

COMPARATIVE TOXICITY STUDY OF CHLOROQUINE AND HYDROXYCHLOROQUINE ON ADULT ALBINO RATS

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Abstract

Expanded use of Chloroquine and hydroxychloroquine drugs for non-malarial disease entities has resulted in prolonged duration of therapy and higher daily dosages leading to cumulative doses greater than those used in antimalarial therapy. The aim of the study is to evaluate and compare the toxic effects of chloroquine and hydroxychloroquine on different organs of albino rats. The study was conducted on 60 normal albino rats divided into 3 groups, the 1st group is the control group that received only distilled water, the 2nd and the 3rd group were given a single daily oral doses equivalent to 1/10th of LD₅₀ chloroquine and hydroxychloroquine respectively. Assessment of liver and kidney functions, and histopathological changes in liver, kidney, and heart in different groups was done. The chloroquine treated group showed significant elevation of serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), total bilirubin (TB), serum creatinine-urea (Cr-U), Creatine Kinase-MB, C-reactive protein and Malonic dialdehyde levels as compared to control and hydroxychloroquine treated group. The histopathological evaluation showed marked hydropic degeneration, vascular congestion, interstitial hemorrhage, and necrosis in the liver, kidney and heart of chloroquine treated group, while hydroxychloroquine treated group showed mild congestion and slight cellular degeneration. Thus, hydroxychloroquine is less toxic and physicians should prescribe it better than chloroquine. Chloroquine if prescribed for therapeutic uses should be taken for short periods.

Keywords: Chloroquine, hydroxychloroquine, comparative, toxicity, rats

Introduction

Chloroquine was first used as an antimalarial agent. It subsequently played an important therapeutic role in various rheumatological diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and other inflammatory and dermatologic conditions. The use of its analogue, hydroxychloroquine, has largely replaced chloroquine in most parts of the world, because of better tolerability at high dosages. Both medications share a similarity in their therapeutic and toxicological aspects (**Pasadhika and Fishman, 2010**).

Expanded use of these drugs for nonmalarial disease entities has resulted in prolonged duration of therapy and higher daily dosages leading to cumulative doses greater than those used in antimalarial therapy (**Rüther et al., 2007**). Many studies revealed that chloroquine has many deleterious effects on many systems. The mechanism of chloroquine toxic effects is still unknown, but some studies reported that it may be due to the formation of some oxidative metabolites, which raises the production of reactive oxygen species (**Al-Jassabi et al., 2011**). Chloroquine accumulates especially in the Kupffer cells of the liver with resultant lysosomal damage including overloading of the liver lysosomes with non-digestible material, and an increase in their size and number (**Schneider et al., 1997**). The reported accumulation of CHQ in lysosomes has an apparent destabilizing effect on lysosomal membranes (**Zhao et al., 2005**).

However, **Ostensen (2006)** and **Michaelides et al. (2011)** reported that chloroquine and hydroxychloroquine are related drugs with different therapeutic and toxic doses. Chloroquine is a safe drug when recommended therapeutic doses are used. Most serious toxicity occurs following accidental or intentional overdose. The acute ingestion of 1–1.5 g (20 mg/kg) will result in toxicity in an adult, and ingestion of 5 g is potentially lethal (**Riou et al., 1988**). Hydroxychloroquine was reported to be less toxic than chloroquine; there are no available data on adverse effects of hydroxychloroquine on male fertility (**Ostensen, 2006**). According to animal studies, chloroquine is 2–3 times more toxic than hydroxychloroquine (**Smith and Klein-Schwartz, 2005**).

Aim of the work

The aim of this work is to evaluate and compare the toxicological effects of chronic exposure to chloroquine and hydroxychloroquine for 6 weeks on adult albino rats.

Material and methods

Drugs: Commercial tablets of chloroquine phosphate (Alexoquine 250 mg tablets) was purchased from Alexandria Company, and Commercial tablets of hydroxychloroquine phosphate (Plaquenil 200-mg film-coated tablets) was purchased from Sanofi Aventis Company.

Animals and Experimental design

A total of 60 normal adult albino rats of either sex weighing between 180 gm and 220 gm were used in this study. Animals were divided randomly into 3 groups with 20 animals in each group:

1. **Group 1: Control group**, treated with distilled water.
2. **Group 2: Chloroquine treated group**, treated with chloroquine.
3. **Group 3: Hydroxychloroquine treated group**, treated with hydroxychloroquine.

