



## Hematological indices and abnormalities in malaria infected albino model treated with *Artemisia annua* extract and chloroquine therapy

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### Abstract

Malaria disease can be categorized as uncomplicated or severe (complicated). In general, malaria is a curable disease if diagnosed and treated promptly and correctly. All the clinical symptoms associated with malaria are caused by the asexual erythrocytic or blood stage parasites. Malaria is caused by the Plasmodium parasite. The parasite can be spread to humans through the bites of infected mosquitoes. Artemisinin derivatives are an important new class of antimalarial agents. These compounds contain endoperoxide bridges which are essential for antimalarial activity. The aim of this study was to evaluate the effect of both Ethanolic and watery extract of Artemisinin against malaria infected mice. Blood was taken from a donor mouse with parasitemia Plasmodium falciparum and diluted using saline to  $5 \times 10^7$  parasitized erythrocytes per ml. Swiss albino mice were infected with 0.2 ml blood., Ethanolic, and water extract of Artemisinin Annua in addition to traditional chloroquine were used in treatment of mice after inoculation with infected blood with plasmodium. In our study there were increase in both red blood cells in Art. Annuna Ethanol Extract Art. Annuna Water Extract and Chloroquine treatment groups with values  $7.48 \pm 0.52$ ,  $8.7 \pm 0.24$ ,  $9.9 \pm 0.51$ , and  $4.05 \pm 0.8$ . This study indicates that the treatment of malaria using *Artemisia annua* results in improved hematological indices.

**Keywords:** Plasmodium; Artemisinin Annua; Hemoglobin

### 1. Introduction

Malaria is a disease of global importance that results in 300-600 million cases annually and an estimated 2.2 billion people are at risk of infection [1]. More than a century after identification of the causative parasites and more than half a century after finding effective drugs and insecticides, the disease as old as humanity itself. Numerically the most important of the life-threatening protozoan disease is malaria, which is responsible for at least 750,000 death a year, mostly in young children in Africa [2]. Malaria infection is initiated upon deposition of sporozoites into a vascular tissue of the skin from the salivary glands of a female mosquito as it probes a blood meal. Sporozoite injected into the blood stream leave the blood vascular system within 40 minutes and subsequently invade the parenchymal cells of the liver. Once merozoites burst from hepatocyte they invade red blood cells (RBCs) and enter the asexual erythrocytic cycle. Invasion of new RBCs. The pathogenic effect of a malarial infection has been considered to be directly related to hemolysis of infected red cell and uninfected cell, liberation of the metabolites of the parasite and the immunologic response of the host to this antigenic material. In persons subjected to repeat attack of malaria anemia is disproportional to the number of red blood cells infected and indicates that non infected red blood cells may become sensitized and be destroyed [3]. Clinical diagnosis is based on the patient's symptoms and on physical findings at examination. The first symptoms of malaria (most often fever, chills, sweats, headaches, muscle pains, nausea, and vomiting) are often not specific and are also found in other diseases (such as the "flu" and common viral infections). The accepted laboratory

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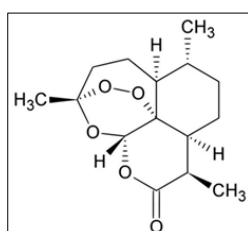
practice for the diagnosis of malaria is the preparation and microscopic examination of blood films stained with Giemsa. The ability to count parasites in sequential blood films enables the response to therapy to be monitored, particularly for *P. falciparum* infections. The primary objective of treatment is to ensure a rapid and complete elimination of the *Plasmodium* parasite from the patient's blood to prevent progression of uncomplicated malaria to severe disease or death, and to chronic infection that leads to malaria-related anemia [4]. Chloroquine was the first drug produced on a large scale for treatment and prevention of malaria infection. Chloroquine has activity against the blood stages of *P. ovale*, *Plasmodium malariae*, and susceptible strains of *P. vivax* and *Plasmodium falciparum* [5].

Artemisinin from the plant *Artemisia annua* (*A. annua*) L, and used as artemisinin combination therapy (ACT), is the current best therapeutic for treating malaria, a disease that hits children and adults especially in developing countries. Traditionally, *A. annua* was used by the Chinese as a tea to treat "fever". More recently, investigators have shown that tea infusions and oral consumption of the dried leaves of the plant have prophylactic and therapeutic efficacy. Currently extracted from *Artemisia annua* (*A. annua*) L., artemisinin is delivered in concert with another antimalarial drug [artemisinin combination therapy (ACT)] as the preferred treatment to slow emergence of drug resistance. Despite these efforts, artemisinin resistance is appearing [6].

