Chloroquine Induced Hepatotoxicity in Developing Albino Rats

A ZAHID *T S ABIDI
*Department of Anatomy, Allama Iqbal Medical College, Lahore, *Ex-Professor of Anatomy, K. E. Medical College, Lahore*
Correspondence to Dr. Aliya Zahid, Assistant Professor Anatomy

Despite the introduction of many new antimalarial drugs, chloroquine is still most widely prescribed drug for prophylaxis and treatment of malaria. It is considered to be the safe antimalarial in pregnant women. This study was carried out to see the effect of chloroquine on developing liver of albino rats. In this study, 24 pregnant female albino rats were used divided in 4 groups. Total gestational period in rats ranges from 20-22 days, which in this study was divided into three trimesters, each of seven days. Using oral dose of chloroquine 700mg/kg body weight in first, second and third weeks of pregnancy, it was found that chloroquine induced hepatic damage in rats exposed to chloroquine during their intrauterine life. Light microscopic examination of liver revealed that hepatic architecture was distorted in all experimental groups. Hepatocytes were enlarged due to degenerative changes in cytoplasm and nuclei. Degenerative changes included microvesicular fatty change progressing to vacuolar degeneration, ultimately leading to necrosis. Parenchyma was infilterated with lymphocytes. These changes were maximum in those animals which were exposed to drug during third trimester of gestation. These observations lead to conclusion that chloroquine produces hepatic damage especially when given during last trimester .
Key words. Chloroquine, hepatotoxicity, albino rats.

Malaria during pregnancy may be associated with significant morbidity and mortality in both mother and fetus[1]. Prevention of malarial infection is an important concern as maternal malaria is associated with poor maternal and perinatal outcome[2]. Chloroquine is the drug of choice for the prophylaxis and treatment of malaria species during pregnancy[3,4].

Chloroquine should be used cautiously in patients with hepatic dysfunction[5]. Higher or more frequent dose schedules may precipitate clinical hepatotoxicity with elevated serum liver transaminase levels [6]. Since this drug concentrates in the liver, 500 times the plasma concentration, it should be used with caution in patients with alcoholism or in conjunction with known hepatotoxic drugs[7]. Administration of chloroquine significantly increases the hepatic and biliary lysosomal enzyme activities[8]. Chloroquine causes decrease in the activities of mitochondrial inner membrane enzymes of hepatocytes such as NADH dehydrogenase, cytochrome c oxidase and succinate dehydrogenase. This in turn causes decrease in ATP synthesis and inhibition of mitochondrial respiration thereby impairing availability and utilization of energy[9].

Chloroquine causes hepatonecrosis and increase in transaminases level[10]. Hepatotoxicity caused by chloroquine may be due to direct toxic effect on hepatocytes causing leakage of lactic dehydrogenase from hepatocytes[11].

Liver cells, especially Kupffer cells are known to accumulate lysosomotropic agents like chloroquine, which can cause overload of liver lysosomes by non-digestible material, increased size and number of liver lysosomes, inhibition of several lysosomal enzymes, increased autophagy and fusion disturbances[12]. Chloroquine lowers the antioxidant enzymes activities in the liver and renders the organ more susceptible to oxidative stress[13]. Everdingen and colleagues[14] reported a case of a 50 years old lady on malarial prophylaxis (Pyrimethamine and chloroquine combination). Her laboratory investigations showed moderately increased serum liver enzyme activities and liver biopsy showed granulomatous hepatitis . In a study, pregnant rats were treated throughout the second half of pregnancy with chloroquine. Their offsprings (sacrificed immediately after birth) showed generalized lipidosis in lungs, liver, pituitary gland, adrenals, spinal cord and hypothalamus[15].

Administration of chloroquine 700mg/kg body wt in pregnant rats for first 15 days of pregnancy resulted in several structural abnormalities. The incidence of hepatomegaly was increased by 30%, the liquification of visceral organs was increased by 15% and a 9% higher incidence of cleft palate, wrist drop, clubfoot and brain liquification was observed[16] .