According to the Research Ethics Committee, Faculty of Medicine, University of Benha, the rats were maintained under standard housing laboratory conditions. Rats were housed in clean well ventilated cages, 12 h light/dark cycles, every 5 rats were housed in a separate cage under strict care and hygiene to keep them in normal and healthy conditions. Free access to food and water were allowed. The animals were kept under supervision of a professional technician. The animals were anesthetized by ether before taking the samples and were eliminated by incineration in Benha university hospital incinerator.

Both tested drugs were dissolved in distilled water as the two drugs are well soluble in water which is an inert substance without any known toxic effects. Each rat received 1/10th of the LD₅₀ orally of each drug daily for 6 weeks. Chloroquine median lethal dose (LD₅₀) in rats is 330mg/kg (**crouzette et al., 1983**), while the oral LD₅₀ of hydroxychloroquine in rats is 1240 mg/kg (**MSDS, 2008**). The dose adjusted every week according to the animal's body

weight. The time of drug administration was fixed for all animals at 12 p.m. and the animals were fasted 4 hours before drug administration. The total experimental period was designated to be 7 weeks; 6 weeks for drug administration and one week period of passive preliminaries in order to acclimatize prior to treatment.

Biochemical estimations

At the end of the 6th week, rats were anaesthetized by ether; blood samples (3 ml) from all groups were drawn from the heart of rats by 5 ml syringes. Serum was separated by centrifugation at 2500 rpm for 15 min and used for the determination of serum glutamate oxaloacetate transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin (TB), serum creatinine-urea (Cr-U). Also, creatine kinase, c-reactive protein and malonic dialdehyde were determined.

Histopathological studies

The rats from all groups were sacrificed after ether anesthesia. Tissues were taken from the kidney, liver and heart for histological assessment. Formalin fixed, paraffin embedded blocks were prepared and 5 microns serial sections were cut and stained with haematoxyline and eosin and examined by light microscope (Benli et al, 2008).

Scoring of histopathological lesions

Five slides were observed from each organ to evaluate the lesion semi-quantitatively by ranking tissue lesion severity (Benli et al, 2008). Ranking was done from 0 to 3 depending on the degree and extent of the alteration as follows: no histopathology changes: (0), histopathological changes in < 20% of fields: (1+), histopathological changes in 20 to 60% of fields: (2+), histopathology changes in >60% of fields: (3+).

Statistical analysis methods

All data were analyzed statistically, using a current SPSS statistical package Version 19 and the data presented as Mean \pm Standard Deviation of Means (S.E.M). Comparison between two groups was performed using t-test and p value was considered statistically significant if ≤ 0.05 , P value >0.05 insignificant.

Results:

Table 1: Effects of chloroquine and hydroxychloroquine on rat serum biochemical parameters.

Group	Biochemical parameters								
	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TB (mg/dl)	Creatinine (μ mol/L)	Urea (mg/dL)	Creatine Kinase-MB (ng/mL)	C-reactive protein (mg/L)	Malonic dialdehyde (μ mol/L)
Control group	42.89 \pm 17.86	41.66 \pm 25.11	271 \pm 44.22	0.78 \pm 0.22	0.41 \pm 1.66	8.01 \pm 0.14	0.9 \pm 0.23	0.3 \pm 0.01	0.4 \pm 0.12
Chloroquine group	422.9 \pm 38.12 *	407.31 \pm 35.5*	362 \pm 34.01	1.44 \pm 0.30	3.54 \pm 0.41*	15.88 \pm 0.21*	10.06 \pm 2.1*	6.4 \pm 1.1*	4.1 \pm 1.02*
Hydroxychloroquine group	143.8 \pm 50.23 *	210.52 \pm 515.4 *	312 \pm 37.3	1.02 \pm 0.09	2.31 \pm 0.22*	13.57 \pm 0.31	6.17 \pm 0.9*	4.3 \pm 0.8*	3.9 \pm 0.9*

Table 2: Histopathological scoring showing effects of chloroquine compared to hydrochloroquine. on rat organs.

Histopathological changes	Group		
	Control group	Chloroquine group	Hydroxychloroquine group
Liver	-		
Hepatoportal and Sinusoidal congestion	-	+++	+
Cloudy swelling and Hydropic degeneration	-	+++	+/-
Cellular necrosis (Nuclear pyknosis, karyorrhexis, karyolysis)	-	+++	-
Inflammatory cellular infiltrate	-	+++	+
Kidneys			
Vascular congestion	-	+++	+
Cloudy swelling and hydropic degeneration	-	+++	+/-
Inflammation	-	+++	+
Interstitial and tubular hemorrhage	-	+++	+
hyper-cellularity of glomeruli	-	+++	-
Focal tubular necrosis	-	++	+
Heart			
Vascular congestion	-	++	++
Cloudy swelling of Cardiac muscle and loss of striation	-	+++	+
Hemorrhage	-	++	+
Cardiac muscle necrosis	-	++	-
Interstitial fibrosis	-	++	-

