

# Morphological Effect of Chloroquine on Developing Ovaries of Rats

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## ABSTRACT

**Aim:** To study the Morphological changes of ovaries in the offspring of female rats exposed to chloroquine during gestation.

**Methods:** In this experimental study 30 female rats and 10 male rats were selected. They were divided into five groups containing 6 rats in each group. Total gestational period in rats is 21 days; it was divided into three trimesters each of 7 days. Chloroquine Phosphate (Resochin, Bayer) in powdered form was used. After dissection ovaries were weighed and observed for detailed morphological changes.

**Results:** Gross appearance of adult female pregnant rats in control and experimental groups were normal and their female offspring at the time of delivery were also normal and active. Morphological evaluation revealed that effects of chloroquine on adult rats organs e.g., eyes, muscles, liver, kidney and cardiovascular system are established. Chloroquine administration during gestational period has adversely affected the paired ovarian weight & relative tissue weight indices at puberty in all the experimental groups. Chloroquine renders the organs more susceptible to subsequent oxidative stress it also causes decrease in activities of catalase.

**Conclusion:** It is concluded that chloroquine which is widely used as anti-malarial drug in our country for the treatment of malaria requires proper evaluation for its possible teratogenic effects.

**Keywords:** Chloroquine, ovary, rat

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## INTRODUCTION

Malaria has been major medical problem in the subcontinent for thousands of years. The overall slide positivity rate of malaria parasite in Pakistan is 3.8%<sup>1</sup>. Malaria is one of the world's most devastating human parasitic infections, afflicting more than 500 million people and causing almost 3 million deaths each year<sup>2</sup>. It caused by four species viz Plasmodium malaria, Plasmodium vivax, Plasmodium falciparum and Plasmodium ovale. The parasites are cyclically transmitted to humans by female mosquitoes of the genus Anopheles. The clinical course is characterized by paroxysm of high fever, chills, anemia and splenomegaly. Plasmodium falciparum often causes serious or fatal complications<sup>3</sup>. Plasmodium falciparum accumulates in the placenta and is associated with dysregulated immune function and poor birth outcomes<sup>4</sup>. Fatal growth retardation in weight and length was found to be associated with maternal malaria<sup>5</sup>. Since malarial parasites are retained in the placenta, placental insufficiency may develop which can result in an increased risk of abortion, still birth, premature delivery and low birth weight<sup>6,7</sup>.

Chloroquine is a synthesized 4 amino quindine<sup>8</sup>. In human, 20mg/kg is a toxic dose, 30mg/kg may be lethal and 40 mg/kg is usually lethal without early intensive therapy in adults. In animals, toxic does is 200-400 mg/kg in mouse and 1050 mg/kg in rat<sup>9</sup>. Chloroquine administration in very high dose for a long duration in some non malarial diseases e.g. rheumatoid arthritis may cause toxic myopathy, retinopathy, peripheral neuropathy, psychiatric illness and cardiomyopathy<sup>10</sup>. The myopathy induced by it is characterized by progressive weakness and atrophy of the proximal muscle<sup>11</sup>. The ocular toxicity is due to accumulation of the drug in the pigmented layers of the eye, particularly the choroids<sup>12</sup>. Prolonged therapy with high doses of it causes hypotension and ECG changes-QRS widening, T-wave abnormality<sup>13</sup>. Chloroquine can cause hemolysis in patients with glucose phosphate dehydrogenase deficiency<sup>14</sup>.

## MATERIALS AND METHODS

This experimental study was conducted in the Department of Anatomy, Sheikh Zayed Postgraduate Medical Institute, Lahore in collaboration with the Department of Zoology, Quaid-e-Azam Campus, University of the Punjab, Lahore. For conception three female rats and one male rat were kept together in a cage for a week and male rat was removed from the cage. Female rats were observed

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daily for signs of pregnancy. Presence of vaginal plug confirmed the pregnancy and was taken as day zero of pregnancy.

After conception male rats were separated and 30 female rats were divided into five groups A, B, C, D and E containing six rats in each group. Total gestational period in rats is 21 days, and in this study, it was divided into three trimesters, each trimester of 7 days. Chloroquine phosphate (Resochin, Bayer) in powdered form was used in this study. Physical appearance, general activity and presence of any congenital anomaly was observed in the offsprings. The shape and colour of ovaries were observed. Detailed examination of ovarian external surface and its cortex and medulla was performed. The body weights of selected 50 female rats were recorded just before they were sacrificed. Paired ovarian weight of each rat was recorded soon after dissection. Relative Tissue weight index was also obtained.