		Plasmodium Species			
		Falciparum	Vivax	Malariae	Ovale
Parasite Life-cycle stages	Ring				
	Trophozoite				
	Schizont				
	Gametocyte				

**Figure 1** Blood smear stained with Giemsa Stain, showing white blood cells and several red blood cells, infected with *Plasmodium*

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**Figure 2** Structure of artemisinin

## 2. Material and methods

25 Male Swiss albino rats, aged 6 weeks old, weighing  $150 \pm 200$  g, raised in Dubai Institute for Environmental Research and Laboratory analysis, Dubai, United Arab Emirates, were used. All animals were housed in standard cages (5 rats/cage), feeding with standard laboratory diet and tap water ad libitum. The experimental animals were housed in air-conditioned rooms at 21-23°C and 60-65% of relative humidity and kept on a 12 h light/12 h dark cycle. The animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals, published by ethics of research committee of Dubai Medical College, Dubai, United Arab Emirates.

**Table 1** Different Groups of induced albino rats and the various treatments

<b>Group I</b>	Food and water given ad libitum, No parasitaemia
<b>Group II</b>	Plasmodium induced and untreated
<b>Group III</b>	Plasmodium induced and treated with aqueous <i>Artemisia annua</i>
<b>Group IV</b>	Plasmodium induced and treated with Ethanolic <i>Artemisia annua</i>
<b>Group V</b>	Plasmodium induced + Chloroquine treatment

### 2.1. Inoculation and Treatment of experimental mice

Blood was taken from a donor mouse with parasitemia, *Plasmodium falciparum* and diluted using saline to  $5 \times 10^7$  parasitized erythrocytes per ml. Swiss albino mice were infected with 0.2 ml blood. On day zero, the individual mouse was inoculated with 0.2ml of infected blood containing  $1 \times 10^7$  Plasmodium parasitized erythrocytes [7].

### 2.2. Confirmation of Parasitemia

Geimsa's stain technique was used to Confirm parasitemia in the blood, a thick film was produced by depositing and spreading drops of the blood sample on a glass slide. The film was allowed to dry thoroughly without any contamination. Then, the slide was immersed in a mixture of 1 drop of Geimsa's stain to each ml of distilled water; washed off and air dried. Finally, the sample was examined under the microscope using the oil immersion objective (x100) [8].

### 2.3. Extraction and Treatment

The treatments dose, 1000 mg/kg/day for aqueous extract and 500 mg/kg/day for hydro alcoholic extract. Chloroquine solutions were prepared by dissolving Chloroquine diphosphate in water (approximately 30 mg/ml) and diluting it to the required concentration for a standard 100- $\mu$ l injection volume.

**Table 2** Watery and Ethanolic Extracts of *Artemisia annua*

<b>Chemical Constituent</b>	<b>Water Extract</b>	<b>Ethanolic Extract</b>
<i>Artemisia annua</i> Ethanol (96%)	12 gm <i>Artemisia annua</i> powder	40 gm <i>Artemisia annua</i> powder
	1000 ml Boiled water	700 ml Ethanol 96%.
	Filtrate was freeze-dried to obtain the dried aqueous extract	Filtrate was evaporated using rotary evaporator

### 2.4. Collection of Blood sample and Hematological analysis

Blood samples were collected and mixed with 2 $\mu$ L of prepared EDTA at room temperature of 36 C. Blood samples were analyzed for hematological indices and. The Red Blood Cell (RBC), and White blood cell (WBC) counts were determined using the hemocytometer. Packed cell volume (PCV) using hematocrit reader [9, 10, 11].

### 2.5. Motor and Muscular activity assessment

Digital Actophotometer was used to study the spontaneous locomotor activity.

## 2.6. Statistics

SPSS software was used, all data were expressed in Mean and Standard deviation. One-way analysis of variance (ANOVA) was used for comparison of groups as appropriate, with a significance level of  $P < 0.05$ .