Histopatological changes

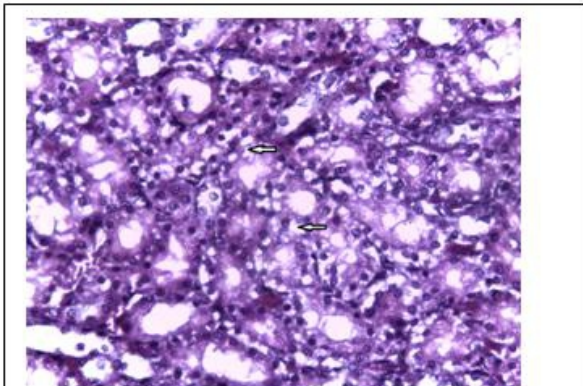


Figure (1): a photomicrograph of a section in Kidney of rat treated with chloroquine shows severe hydropic degeneration (Hx.&E. X200).

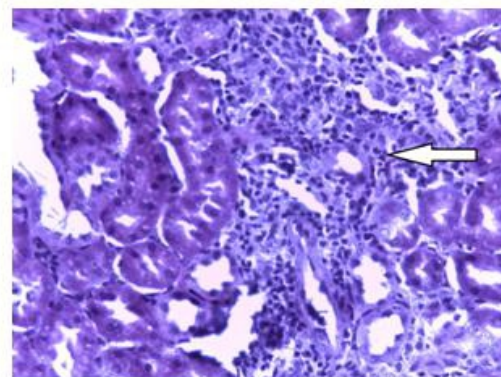


Figure (2): a photomicrograph of a section in Kidney of rat treated with chloroquine shows severe inflammation and severe hydropic degeneration (Hx.&E. X200).

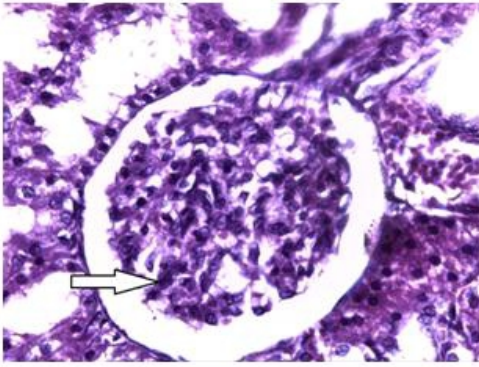


Figure (3): a photomicrograph of a section in Kidney of rat treated with chloroquine shows severe hyper -cellularity of glomeruli (Hx.&E. X400)

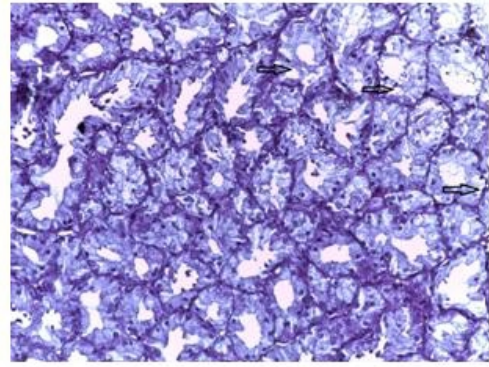


Figure (4): a photomicrograph of a section in Kidney of rat treated with hydrochloroquine shows mild cloudy swelling (Hx.&E. X200).

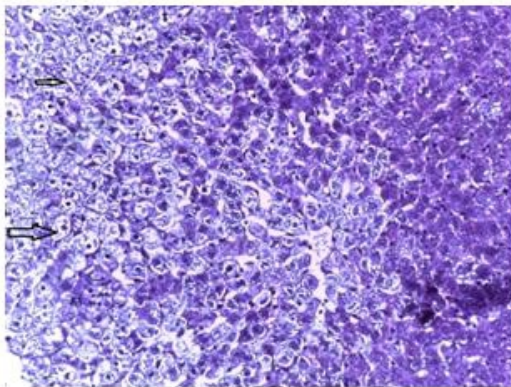


Figure (5): a photomicrograph of a section in liver of rat treated with hydrochloroquine shows mild cloudy swelling (Hx.&E. X200).