Materials & methods

Animals In this experimental study, twenty four adult female rats and eight adult male rats of Albino Wistar strain were used. Weight of female rats used was between 250-300gms and male rats was between 300-350gms. They were obtained from National Institute of Health, Islamabad.

Acclimatization:

Animals were kept in the Animal House of Post Graduate Medical Institute, Lahore. For acclimatization, they were kept without treatment for 15 days. They were provided with Chick feed no 3 and tap water ad libitum. Male and female rats were kept in separate cages. Care was taken regarding optimal light and temperature.

Conception: For conception three female rats and one male rat were kept together in a cage for a week and then male rat was removed from the cage. Female rats were observed daily for signs of pregnancy which was

confirmed by presence of vaginal plug, increase in weight and abdominal girth . Presence of vaginal plug was taken as day one of pregnancy .

Experimental Groups: After conception male rats were separated and 24 female rats were divided into four groups A, B, C and D, containing six animals each. Total gestational period in rats ranges from 20-22 days, which in this study was divided into three trimesters, each of seven days. The rats were weighed and marked. They were placed in their respective cages which were labeled by tags. Chloroquine phosphate in powdered form was used in this study.

Group A. This was a control group containing 6 animals each which were fed on normal diet throughout pregnancy. They were allowed to complete their gestational periods without drug intake.

Group B, C and D: Each containing 6 animals, were given oral dose of chloroquine 700mg/kg body weight during first, second and third trimester of pregnancy, respectively

After the control and experimental groups had delivered, their offsprings were selected at random (about 5/adult rat). Before dissecting the offsprings they were weighed and observed for any gross abnormality. Newborn rats on day one were anaesthetized by cotton pledged soaked with chloroform. After 3-5 minutes while the rats were still breathing, a ventral midline abdominal incision was made to expose abdomen. Liver was then dissected out and was placed on blotting paper to make it free of surrounding fluid. In control group, the external surface of liver was smooth and shiny. The colour of liver was reddish brown. In group B, the external surface of liver was smooth and glistening but size was decreased as compared to control group. In group C and D, liver was small and external surface was dull. Liver was weighed and observed for any gross change and then fixed in 10% formaldehyde solution for at least 12 hrs. The livers were processed in an autoprocessor. Blocks were made, cut, stained and mounted.

Histological Parameters:

Sections were stained with haemotoxylin and eosin stains and following parameters were studied:

1. General architecture of hepatic lobule
2. Morphology of hepatocytes
   - Change in size
   - Presense of necrosis or vacoulation
   - Nuclear changes

Morphometric Analysis :Morphometric analysis was performed with the help of an objective micrometer. Following were measured:

1) Size of hepatocyte in a field taken at random
2) Size of hepatocyte nuclei selected at random

Biostatistical Analysis :

Data was collected and appropriately compiled. Mean of all variables were expressed as Mean±Standard deviation. Difference in mean values of control and experimental groups were analysed using student's t-test (two tailed) and compared for significance using two tailed probability points of the t distribution.

Results :

Histological Observations :

Control Group A: This group showed normal hepatic architecture comprising of hepatic lobules with a central vein in the center and portal triads at the periphery. Hepatocytes were arranged in form of radiating cords around the central vein. Sinusoids were present. Hepatocytes were polyhedral in shape and their mean diameter was 11.45 microns. Each hepatocyte contained a vesicular nucleus with 1-2 nucleoli. Mean diameter of nuclei in group A was 4.32 microns.

Table 1. Effect of chloroquine on different histological parameters of newborn rat liver (n=120).

Parameters		Groups			
		A	B	C	D
Hepatocyte (microns)	size	11.45	**11.85 ± 1.14	***19.28 ±3.55	***19.98 ±3.64
Hepatocyte nucleus (microns)	size	4.32 ±0.92	*4.58 ±0.64	***7.65 ±1.70	***7.86 ± 1.37

All values are expressed as : Mean±SD, * P<0.05 difference significant, ** P<0.01 difference considerably significant, *** P<0.001 difference very significant, (P< 0.01). Nuclear size was also increased measuring 4.58 micron ,(P<0.01). Mild degenerative changes were seen in hepatocytes. Parenchymal inflammation was mild to moderate .

