

## ELECTROLYTE PROFILE OF MALARIA INFESTED WISTAR ALBINO RATS TREATED WITH THE CRUDE EXTRACT OF ARTEMISIA ANNUA AND ARTEMISININ COMBINATION THERAPY.

Ekam V. S.\*, Odio S. B., Ekam N. V. and Ihe S. O.

Department of Biochemistry, P.M.B 1115 University of Calabar, Calabar.

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### \*Corresponding Author

Ekam V. S.

Department of Biochemistry,  
P.M.B 1115 University of  
Calabar, Calabar.

### ABSTRACT

Effects of the crude extract of *Artemisia annua* on the serum electrolyte level of wistar albino rats was assessed in this study. It involved twenty four (24) wistar rats, weighing between 180-220g and distributed into four groups of six rats each. Group one served as the normal control and received 0.2ml of distilled water only, group two served as malaria control and was given 0.2ml of distilled water (0.4mg/kg body weight), group three was infected and treated with (1.143mg/kg body weight) ACT, group four was infected and treated with (300mg/kg body weight) crude extract of *Artemisia annua*. The treatment was done twice daily and lasted for 3 days, which was after

three days of malaria parasite inoculation. The rats were fasted overnight after the last administrations and were sacrificed under chloroform inhalation anesthesia. Blood sample was collected for analysis by cardiac puncture into treated sample bottles. The results shows that the administration of crude extract of *Artemisia annua* produced a significant change ( $p < 0.05$ ) in potassium and Bicarbonate ions but no significant change ( $p > 0.05$ ) in sodium and chloride ions while ACT shows significant increase ( $p < 0.05$ ) in bicarbonate ion concentration but shows insignificant difference ( $p > 0.05$ ) in sodium ion, potassium ion and chloride ion. It may therefore be concluded that both ACT and extract *Artemisia annua* have no negative effect on serum Electrolyte level in treating malaria.

**KEYWORDS:** Electrolyte, *Artemisia annua*, Plasmodium, Artemisinin, Phytochemicals, Serum.

## INTRODUCTION

Electrolyte profile is a test, which includes the measurement of positively or negatively, charged molecules called ions that are found in the bloodstream and in other fluids throughout the body, an imbalance of these electrolytes in the blood can lead to several metabolic disorders. Electrolytes produce an electrically conducting solution when dissolved in a polar solvent such as water. The dissolved electrolytes separate into cations and anions and are dispersed uniformly through the solution. Electrically, such a solution is neutral. Electrolytes include most soluble salts, acids, and bases. Electrolyte solutions can also result from the dissolution of some biological (e.g. DNA) and synthetic polymers (e.g. polystyrene sulfonate) termed polyelectrolytes, which contain charged functional groups. A substance that dissociates into ions in solution acquires the capacity to conduct electricity. In the serum, the concerned electrolytes include potassium ion ( $K^+$ ), chloride ion ( $Cl^-$ ), bicarbonate ion ( $HCO_3^-$ ), and sodium ion ( $Na^{2+}$ ). This study is therefore aimed at investigating the impact of the extract of *Artemisia annua* on serum electrolyte profile.

Medicinal plants have been in use for many decades, and have served as a lead in pharmaceutical industries across the globe. Developing countries have been using plants as their main source of medicine for ages. Reports have shown that 80% of the populations in Asian and African countries presently use herbal medicine for some aspect of primary health care. Studies in the United States and Europe have shown that their use of medicinal plants is increasingly becoming more common in recent years as scientific evidence has become more widely available. Plants are the oldest known primary health care products and the knowledge of their use varies depending on the medicinal and historical background of each country (Sumner and Judith, 2000). *Artemisia annua* has been implicated in treating certain medical complications in history. In traditional Chinese medicine, *Artemisia annua* is used to treat fever and Research to develop anti malaria drugs led to the discovery of artemisinin, which is extracted from *Artemisia annua*, a herb traditionally used for the treatment of fever (Jansen, 2006). The proposed mechanism of action of artemisinin involves cleavage of endoperoxide bridges by iron, producing free radicals which damage biological macromolecules causing oxidative stress in the cells of the parasite (Cumming *et al.*, 1997). This enables herbal medicine but also gives them the same potential to cause harmful side effects (Stepp *et al.*, 2001). Malaria is an intermittent and remittent fever caused by a protozoan (parasite) that invades the red blood cells. The parasite is transmitted by the female anopheles mosquitoes in many tropical and sub tropical regions. The parasite belongs to the genus *Plasmodium*.