Ovaries were dissected out and placed on blotting paper to make it free from surrounding fluid. Ovaries were weighed and observed for detailed morphological evaluation. Other organs were preserved for further study by the Department. All male offsprings were also preserved for further studies.

## RESULTS

Mean body weight of adult female rat offsprings at puberty of group A1 (control) was 141.10gms ( $\pm 5.76$ ), while mean body weights of adult female rat offsprings at puberty of experimental groups B1, C1, D1 & E1 were found to be 119.50gms ( $\pm 6.85$ ), 110gms ( $\pm 7.81$ ), 106.90gms ( $\pm 4.77$ ) and 105gms ( $\pm 5.27$ ) respectively. The mean body weight of adult female rats in experimental groups B1, C1, D1 and E1 was found to be significantly reduced as compared to control group A1 ( $p < 0.01$ ). The comparison of mean body weight of experimental groups C1, D1 and E1 was also reduced significantly as compared to experimental group B1 ( $p < 0.01$ ) while the difference of mean body weight between experimental groups C1, D1 and E1 was not significantly reduced ( $p > 0.05$ ).

The mean weight of paired ovaries of group A1 (control) was found to be 0.27gms ( $\pm 0.02$ ). The mean weights of paired ovaries in experimental groups B1, C1, D1 & E1 were found to be 0.19gms ( $\pm 0.03$ ), 0.18gms ( $\pm 0.04$ ), 0.17gms ( $\pm 0.03$ ), 0.12gms ( $\pm 0.02$ ) respectively (Table 1). The mean weight of paired ovaries in experimental groups B1, C1, D1 and E1 was significantly reduced as compared with control group A1 ( $p < 0.01$ ). The mean paired ovarian weight in experimental group E1 was significantly reduced as

compared with experimental groups B1, and D1 ( $p < 0.01$ ). The difference in the mean paired ovarian weight was not significant between groups B1 vs C1, B1 vs D1 and C1 vs D1 ( $p > 0.05$ ) (Table 2). The mean relative tissue weight index for group A1 (control) was calculated to be 0.19 ( $\pm 0.02$ ). The mean relative tissue weight index for group B1 (experimental) was calculated to be 0.15 ( $\pm 0.02$ ). The mean relative tissue weight index for group C1 (experimental) was calculated to be 0.16 ( $\pm 0.04$ ).

## DISCUSSION

Animal studies revealed disturbances in structure and functions of endocrine glands<sup>15</sup> and abnormalities of testicular functions<sup>15</sup>. In rats alternation of estrous cycle due to disturbance of hypothalamic-pituitary-ovarian axis have also been reported<sup>16</sup>. Results of the present research study showed that mean body weight of female rat offsprings at puberty in experimental groups B1, C1, D1 and E1, which were exposed to chloroquine during different gestational periods was significantly reduced when compared with control group A1 ( $P < 0.01$ ). The mean body weight of experimental groups C1, D1 and E1 was also reduced significantly compared with experimental group B1 ( $P < 0.01$ ), in contrast this difference was statistically non-significant between experimental groups C1 vs D1, C1 vs E1 and D1 vs E1 ( $P > 0.05$ ).

These results indicate that prenatal exposure to chloroquine affected the postnatal growth of experimental animals, which resulted in the reduction of the body weight at puberty. The mean paired ovarian weights of experimental groups B1, C1, D1 & E1 were significantly reduced as compared with control group A1 ( $P < 0.01$ ). The comparison of mean paired ovarian weights among the experimental groups showed that reduction was more marked in experimental group E1, which was exposed to chloroquine during all the three trimesters. Although the reduction of mean paired ovarian weight was observed in all the experimental groups but comparison revealed non-significant reduction in groups B1 vs C1, B1 vs D1 & C1 vs D1 ( $P > 0.05$ ). Similar results were observed by statistical calculations of relative tissue weight indices. There was no sign of inflammation in the cortex & medulla of control group A1, while mild inflammatory changes (lymphocytic infiltration) was present in the ovaries of experimental groups B1 and C1. These inflammatory changes were moderate in experimental groups D1 and E1. These inflammatory changes seen in experimental groups were statistically significant when compared with control groups A1 ( $P < 0.0001$ ).

## CONCLUSION

The results of the present research work indicate that chloroquine administration during various periods of intrauterine development produced deleterious effects on the gross and histological structure of ovaries or rats, which were examined at puberty (70 days of age). Although deleterious effects were observed with chloroquine administration during 1st, 2nd and 3rd week of gestation in experimental groups B1, C1 & D1 respectively, but the adverse effects were more marked in experimental group E1 which was exposed during all the three trimesters. So it is suggested that the use of chloroquine during pregnancy must be done with caution.

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