gave the highest mean WBC count; followed by group 4 and the least WBC count was shown by group 3 on days 3 and 5 post treatment. The standard control exhibited better WBC counts than any of the extract-treated groups. The extract-treated groups caused an increase in WBC count of mice showing that the extract had the capability of enhancing

the immune system and hence its function of defending the body against infectious diseases and foreign substances.

The packed cell volume (PCV) percentage of the red blood cells is a biological marker for diagnosis of malaria. A marked decrease in a patient's PCV is one of the indications of severe malaria (Jelkmann, 2004). Among the extract-treated groups, group 5 had the highest mean PCV, followed by group 4 and the least PCV was in group 3 on days 3 and 5 post treatment. The standard control gave higher values on days 3 and 5 post treatment than the extract-treated groups. The evidence that the extract-treated groups caused an increase in PCV of mice revealed that the extract had the capacity to protect the integrity of erythrocytes by boosting their volume percentage in the blood. Haemoglobin (Hb) pigment is a high molecular weight compound found within the erythrocytes and when bound to oxygen (Strippoli *et al.*, 2010), a complex known as oxyhaemoglobin is formed. The highest mean Hb concentration was shown by group 5; followed by group 4 and the least Hb concentration was shown by group 3. The same trend followed on day 5 post treatment. The standard control produced an appreciable mean Hb concentrations on days 3 and 5 post treatment when compared with the positive control. The extract-treated groups caused an increase in Hb concentration compared to the positive control showing that the extract had the ability to protect the Hb contents of the RBCs in the body system for good oxygen transport.

The time-dependent effects of the treatment produced greater effect on day 5 than day 3 post treatment for all the haematological parameters. There is positive significant ( $p < 0.01$ ) correlation between the values for all the haematological parameters. The results of the haematological studies of this research work agree with the findings of Odeghe *et al.* (2012) who reported that

graded doses of methanol extract of *Anthocleista grandiflora* stem bark administered to mice infected with *Plasmodium berghei* showed significant ( $p < 0.05$ ) increase in PCV, Hb, WBC and platelet counts compared to the positive control. Moreso, Ugwu *et al.* (2013) recorded that the haematological parameters-PCV, Hb, and RBC count increased significantly ( $p < 0.05$ ) in mice infected with malaria and treated with 180 mg/kg body weight of ethanol leaf extract of *Moringa oleifera* compared to the positive control.

Potassium ( $K^+$ ), sodium ( $Na^+$ ) and calcium ( $Ca^{2+}$ ) ions play a vital role in muscle contraction and relaxation. The result of the effects of the extract on  $K^+$  electrolyte concentration of malaria infected mice showed that group 3 had the highest  $K^+$  concentration and the least  $K^+$  concentration was exhibited by group 5 on days 3 and 5 post treatment. The standard control showed higher  $K^+$  concentrations than the extract-treated groups on days 3 and 5 post treatment. The result of the effect of the extract on  $Na^+$  concentration showed that group 3 showed the least  $Na^+$  concentration; followed by group 4 and the highest concentration by group 5 on days 3 and 5 post treatment. The standard control showed lower  $Na^+$  concentrations than the extract-treated groups on days 3 and 5 post treatment. The extract treatment caused increase in  $Na^+$  concentration and decrease in  $K^+$  concentration revealing that the administration of the extract could lead to loss of water (dehydration) or damaged kidney tissues. The time-dependent effect of the treatment showed greater effect on day 5 than day 3 of post treatment. There was negative non-significant ( $p > 0.05$ ) correlation between the serum  $Na^+$  and  $K^+$  concentrations. These findings correlate with those of Arise *et al.* (2013) who noted that there were significant decreases in serum electrolytes (bicarbonate, chloride, sodium and potassium) of malaria infected mice treated with some extracts for 7 and 14 days. In view of this, they suggested that prolonged administration may potentiate some level of toxicity.

## CONCLUSION

The haematological parameters were greatly improved in malaria-infected mice after administration of the seed extract of *Picralima*

*nitida*; an evidence that it protected the integrity of the blood cells. Alterations in the serum electrolytes ( $K^+$  and  $Na^+$  ions) is an evidence that the extract interfered with the electrolyte balance in the body. So, care should be taken in administering this extract so that serious electrolyte imbalance or disorders do not ensue owing to the drug's administration. The findings in this study have proven *Picralima nitida* seed extract as good anti-malarial decoction, as used in traditional medicine. Other indices of malaria infection are to be investigated and possibly the active ingredient responsible for this anti-malaria activity be isolated for further analysis.

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