

# Histological Effect of Chloroquine on Developing Ovaries of Albino Rats

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## ABSTRACT

**Objectives:** To study the Histological features of ovaries in the offspring of female rats.

**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 trimester each of 7 days. Chloroquine Phosphate (Resochin, Bayer) in powdered form was used.

**Results:** Gross appearance of adult female pregnant rats in control and experimental groups were normal and their female offsprings at the time of delivery were also normal and active. Histological evaluation revealed that lining epithelium of control and experimental groups was similar and no difference was observed. Chloroquine administration during gestational period 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 affects female offspring's weight if used during gestational period, so Chloroquine administration during pregnancy must be used with caution.

**Key words:** Effect of chloroquine, ovaries, albino rats

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## INTRODUCTION

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>1</sup>. Malaria is defined as an acute or chronic disease caused by four species viz Plasmodium malariae, 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>2</sup>.

Women living in endemic areas demonstrated an increased prevalence in the severity of malarial infection during pregnancy, particularly in the second trimester<sup>3</sup>. Placental malaria in pregnant women significantly increased the prevalence of anemia in women regardless of gravidity or age. In addition, the mean infant birth weight was lower and the percentage of pre-term deliveries and low birth

weight babies were higher in primigravidae and women less than 20 years of age who had placental malaria<sup>4</sup>.

Chloroquine is a synthesized 4 amino quindine<sup>5</sup>. In human, 20mg/kg is a toxic dose, 30 mg/kg may be lethal and 40mg/kg is usually lethal without early intensive therapy in adults. In animals, toxic doses is 200-400 mg/kg in mouse and 1050 mg/kg in rat<sup>6</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>7</sup>. The myopathy induced by it is characterized by progressive weakness and atrophy of the proximal muscle<sup>8</sup>. The ocular toxicity is due to accumulation of the drug in the pigmented layers of the eye, particularly the choroids<sup>9</sup>. Prolonged therapy with high doses of it causes hypotension and ECG changes-QRS widening, T-wave abnormality<sup>10</sup>. Chloroquine can cause hemolysis in patients with glucose phosphate dehydrogenase deficiency<sup>11</sup>.

## MATERIALS AND METHODS

This experimental study was conducted in the Department of Anatomy, Shaikh Zayed Postgraduate

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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 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.

**Group A:** This was a control group containing 6 pregnant rats, which were fed on normal diet. They were allowed to complete their gestation without drug intake.

**Group B (Experimental):** This group contained 6 pregnant rats and was given single oral dose (through Naso gastric tube) of chloroquine 750 mg/kg body weight during first trimester, on day 4 of gestation.

**Group C (Experimental):** This group contained 6 pregnant rats and was given single oral dose (through Naso gastric tube) of chloroquine 750 mg/kg body weight during second trimester of pregnancy, on day 11 of gestation.

**Group D (Experimental):** This group contained 6 pregnant rats and was given single oral dose (through Naso gastric tube) of chloroquine 750 mg/kg during third trimester of pregnancy, on day 18 of gestation.

**Group E (Experimental):** This group also contained 6 pregnant rats and was given single oral dose (through Naso gastric tube) of chloroquine 750 mg/kg body weight during all the trimesters of pregnancy i.e. day 4, 11 & 18 of gestation.

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. The following parameters were observed in the ovaries.

## RESULTS

Each ovary was cut in the centre and one tissue block from each ovary was made. Five sections from each block were taken and stained with H&E (Haematoxylin and Eosin). The following parameters were studied and compared with the normal

histological parameters of ovary of control group. The following parameters were studied:

**Lining epithelium of the ovary:** Type of the lining epithelium of the ovary was observed

**Inflammation and Congestion:** Whether the inflammation and congestion in the cortex and medulla was present or not.

**3. Fibrosis:** Whether fibrosis was present or not.

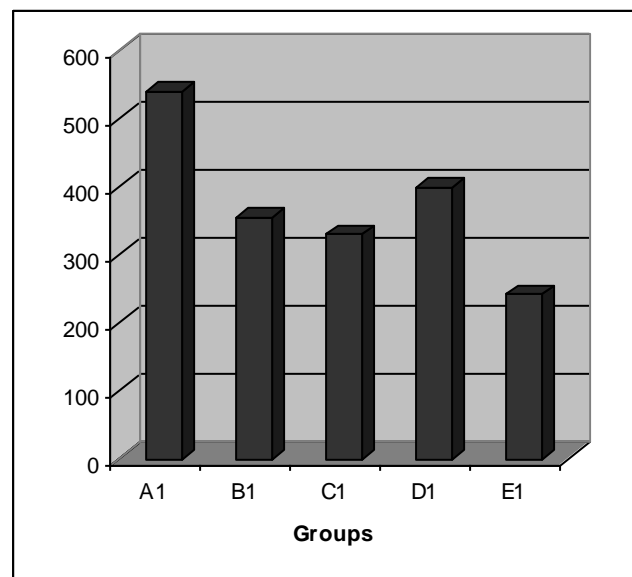
The following measurements in  $\mu\text{m}$  were performed in all the groups with ocular micrometer:-

1. Diameter of graafian follicle ( $\mu\text{m}$ )
2. Diameter of secondary oocytes ( $\mu\text{m}$ )

The histological appearance of ovaries in control group A was normal. The lining epithelium was simple cuboidal and cortex contained primordial follicles. The Graafian follicles were normal, follicular antrum, zona granulosa, theca interna and externa were found to be intact and normal. Medulla contained blood vessels and smooth muscle cells and no sign of inflammation and fibrosis was seen. However mild congestion in the medulla was observed (Fig 1 & 2).

