Original Article

# Hematoxicity of Amodiaquine in Sprague-Dawley Rats

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## ABSTRACT

**Objective:** The use of amodiaquine (AQ) and its associated toxic effect has been a major public health concern since cases of life-threatening agranulocytosis and hepatic toxicity were reported during its prophylactic use. The objective of this study was to evaluate the hematological safety profile of AQ therapy. **Materials and Methods:** Sprague-Dawley rats were randomly distributed into four groups ($n$=5). Group 1 was the control, while groups 2, 3, and 4 received AQ treatment for 14 days at varying doses of 5 mg/kgBW, 10 mg/kgBW, and 15 mg/kgBW daily, respectively. **Results:** Following treatment, hematological variables were comparable in all groups ($P$>0.05). **Conclusion:** This study provides evidence to support the use of AQ in the treatment of uncomplicated malaria. However, to prevent emergence of local drug resistance, it should be used as part of a combination therapy. Monitoring for adverse effects is suggested.

**Key words:** Amodiaquine, hematological parameters, malaria, toxicity

## INTRODUCTION

Amodiaquine (AQ), a 4-aminoquinoline related to chloroquine (CQ), is commonly used as an antimalarial and anti-inflammatory agent.[1] It is used as prophylaxis as well as chemotherapy in acute malarial attacks in nonimmune subjects. Although resistance to AQ has been reported, it remains effective against some chloroquine-resistant strains (CRS). AQ has also been tried with variable success in the treatment of giardiasis, hepatic amoebiasis, lepra reactions, lupus erythematosus, rheumatoid arthritis, and urticarial.[2]

AQ, a congener of CQ, was withdrawn from use in some parts of the world because of fatal side effects, notably agranulocytosis and hepatitis, which occurred mainly in nonimmune adults taking the drug for prophylaxis.[3] AQ has been reported to cause direct bone marrow stem cell toxicity,[4] whereas other studies have detected little direct toxicity to peripheral cells at therapeutic concentrations.[5] Polymorphonuclear toxicity, however, has been observed in the presence of AQ-specific serum components, indicative of an indirect immunological mechanism for the agranulocytosis. The mechanism underlying the well-known side effects of AQ (agranulocytosis and hepatitis) is direct toxicity or immune-mediated hypersensitivity.[6]

Despite it being no longer recommended in the United States and some other parts of the world for chemoprophylaxis of *Plasmodium falciparum* malaria because of its associated hepatic toxicity and agranulocytosis, its inexpensiveness and substantial activities in CRS has encouraged its use in endemic areas with few alternatives to be debated and re-evaluated.[3] This study extensively evaluated the hematological safety profile of AQ treatment.

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## MATERIALS AND METHODS

### Animals

Sprague-Dawley rats were used for the experiment. They were housed in standard rat cages under laboratory

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conditions with 12:12-hour light/dark cycle at 25°C±2. The animals were allowed to acclimatize for two weeks.[7]

### Treatment

Rats were randomly distributed into four groups ($n$=5). Group 1 was the control, while groups 2, 3, and 4 received AQ treatment (Camoquine®, Parke-Davis Laboratories, United Kingdom) for 14 days at varying doses of 5 mg/kgBW (low dose), 10 mg/kgBW (normal dose), and 15 mg/kgBW (high dose) daily, respectively. All rats were allowed free access to standard rat chow and water.

### Hematological evaluation

Blood samples were collected and dispensed into tubes containing lithium-heparin anticoagulant. Red blood cells and total white blood cell were counted by Neubauer's improved hemocytometer using Hyem's and Turks solution as a diluting fluid, respectively. Differential white blood cell (DWBC) count was estimated by standard laboratory method using Lieshman's stain. Hemoglobin was estimated by Shalis method. Packed cell volume was estimated using a microhematocrit reader after centrifuging the blood sample at 3 000 rpm for 30 minutes.[8] Mean cell volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated respectively using standard formula described by Dacie and Lewis[9] and Joshi *et al*.[10]

### Statistical analysis

Data are expressed as mean±standard deviation (SD). Statistical analyses were done by ANOVA, followed by Duncan's multiple range test for pairwise comparison. Analyses of data were done using the SPSS software (SPSS Inc., Chicago, USA). $P<0.05$ was set as the level of significance.

### Ethics

All animals received humane care in compliance with the institution's guideline and criteria for humane care as outlined in the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

## RESULTS

Hematological changes in test groups and the control group are presented in Table 1. Hematological variables were comparable in all groups ($P>0.05$).

Table 2 shows the effect of AQ on red blood indices. There were no significant differences in MCV, MCH, and MCHC in all groups ($P>0.05$).

DWBC counts are shown in Table 3. There were no significant differences in all groups ($P>0.05$).