Malaria causes symptoms that typically include fever, fatigue, vomiting, and headaches. In severe cases it can cause yellow skin, seizures, coma, or death. Humans can be infected by 5 species of *Plasmodium* which are *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. The species *P. knowlesi* rarely causes disease in humans. Malaria is typically diagnosed by the microscopic examination of blood using blood films or with antigen-based rapid diagnostic tests; methods that use the polymerase chain reaction (PCR) to detect the parasite's DNA have been developed, but are not widely used in areas where malaria is common due to their cost and complexity (Nadjm and Behrens, 2012). Despite the need, no effective vaccine exists, although efforts to develop one are ongoing. However, the recommended treatment for malaria is a combination of anti-malarial medications that includes an artemisinin (Caraballo, 2014) and its derivatives. This is because of the insolubility and low bioavailability of artemisinin, hence the concentrations of artemisinin alone are considered insufficient to treat malaria (Jansen FH, 2006).

In spite of the global proliferation of anti malarial drugs, there have been no substantial studies geared towards the assessment of the effect of these drugs on serum electrolyte profile. It therefore became necessary to evaluate the electrolyte profile of malaria infested wistar albino rats treated with the crude extract of *Artemisia annua* and artemisinin combination therapy (ACT).

## MATERIALS AND METHODS

**Collection and preparation of materials:** The anti malarial drug zymal® (Artemether Tablet 80mg + Lumenfantrine Tablets 480mg) manufactured by Innova Cap Tab, Pharmaceutical Co Ltd, 81-B EPIP, Phase-I, Jharmajri, Baddi (H.P) India. Manufacturing Lic. No.: MNB/06/394, 5% dextrose water was bought from Turtle Bay pharmacy in Calabar and Distilled water were used for the study. The *Artemisia annua* plant was gotten from the biotechnology farm operated by Prof. Ebiamadon Andi Brisibe, Professor of Biotechnology of the Department of Genetics and Biotechnology, Faculty of Science, University of Calabar.

**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 rats weighing between 180 - 220g were used for this study, they were purchased from the animal house 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 weight as shown in table 1.

**Inoculation:** The infection of the recipient rats was initiated by injecting the parasite preparation gotten from the Calabar office of Roll Back Malaria to healthy test rats via intramuscular route as described by David et al (2004) and Peter and Anatoli (1998). 2ml of Plasmodium falciparum sample received from the Calabar office of Roll Back Malaria with a parasite load of 161.5 was diluted with 5% dextrose water using a dilution factor of 1:4 (Shakya et al., 2012). 0.5ml per kilogram 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).

**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 soaked 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 40°C. 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 investigation of the electrolyte profile. 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 sterile test tubes.

### Electrolyte Analysis

The samples were analyzed in a spectrophotometer within the visible range.

The parameters to be assayed includes: potassium ion ( $K^+$ ), chloride ion ( $Cl^-$ ), bicarbonate ion ( $HCO_3^-$ ), sodium ion ( $Na^+$ ).

**Statistical analysis:** Data was expressed as mean  $\pm$  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 the results of the electrolyte profile,  $K^+$  (mmol/L),  $Cl^-$  (mmol/L),  $HCO_3^-$  (mmol/L),  $Na^+$  (mmol/L).