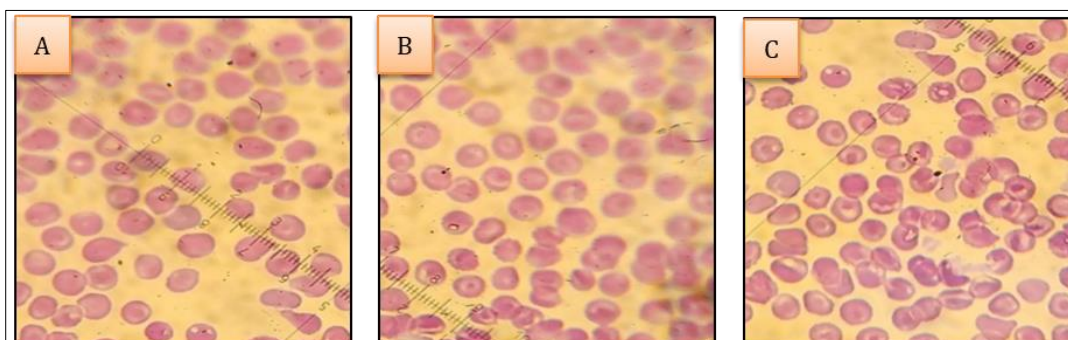
## 3. Results and discussion

Numerous medicinal plants contain various compounds which serve as possible drug sources for human disease management [12]. A perfect antimalarial treatment exhibits selectivity and therapeutic activity on malaria parasites without consequential toxicity on the host. The majority of the known antimalarials such as CQ, mefloquine, and recently artemisinin products have been discovered using rodent malaria model [13].

Anemia is one of the symptoms of malaria. Anemia levels are directly proportional to the severity of malaria. Human and murine model have a close linkage in anemia expression due to similarity in biological makeup [14]. Packed cell volume values are inversely proportional to anemia because of malaria [15].

**Table 3** The Red Blood Cell (RBC) and Blood Indices in all groups

Groups CBC	No parasitaemia Group	Plasmodium induced and untreated	Art. Annuna Ethanol Extract	Art. Annuna Water Extract	Chloroquine	P value
WBCs	8,42 ± 183	69.1 ± 7.36	45.98 ± 3.8	49.08 ± 0.4	45.2 ± 2.5	$P < 0.005$
RBCs 106/UL	5,2 ± 0.43	4.05 ± 0.8	7.48 ± 0.52	8.7 ± 0.24	9.9 ± 0.51	$P < 0.005$
Hg (g/dL)	15 ± 0.3	7.28 ± 1.04	10.42 ± 0.63	10.2 ± 0.23	10.2 ± 0.16	$P < 0.005$
HCT %	48.2 ± 1.8	31.66 ± 1.34	36.28 ± 0.29	36.3 ± 0.21	37.2 ± 0.63	$P < 0.005$
MCV (FL)	93 ± 2.5	50.12 ± 0.86	51.18 ± 1.68	51.9 ± 0.90	52.3 ± 0.64	$P < 0.005$
MCH (Pg)	29 ± 2.8	17.02 ± 0.45	16.98 ± 0.29	17.2 ± 0.25	18.2 ± 0.52	$P < 0.005$
MCHC (g/dL)	31 ± 2	33.92 ± 0.92	36.6 ± 0.28	36.5 ± 0.56	37.1 ± 0.14	$P < 0.005$



**Figure 3** Representative images of Giemsa-stained Blood from Plasmodium induced and treated with Ethanolic *Artemisia annua* (A, B and C)

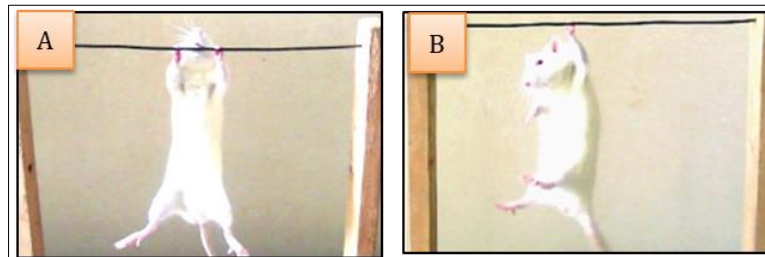
**Table 4** Muscular Activity in all groups per seconds

Groups	No parasitaemia Group	Plasmodium induced and untreated	Art. Annuna Ethanol Extract	Art. Annuna Water Extract	Chloroquine	P value
Before	89.9 ± 5.4	149.6 ± 5.4	148.0 ± 7.3	146.8 ± 2.7	127.2 ± 11.0	$P < 0.005$