**Table 1: Blood cells counts, packed cell volume, and hemoglobin concentrations in experimental groups**

| Variables | Group 1 Control | Group 2 Low dose | Group 3 Normal dose | Group 4 High dose | *P* values | | |
|---|---|---|---|---|---|---|---|
| | | | | | 1 *vs* 2 | 1 *vs* 3 | 1 *vs* 4 |
| Red blood cell count (×10$^{12}$/l) | 6.28 ± 3.18 | 6.92 ± 1.95 | 5.89 ± 0.47 | 7.51 ± 0.425 | 0.711 | 0.793 | 0.416 |
| Packed cell volume (%) | 44.83 ± 3.42 | 42.30 ± 3.85 | 43.90 ± 7.76 | 45.20 ± 1.83 | 0.304 | 0.812 | 0.837 |
| Hemoglobin count (g/dl) | 15.60 ± 0.94 | 13.63 ± 1.05 | 11.53 ± 1.95 | 14.60 ± 1.12 | 0.162 | 0.300 | 0.164 |
| Total white blood cell count (×10$^{9}$/l) | 7.10 ± 1.12 | 5.43 ± 2.97 | 7.60 ± 5.61 | 5.17 ± 2.33 | 0.274 | 0.850 | 0.133 |
| Platelet count (×10$^{9}$/l) | 449.67 ± 52.90 | 487.00 ± 21.30 | 371.67 ± 158.00 | 484.33 ± 113.00 | 0.182 | 0.327 | 0.551 |

**Table 2: Blood indices in experimental groups**

| Variables | Group 1 Control | Group 2 Low dose | Group 3 Normal dose | Group 4 High dose | *P* values | | |
|---|---|---|---|---|---|---|---|
| | | | | | 1 *vs* 2 | 1 *vs* 3 | 1 *vs* 4 |
| MCV (fl) | 71.39 ± 8.77 | 61.13 ± 7.65 | 74.53 ± 5.41 | 60.18 ± 7.63 | 0.084 | 0.515 | 0.063 |
| MCH (pg) | 24.84 ± 4.70 | 19.70 ± 2.68 | 19.58 ± 3.80 | 19.47 ± 2.91 | 0.066 | 0.087 | 0.061 |
| MCHC (%1 g/100ml) | 34.80 ± 4.25 | 32.22 ± 2.01 | 26.26 ± 7.69 | 32.30 ± 2.91 | 0.255 | 0.062 | 0.309 |

MCV: Mean cell volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration

**Table 3: Differential white blood cell counts in experimental groups**

| Variables | Group 1 Control | Group 2 Low dose | Group 3 Normal dose | Group 4 High dose | *P* values | | |
|---|---|---|---|---|---|---|---|
| | | | | | 1 *vs* 2 | 1 *vs* 3 | 1 *vs* 4 |
| Neutrophils (%) | 12.67 ± 2.68 | 10.67 ± 6.62 | 7.67 ± 4.92 | 8.33 ± 4.20 | 0.549 | 0.081 | 0.088 |
| Eosinophils (%) | 1.67 ± 0.74 | 3.13 ± 1.41 | 3.33 ± 0.74 | 2.33 ± 0.74 | 0.081 | 0.070 | 0.195 |
| Basophils (%) | 0.33 ± 0.33 | 0.33 ± 0.74 | 0.35 ± 0.72 | 0.40 ± 0.74 | 1.000 | 0.966 | 0.884 |
| Monocytes (%) | 7.00 ± 1.30 | 7.67 ± 2.68 | 7.88 ± 0.74 | 8.00 ± 1.30 | 0.629 | 0.224 | 0.258 |
| Lymphocytes (%) | 77.67 ± 4.16 | 78.67 ± 8.30 | 79.33 ± 1.99 | 81.00 ± 2.24 | 0.816 | 0.443 | 0.153 |

## DISCUSSION

Hematological changes in all the treated groups when compared with those of the control revealed that AQ has no significant effect on hematological parameters. Similarly, red blood indices were comparable in all groups. Although there were marginal changes in hematological variables and red cell indices, these were not statistically significant. This is in keeping with previous studies.[11,12] The results observed from this study suggest that AQ does not cause inhibition of hemopoiesis, reduction of growth factors, and other food utilization parameters associated with hemopoiesis or hemolysis.

This study also observed that there were no statistically significant changes in DWBC of all groups following AQ treatment. This is in consonance with previous studies[11,12] that observed marginal and within normal limits of hematological profile with no agranulocytosis in patients who received AQ treatment. However, this is in contrast with other studies[3] that reported agranulocytosis in adults taking AQ for prophylaxis. The variation observed might be due to the duration of treatment, as cases that reported AQ-induced agranulocytosis were associated with its long-term use as prophylaxis. Pharmacogenetic polymorphism might also be a contributing factor.

Results from this study corroborate previous studies[13-19] that reported the safety and efficacy of AQ treatment. This shows that AQ poses no hematological toxicity and thus supports its continued use in the treatment of uncomplicated malaria. However, we suggest that it is used as part of a combination therapy, preferably artemisinin-based combination therapy,[20] to prevent the development of local drug resistance. Monitoring for rare adverse effects is recommended.

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