Group B: This group received chloroquine only during its first week of intrauterine life. They showed mild distortion of hepatic architecture. Hepatocytes were slightly increased in size measuring 11.85 microns

Group C: This group was exposed to chloroquine only during second week of intrauterine life. There was disturbed liver architecture. Hepatocytes were increased in size measuring 19.28 microns (P<0.001). Nuclear size was also increased measuring 7.65 microns. There was increase in number of multinucleated hepatocytes. Number of necrotic foci were increased. Parenchymal inflammation was marked. Degenerative changes were seen in hepatocytes.

Group D: This group was exposed to chloroquine only during third week of gestation . Hepatic architecture was completely distorted. Hepatocytes were enlarged measuring 19.98 microns in diameter (P<0.001). Number of necrotic foci were increased. There was marked parenchymal inflammation .

Fig 1: Hepatocyte size in control and experimental groups

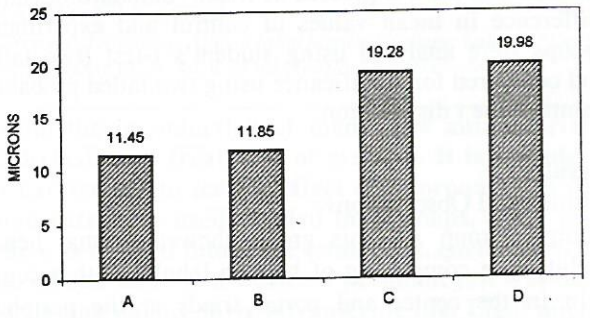


Fig 2 . Effect of chloroquine on hepatocyte nucleus size

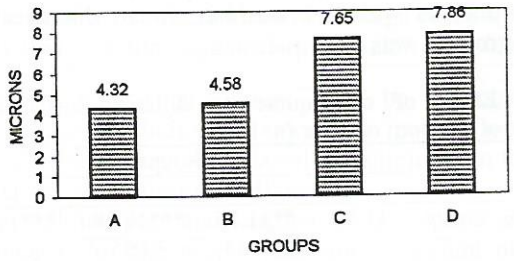


Fig. 3. Microscopic structure of liver from control group A.

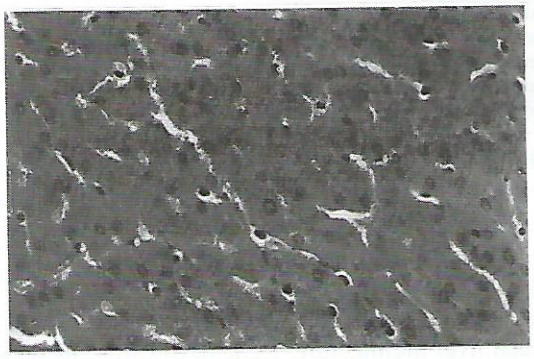


Fig. 4. Microscopic structure of liver from group B

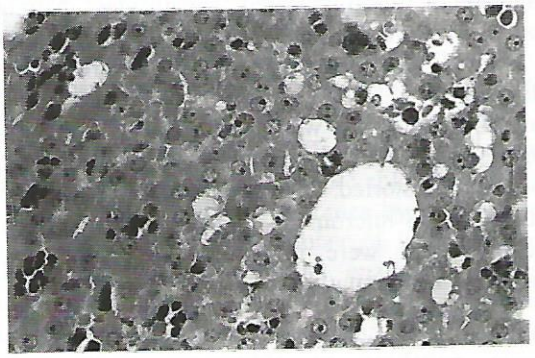


Fig.5. Microscopic structure of liver from group C.