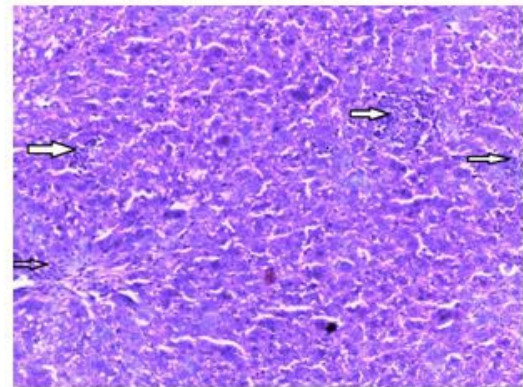


Figure (6): a photomicrograph of a section in liver of rat treated with hydrochloroquine shows mild inflammatory changes (Hx.&E. X200).

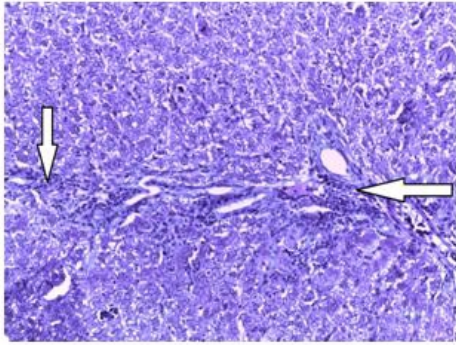


Figure (7): a photomicrograph of a section in liver of rat treated with chloroquine shows sever inflammatory changes (Hx.&E. X200).

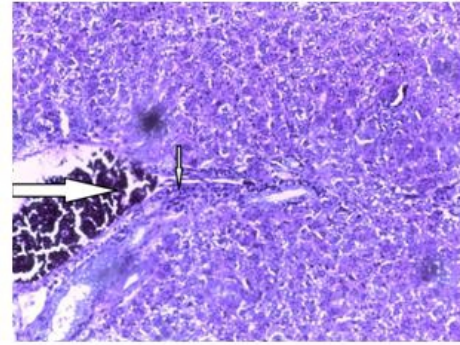


Figure (8): a photomicrograph of a section in Liver of rat treated with chloriquine shows severe inflammation and congestion (Hx.&E. X200)

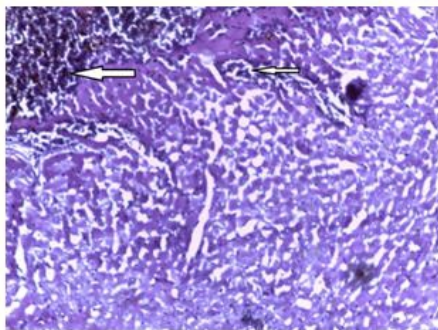


Figure (9): a photomicrograph of a section in heart of rat treated with hydrochloroquine shows moderate congestion and mild haemorrhage (Hx.&E. X200).

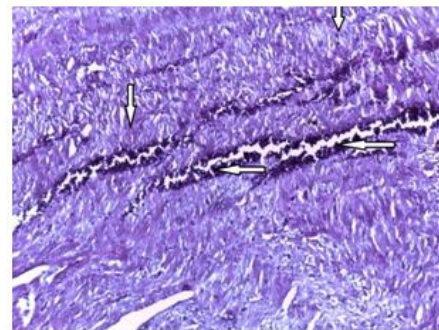


Figure (10): a photomicrograph of a section in heart of rat treated with chloroquine shows moderate congestion and interstitial fibrosis (Hx.&E. X200)

Discussion

Most antimalarial agents had been shown to be toxic. Chloroquine was first used as an antimalarial agent. It subsequently played an important therapeutic role in various rheumatological diseases. The use of chloroquine analogue, hydroxychloroquine, has largely replaced chloroquine in most parts of the world, because of better tolerability at high dosages (**Adeeko and Dada, 1998; Pasadhika and Fishman, 2010**).

So, physicians uses chloroquine and hydroxychloroquine by large doses in treating various rheumatological diseases. Systemic side effects of hydroxychloroquine and chloroquine include disturbances in hepatic and renal function (**Lee, 2005; Ling Ngan Wong, 2008**).

The results of the present study indicated that Chloroquine induced marked lesions in the liver, kidney, and heart, as shown by pathologic study. The lesions appeared on the form of hydropic degeneragtion, congestion, inflammation, necrosis and atrophied glomeruli. Hydroxychloroquine treated group showed mild changes in organ structure as compared to chloroquine group. Chloroquine produced statistically significant increase in the level of biochemical parameters as compared to control group and hydroxychloroquine treated rats.

The histopathologic changes are in agreement with the biochemical results. The pathological changes of kidney, liver and heart in rats treated by chloroquine are more severe than that occurred with the rats treated with hydrochloroquine. Chloroquine seems to have toxic effect as a powerful membrane destabilizer, as well as direct effect on organs (**Riou et al., 1988**).