**Effect of treatment on Serum Potassium level:** The serum potassium level (mmol/L) showed a significant decrease in the malaria control group ( $4.83 \pm 0.13$ ) and ACT treated group ( $5.40 \pm 0.35$ ) compared to the normal control group ( $5.60 \pm 0.27$ ), whereas the Artemisia treated group ( $5.98 \pm 0.19$ ) showed a significant increase when compared to the normal control group. Potassium level of the Artemisia treated group was significantly higher ( $P < 0.05$ ) than the ACT treated group.

**Effect of treatment on serum chloride level:** Total chloride level (mmol/L) for malaria control group ( $100.50 \pm 0.62$ ) ACT treated group ( $101.33 \pm 0.49$ ) and Artemisia treated group ( $99.67 \pm 0.67$ ) showed no significant difference ( $P > 0.05$ ) from normal control group ( $101.00 \pm 0.73$ ). These was no significant different ( $P > 0.05$ ) between the test groups.

**Effect of treatment on serum Bicarbonate level:** The serum Bicarbonate level (mmol/L) of the malaria control group ( $23.00 \pm 0.37$ ). ACT treated group and Artemisia annua treated group ( $23.33 \pm 0.49$ ) were significantly increased ( $P < 0.05$ ) compared to the normal control

group ( $22.00 \pm 1.18$ ). Bicarbonate level of the ACT treated group was significantly higher ( $P < 0.05$ ) than the Artemisia treated group.

**Effect of treatment on serum sodium level:** Total sodium level (mmol/L) for malaria control group ( $144.83 \pm 1.47$ ) ACT treated group ( $146.67 \pm 0.42$ ) and Artemisia annua treated group ( $147.50 \pm 0.85$ ) showed no significant difference ( $P > 0.05$ ) from normal control group ( $143.50 \pm 0.89$ ). There was also no significant difference ( $P > 0.05$ ) between the test groups.

**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 <i>A. Annua</i> treated

**Table 2: Serum electrolyte baseline values**

The total mean serum level of electrolytes in wistar rats after administration of ACT and crude extract of *Artemisia annua* for 3 days are presented in the table below in (mmol/L).

Group/No	Groups	Na <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>
1	Control	$145.50 \pm 0.89$	$5.60 \pm 0.27$	$101.00 \pm 0.73$	$14.83 \pm 0.48$
2	Malaria control	$144.83 \pm 1.47$	$4.83 \pm 0.13^*$	$100.50 \pm 0.62$	$23.00 \pm 0.37^*$
3	Malaria + ACT	$146.67 \pm 0.42$	$5.40 \pm 0.35$	$101.33 \pm 0.49$	$23.33 \pm 0.49^*$
4	Malaria + extract of <i>Artemisia annua</i>	$147.50 \pm 0.85$	$5.98 \pm 0.19^a$	$99.67 \pm 0.67$	$22.00 \pm 1.18^*$

Values are expressed as mean  $\pm$ SEM, n = 6

\* = significantly different from control  $P < 0.05$

a = significantly different from malaria control  $P < 0.05$

b = significantly different from malaria + ACT  $P < 0.05$

## DISCUSSION

Several studies on medicinal plants such as *Artemisia annua* have revealed that they contain certain phytochemicals and related components which have great potentials to treat human diseases (Edet, *et al.*, 2009). The use of medicinal plants in the management of various metabolic disorders is attributed to their rich phytochemical constituents (Trease and Evans, 2002). The extract when administered to malaria infested rats' causes changes in some of the

electrolyte profile as compared with the control, ( $P < 0.05$ ). Sodium and chloride ions show no significant difference ( $P > 0.05$ ) from the control, while potassium and bicarbonate ions shows significant difference as compared to the control. This result is inconsistent with earlier reports by Nurul, *et al.*, (2013), on biochemical and haematological analysis, which shows no significant, changes in potassium ions. The changes observed in this study may be attributed to the bioactive constituents identified to be present in this plant. Therefore, the leaf extract of this plant may have impact in the treatment of disorders such as malaria.

## CONCLUSION

In conclusion, both ACT and extract of *Artemisia annua* have no negative effect on serum electrolyte level in treating malaria. The extract should be recommended for those who cannot afford the refined ACT drugs.

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