After Treatment	92.4 ± 5.4	69.2 ± 17.1	152.4 ± 5.7	134.4 ± 45.1	125.4 ± 10.5	P< 0.005
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**Table 5** Motor Activity in all groups per seconds

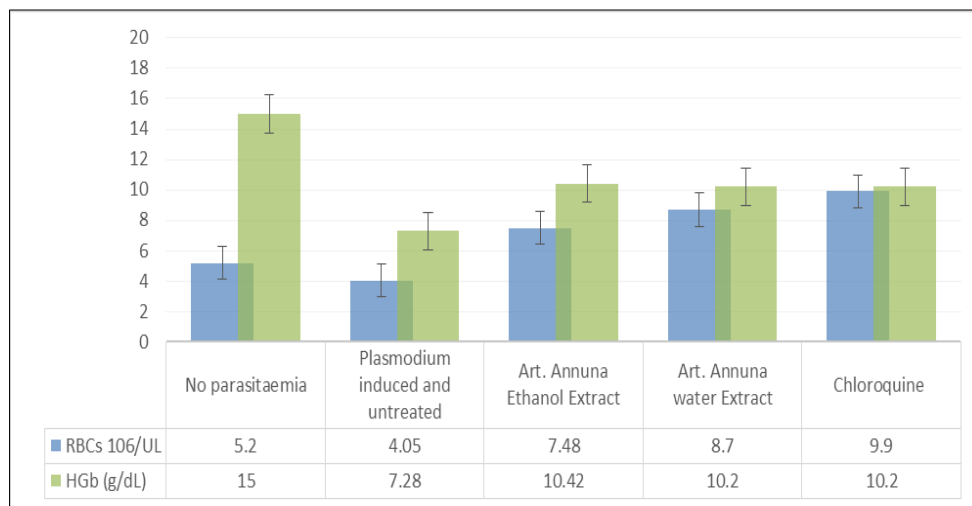
Groups	No parasitaemia Group	Plasmodium induced and untreated	Art. Annuna Ethanol Extract	Art. Annuna Water Extract	Chloroquine	P value
Before	70.1 ± 4.1	67.4 ± 10.78	66.8 ± 15.12	68.2 ± 4.1	64.4 ± 4.3	0.506
After Treatment	71.8 ± 3.12	52.4 ± 10.64	81.2 ± 5.3	78.8 ± 6.5	75.4 ± 3.5	0.000



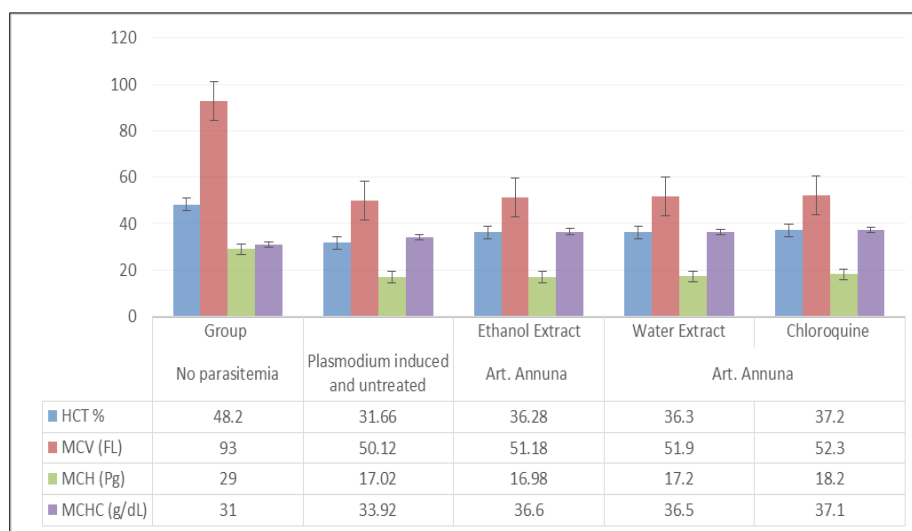
**Figure 4** Muscular activity for all groups



**Figure 5** Motor activity test for all groups



(X-axis represent groups, Y-axis Mean Values of RBC and Hg)

**Figure 6** Mean value of Red Blood Cell and Hg in all groups

(X-axis represent groups, Y-axis Mean Values of RBC indices)