The results of the present study showed that chloroquine has deleterious effects on the structure of the liver; 90% of chloroquine treated rats had severe inflammation and congestion and this result was statistically significant as compared with control group, only 20% of hydroxychloroquine treated rats have Inflammation and congestion and this results was statistically significant.

Colombo and Bertini (1988) argued that the biological and pharmacological actions of chloroquine are directly related to its interaction with lysosomal membranes. Chloroquine, however, decreases the density of hepatocyte lysosomes. this could result from the fact that hepatocyte lysosomes accumulate Chloroquine to high extent in the liver.

In the present study chloroquine treated rats showed severe hyper -cellularity of glomeruli in 50 % of rats and this result was statistically significant as compared with control group and hydroxychloroquine group, while in hydroxychloroquine treated rats only 10% of treated rats had abnormalities in glomeruli.

The accumulation of Chloroquine in tissues may result from inhibition of anti-malarial microsomal metabolism in kidney cells and potentiate its uptake in lysosomes in the cytoplasm. Chloroquine, which is also deposited in the adrenal glands may indirectly affect kidney function by modulating the secretory patterns of aldosterone to cause a reduction in tubular Na⁺ handling. The deposition of CHQ in the epithelial cells of the kidney may result in a possible interference with ion movements (**Cooper and Magwere, 2008**).

Currently, there are only few investigations on the effect of Chloroquine on kidney morphology. Chloroquine may exert its renal effects indirectly via histopathological and ultrastructural cardiac damage through reductions in glomerular filtration rate (**Teixeira et al., 2002**).

The results of the present study indicated that short term chronic administration of chloroquine can cause necrosis of cells of rats' organs, hydroxychloroquine treated rats also showed these changes but to lesser extent than chloroquine group. The results of the present study revealed that 70% of chloroquine treated rats had interstitial tissue fibrosis compared to only 20 % of hydroxychloquine group and this difference was statistically significant. These results are agreed with **Jordan et al. (1999)** who reported that chloroquine is 2-3 times more toxic than hydroxychloroquine in animal studies.

Bercovici (1982) in a review of published cases found that chloroquine leads to increase the accumulation of cell associated epidermal growth factor (EGF) and inhibition of mitogenic activity of EGF and may lead to interstitial fibrosis.

These findings in the study agreed with the work of **Izunya et al. (2011)** who found that chronic oral administration of chloroquine may cause cytoplasmic vacuolation, nuclear enlargement and apoptosis of cells.

Chloroquine is 60% bound to plasma proteins and equally cleared by the kidney and liver, the toxicity of chloroquine is partially related to its transiently high whole blood concentration presents early in the distribution phase (**Looaresuwan et al., 1986**).

For biochemical myocardial parameters profile, Creatinine kinase-MB, C-reactive protein and Malonic dialdehyde were significantly elevated in the chloroquine treated group compared to control. Also, these differences were statistically significant in comparison to hydroxychloroquine treated group. The increased cardiac markers could be directed to the reported pathological degeneration and necrosis in the myocardium. Chloroquine treated rats showed moderate congestion and interstitial fibrosis of cardiac muscle. Comparison between the two tested drugs showed that chloroquine has more toxic effect on heart than hydroxychloroquine, as 85% of chloroquine treated rats had fibrosis compared to only 10% in hydroxychloroquine treated group. This result was statistically significant.

Death from chloroquine toxicity seems to be related to cardiac toxicity from chloroquine's action on the cardiac conduction system and myocardium. (Clemessy et al., 1995). Long-term chloroquine can cause cardiac complications, such as conduction disorders and cardiomyopathy (restrictive or hypertrophic), by structural alteration of the interventricular septum (Cervera et al., 2001).

Conclusion

In conclusion, the risk of toxicity with chloroquine appears to be significantly higher than with hydroxychloroquine. For this reason, chloroquine should only be considered if hydroxychloroquine has failed to control the disease adequately. It is recommended that all patients taking chloroquine should be counseled on the risk of liver, kidney and heart toxicity and examination for renal and liver function should be arranged. The maximum dosage of hydroxychloroquine should not exceed 6.5mg / kg body weight (typically 200-400 mg daily).

Recommendations

Hydroxychloroquine is lesser toxic than chloroquine to a great extent. So hydroxychloroquine must replace chloroquine. Also, liver and kidney function should be done periodically for patients receiving hydroxychloroquine and chloroquine for prolonged time. Further investigation on the toxicity of chloroquine and hydroxychloroquine should be done. Finally, results will be disseminated to rheumatology department in faculties of medicine.

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