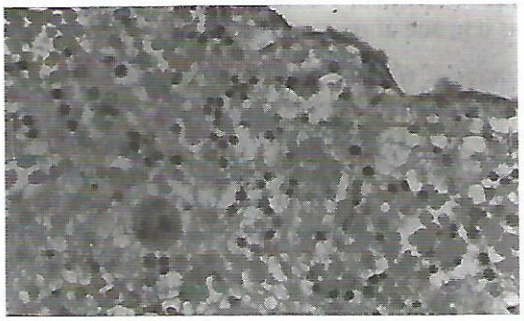
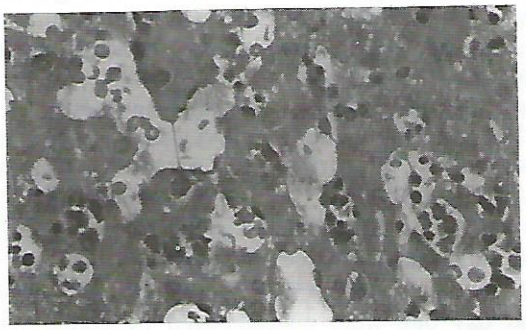


Fig. 6. Microscopic structure of liver from group D.



Discussion

The results of present research have revealed that chloroquine induces hepatic damage in rats exposed to chloroquine during their intrauterine life. Livers from experimental animals showed significant decrease in weight as compared to their control. Decrease in liver weight was more marked in groups that were exposed to chloroquine during second and third week gestation .The decrease in weight could be explained on embryological basis. In rats, development of liver bud and hepatic cords occur on 10^{th} to 12^{th} day of gestation[17]. During the last 3 days before birth in the rat, the liver undergoes a striking acceleration of growth. During this time glycogen deposition in hepatocytes increases dramatically, the volume of individual parenchymal cells triples and the total protein content of the liver increases twofold[18]. Chloroquine administration during this period interfered with the development and growth of hepatocytes and caused destruction of already formed parenchymal tissue. Lowering of antioxidant enzymes activities by chloroquine in liver renders the organ more susceptible to subsequent oxidative stress.

Chloroquine induces biochemical changes in liver[10,13,19]. Biochemical changes lead to metabolic disturbances which in turn can cause degenerative and structural changes exhibited as fatty change, change associated with cellular swelling or hyaline degeneration ,ultimately leading to necrosis accompanied by nuclear changes in cell[20,21]. In addition to above mentioned mechanisms, chloroquine has a direct toxic effect on hepatocytes causing leakage of lactic dehydrogenase from hepatocytes which is confirmed by increase in lactic

dehydrogenase level[11]. Chloroquine causes hepatonecrosis and increased transaminase levels in rats at dose of 970mg/kg body weight[10].

Chloroquine stimulates the synthesis of nitric oxide in endothelial cells. Nitric oxide is a free radical that acts as mediator of tissue injury [22]. Nearly all the cells of liver including hepatocytes, Kupffer cells, stellate cells and endothelial cells have capacity to generate nitric oxide. Nitric oxide and its oxidation products such as peroxynitrite contribute to the process of tissue injury by directly damaging the tissue or by initiating additional immunologic reactions that result in damage[23]. Increase in size of hepatocytes and decrease in liver weight may be due to incomplete formation of trabeculae during development.

Degeneration and necrosis of parenchymal tissue is another reason for decrease in organ weight. The increase in inflammatory cells in all experimental groups reflects an attempt to remove necrotic debris.

Conclusion

It is concluded from this study that chloroquine causes hepatic damage in rats exposed to chloroquine during their intrauterine life. So its use during pregnancy should be discouraged.