**Figure 7** Mean value of Blood indices in all groups

In our study there were increase in both red blood cells in Art. Annuna Ethanol Extract Art. Annuna Water Extract and Chloroquine treatment groups with values  $7.48 \pm 0.52$ ,  $8.7 \pm 0.24$ ,  $9.9 \pm 0.51$ , and  $4.05 \pm 0.8$ . In our study there were increase in hemoglobin in Art. Annuna Ethanol Extract Art. Annuna Water Extract and Chloroquine treatment groups with values  $10.42 \pm 0.63$ ,  $10.2 \pm 0.23$ , and  $10.2 \pm 0.16$ . In our study there was increase in PCV in both Art. Annuna Ethanol Extract Art. Annuna Water Extract and Chloroquine treatment groups with values  $36.28 \pm 0.29$ ,  $36.3 \pm 0.21$ , and  $37.2 \pm 0.63$  this agrees with Ovukporage [18] reported that malaria parasite reduces red blood cell count, Packed Cell Volume (PCV) and Hemoglobin (Hb) concentration. Previous studies have reported that dehydroartemisinin, a derivative of artemisinin significantly elevated the packed cell volume [19], while artesunate and dehydroartemisinin has been reported to cause no significant effect on RBC and Hb. The elevated levels of hemoglobin in the A. annua treated group could be because of increase in number or sizes of the RBCs [20]. CQ is a blood schizonticidal drug without activity against the liver stage [21]. Accordingly, the parasite enters the host's red blood cell and digests hemoglobin (Hb) in its acidic food vacuole (FV) as the dominant source of nutrition. Hb is a multi-subunit protein with an iron-containing heme group found in erythrocytes. After Hb digestion in the FV of the parasite, Hb is degraded to amino acids and heme (ferriprotoporphyrin IX) as a toxic by-product. The parasite needs these amino acids for its growth, and several malarial protease enzymes are involved in this degradation process. Plasmodium parasites do not have any enzymes to metabolize toxic heme, which is responsible for the parasite's death. Nonetheless, the malaria parasite has developed a special process for the detoxification of heme. For this purpose, the malaria parasite converts heme to hemozoin as an insoluble crystalline form (malaria pigment) by heme polymerization. Hemozoin is not toxic for the parasite, and its formation is an essential mechanism for the detoxification and survival of the parasite. Generally, small molecules with a 4-aminoquinoline moiety such as CQ and its analogues present antimalarial activity through the prevention of hemozoin formation which is resulted to the parasite's death [22] this agree with our study in which there was increase in both Hemoglobin and red blood cells count in chloroquine treated animals with mean values  $9.9 \pm 0.51$ ,  $10.2 \pm 0.16$  respectively. Table 3, Figures (3, 6, 7).

Muscular Activity after treatment in no parasitemia Group, Plasmodium induced and untreated, Art. Annuna Ethanol Extract, Art. Annuna water Extract and Chloroquine were  $92.4 \pm 5.4$ ,  $69.2 \pm 17.1$ ,  $152.4 \pm 5.7$ ,  $134.4 \pm 45.1$  and  $125.4 \pm 10.5$ ; respectively ( $p < 0.005$ ) table 4. Motor Activity after treatment in no parasitemia Group, Plasmodium induced and untreated, Art. Annuna Ethanol Extract, Art. Annuna water Extract and Chloroquine were  $71.8 \pm 3.12$ ,  $52.4 \pm 10.64$ ,  $81.2 \pm 5.3$ ,  $78.8 \pm 6.5$  and  $75.4 \pm 3.5$ ; respectively ( $p < 0.005$ ) table 5, figures (4, 5).

#### 4. Conclusion

The hematological changes in malaria infested albino mice administered with extract of *Artemisia annua* and artemisinin was investigated in this research. The results of this study indicate that the treatment of malaria using *Artemisia annua* results in improved hematological indices. Therefore, there is a need to further phytoresarch especially on plants which have already been shown to have antimalarial activities.



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## Compliance with ethical standards

### *Acknowledgments*

Authors would like to thank Dubai Medical College for support. Our gratitude also goes to Dubai Institute for Environmental Research and Laboratory Analysis for supplying mice.

### *Disclosure of conflict of interest*

No conflict of interest.

### *Statement of ethical approval*

This work was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of Dubai Institute for Environmental Research and Laboratory Analysis. The animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals, published by ethics of research committee of Dubai Medical College, Dubai, United Arab Emirates.

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