The following observations were seen in the histological evaluation of ovaries of the experimental groups.

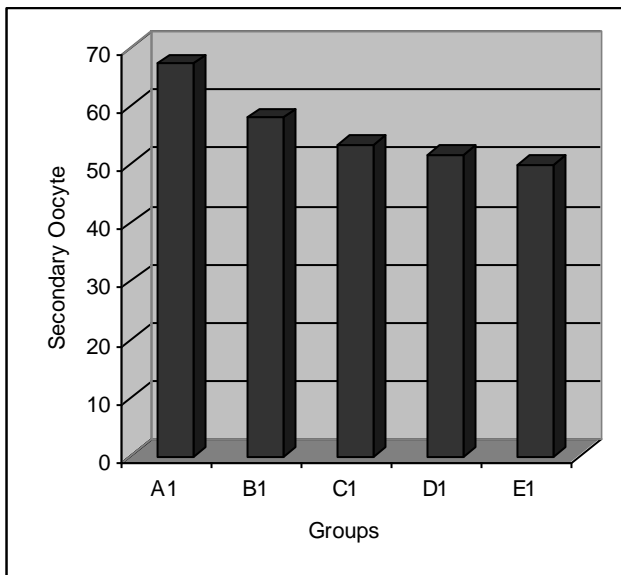
Fig.1: Effect of chloroquine on the mean diameter of graafinfollicles of control group A1 and experimental group B1, C1, D1 and E1 or rats



**Lining Epithelium:** The lining epithelium of ovary in experimental group B was same as compared with control group A. The lining epithelium of ovaries in experimental groups C, D and E was changed from that of control group A and experimental group B, it became flattened which was statistically significant ( $p < 0.0001$ ).

**Congestion and Inflammation in the Cortex and Medulla of the Ovaries:** Histological examination of the ovaries revealed mild congestion in the cortex and medulla of the ovaries of control group A and experimental groups B and C. This difference was statistically non-significant ( $p>0.05$ ). Inflammation was not seen in the cortex and medulla of ovaries in control group A while mild inflammation was seen in the experimental groups B and C which was statistically significant ( $p<0.0001$ ) when compared with control group A.

Fig.2: Effect of chloroquine on the mean diameter of secondary oocyte of control group A1 and experimental groups B1, C1, D1 and E1 or rats



## DISCUSSION

The present study was designed to observe the effects of chloroquine on the gross and microscopic structure of ovaries of albino rats exposed during different periods of intrauterine development. Alteration of oestrus cycle in rats was associated with chloroquine administration with disturbances on hypothalamic-pituitary-ovarian axis. Since this drug is also frequently used for the treatment of malaria during pregnancy so concern remained there to evaluate its safety or otherwise during intrauterine developmental period and lactation. On animal studies congenital anomalies have been reported. Congenital internal ear defects are also reported in humans.

Our results match with the results of study conducted by Sharma A and Rawat AK<sup>12</sup> who observed that chloroquine administration during gestational period resulted in reduction of 40% body weight of albino rats at birth. Similar reduction on

neonatal and post-weaning body and organ weights of albino rats have also been proved in research study conducted by Mgbodile MU<sup>13</sup>.

Our study results also match with results of study conducted by Phillips-Howard PA and Wood D<sup>14</sup> who proved that Chloroquine accumulates in the cells of cultured rat embryo and inhibited protein synthesis and also inhibited the proteolytic activity of yolk sac. The size of the graafian follicle and secondary oocytes depend on the secretion of gonadotropin.

In one study it has been shown that with the application of organo-carbons the size of graafian follicle and secondary oocytes was reduced in wild mice and in these animals levels of oestrogen was also low<sup>15</sup>.

Our results are in contrast with the results proved by Ghigo D et al<sup>16</sup> who state after their research study that Chloroquine stimulate nitric oxide synthesis in murine, porcine and human endothelial cells. Difference in our observation and their statement may be difference in genetic variation in animal species.

Laskin JD et al<sup>17</sup> explained that nitric oxide and its oxidative products such as peroxynitrite contribute to the process of tissue injury by directly damaging the tissue or by initiating additional immunological reactions. Inhibition of basic fibroblast growth factor with Chloroquine on cultured human microvascular endothelial cells has also been proved by Inyang AL et al<sup>18</sup>.

Our results do not match with results of research study conducted by Dass EE and Shah KK<sup>19</sup> who observed hepatocellular necrosis in adult rats exposed to Chloroquine at a dose of 970 mg/kg body weight. Although cellular necrosis was not seen in the ovaries in our research work, instead cellular to collagenous fibrosis was present indicating some sort of damage to developing ovaries of rats by Chloroquine which ended up in fibrosis evident at puberty.

## 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 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> 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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