References

1. Wong RD, Murthy AR, Mathisen GE, Glover N Thornton PJ. Treatment of severe falciparum malaria during pregnancy with quinidine and exchange transfusion . Am J Med 1992; (5): 561-2
2. Pergament E, Kelly O, Kristin DM. Travel and pregnancy . Risk news letter1998 ; Vol 6#3 .
3. Diro M, Beydon SN. Malaria in pregnancy. South Med J 1982; 75(8): 959-62,68 .
4. Rose SR. Pregnancy and travel. Emerg Med Clin North Am 1997; 15(1): 93-111 .
5. Mycek MJ, Harvey RA, Champe PC. Chemotherapy of malaria. In: Lippincott's Illustrated Reviews Pharmacology 2000. 3rd ed, Lippincott Raven, USA. pp 351-52 .
6. Goodman L, Gilman AG. Drugs used in the chemotherapy of protozoal infections. In: The Pharmacological Basis of Therapeutics 1996. 9th ed McGraw Hill Companies Inc USA pp 965-970
7. Craig CR, Stitzel RE, 1997. Antimalarial drugs. In: Modern Pharmacology with clinical applications 1997.5thed .Little Brown and Company Inc USA. pp 643-44.
8. Hideaki F, Kyoshi O, Nagakawa JI, Osamu T,Yoshiharu Y.Effect of rabeprazole, a gastric proton pump inhibitor , on biliary hepatic lysosomal enzymes in rats. Jpn J Pharmacol 1998; 76(3): 279-288.
9. Deepalakhsmi PD, Parasakthy K, Shanthi S, Devaraj NS. Effect of chloroquine on rat liver mitochondria .Indian J Exp Biol 1994; 32 (11): 797-9
10. Dass EE, Shah KK.Paracetamol and conventional antimalarial drugs induced hepatotoxocity and its protection by methionine in rats. Indian J Exp Biol 2000; 38 (11): 1138-42 .
11. Akesson A, Akesson B. Hepatotoxic effects of antirheumatic drugs in cultured rat hepatocytes.Scand J Rheumatol 1984, 13(3):198-202.
12. Schneider P, Korolenko TA, Busch U.A review of drug induced lysosomal disorders of the liver in man and laboratory animals . Micros Res Tech 1997; 15, 36(4) : 253-75 .
13. Magwere T, Naik YS, Hasler JA. Effects of chloroquine treatment on antioxidant enzymes in rat liver and kidney. Free Radic Biol Med 1997; 22 (1-2) :321-7 .
14. Everdingen-Bongers JJ, Janssen P, Lammens M, Stricker H, Bravenboer.Granulomatous hepatitis attributed to combination pyrimethamine-chloroquine. Ned Tijdschr Geneeskd 1996; 10, 140 (6) :320-2 .
15. Lullman RR, Stoermer B.Generalized lipidosis in newborn rats and guinea pigs induced during prenatal development by administration of amphiphilic drugs to pregnant animals. Virchows Arch B cell Pathol Incl .Mol Pathol. 1982, 39 (1):59-73 .
16. Sharma A, Rawat AK.Toxicological consequences of chloroquine and ethanol on developing fetus. Pharmacology Biochemistry and Behaviour 1989; Vol 34 .pp77-82 .
17. Godlewski G, Gaubert CR , Rouy S, Prudhomme M. Liver development in the rat and in man during embryonic period. Micros Res Tech.1997; 39 (4): 314-27.
18. Peavy DE, De Sante DC. Protein degradation in cultured fetal hepatocytes. Biochem J 1990; 267:671-7 .
19. Achudume AC, Adepoju AY, Abu KA, Olanrewaju IG. Experimental analysis of gossypol and chloroquine interaction in serum and in liver of rat. Indian J Exp Biol 1998; 36(10):1020-2
20. Robin SL, Corten RS, Kumar V. Cellular injury and adaptation. In: Pathological basis of disease 1984 .3rd ed .WB Saunders Company, Philadelphia. pp 1-39 .
21. Walter JB , Israel MS .Cell response to injury . In: General Pathology 1987. Churchill Livingstone, Edinburgh. pp 57-67 .
22. Ghigo D, Aldierri E, Todde R, Costamagna C, Garbarino G, Bosia A .Chloroquine stimulates nitric oxide synthesis in murine porcine and human endothelial cells . J Clin Invest 1998; 1, 102(3): 595-605 .
23. Laskin JD, Heck DE, Gardner CR, Laskin DL.Prooxidant and antioxidant functions of nitric oxide in liver toxicity . Antioxid Redox Signal 2001; 3 (2): 261-271.