

## Chloroquine Induced Oxidative Stress in Male Albino Mice: RCT

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### ABSTRACT

Many drugs have been found to induce oxidative stress. Oxidative stress is responsible for a large number of diseases. Chloroquine is one of the drugs, which can induce oxidative stress, when it is given at higher dose.

**Purpose:** To find the effect of chloroquine as stress inducer on albino mice.

**Study Design:** Randomized clinical trial.

**Methodology:** Sixty male albino mice were taken into this randomized controlled study. Those were divided into two groups of 30 each. Group A was the control group while group B mice were given single oral dose of 970 mg/kg of body weight of chloroquine on 9<sup>th</sup> day of experiment. Terminal intracardiac blood sample was obtained on 17<sup>th</sup> day of experiment.

**Statistical analysis:** SPSS version 23 was used for data analysis.

**Results:** When results of group B were compared with those of group A, there was highly significant ( $p= 0.000$ ) rise in serum malondialdehyde level and highly significant ( $p= 0.000$ ) decrease in serum glutathione peroxidase level.

**Conclusion:** It was concluded that Chloroquine induces oxidative stress when it is given at the dose of 970 mg/kg of body weight in mice.

**Keywords:** Chloroquine, Oxidative Stress and Malondialdehyde.

### INTRODUCTION

Oxidative stress is a process in which imbalance occurs between the production and removal of reactive oxygen species.<sup>1</sup> Reactive oxygen species attack the cell membranes and disrupts the structure of DNA and intracellular proteins.<sup>2</sup> As a result, reactive oxygen species induced damage causes various diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease.<sup>3</sup>

Many drugs have been found to induce oxidative stress by generation of reactive oxygen species, during their detoxification process in the liver.<sup>4</sup> Drugs which can induce hepatotoxicity due to oxidative stress induction include sulphonamides, isoniazid, methyl dopa, methotrexate and amiodarone etc.<sup>5</sup> Chloroquine is one of the drugs, which when given at higher dose can induce oxidative stress.<sup>6</sup>

Chloroquine is a commonly used antimalarial drug in developing countries due to its easy availability and cost effectiveness.<sup>7</sup> It is also used to treat systemic lupus erythematosus and rheumatoid arthritis due to its anti-inflammatory actions.<sup>8</sup> The safe therapeutic dose in humans have been advised to be less than 6 gm/ kg of body weight per day. Chloroquine has the potential to generate reactive oxygen species, when given at higher dose.<sup>9</sup>

These reactive oxygen species attack the lipids of the cell membrane and induce oxidative stress by lipid peroxidation process.<sup>10</sup> Lipid peroxides are the highly reactive substances which are generated in lipid peroxidation process. These lipid peroxides disrupt the structure of DNA and proteins.

As a result of lipid peroxidation process, many secondary metabolites are generated such as malondialdehyde. Malondialdehyde has the ability to form adducts with proteins and DNA. These adducts cause increased intermolecular and intramolecular cross linking

between proteins and DNA.<sup>11</sup> Membrane loses its integrity and becomes more permeable.<sup>12</sup>

Antioxidants such as glutathione peroxidase has the ability to scavenge free radicals and hence terminate lipid peroxidation process.<sup>12</sup> Due to the utilisation of antioxidants in oxidative stress, their level in serum is decreased. Lipid peroxidation is assessed by increased serum levels of malondialdehyde<sup>13</sup> and decreased serum levels of glutathione peroxidase.<sup>14</sup>

**objectives:** To find the effect of chloroquine as stress inducer on albino mice.

### METHODOLOGY

The study was conducted from March 2017 to December 2018 at Akhtar Saeed Medical and Dental College, Lahore. In this randomized controlled trial study, sixty male albino mice were taken from University of Veterinary Sciences, Lahore. The average weight of mice was 28 gm and age was 8-10 weeks. They were divided by lottery method into two separate groups i.e group A and group B. Each group contained 30 mice. Mice of each group were kept in separate cages. They were acclimatized for about 1 week before the start of the experiment. They were kept at 26 ± 2 °C with 12 hour light dark cycle.<sup>8</sup> The humidity was maintained at 55 %. Mice had free access to food and water.

**Group A:** (Control, n=30): was not administered chloroquine.

**Group B:** (Chloroquine, n=30): was administered a single oral dose of chloroquine (970 mg/kg of body weight) at 9<sup>th</sup> day of experiment.

**Statistical Analysis:** SPSS version 23 was used for data analysis. Serum malondialdehyde and glutathione peroxidase between groups were presented as mean ± SD. Student t-test was applied with  $p\text{-value} \leq 0.05$  taken as significant.

## RESULTS

Mean values of serum malondialdehyde and glutathione peroxidase between groups A and B were compared, using student's t test. Group B, in which chloroquine was given, depicted highly significant ( $p=0.000$ ) rise in serum malondialdehyde and highly significant ( $p=0.000$ ) decrease in serum glutathione peroxidase as shown in table-1.

Table-1: Comparison of Serum Malondialdehyde & Glutathione Peroxidase Between Groups

Parameters	Group A (n=30)	Group B (n= 30)	p- value
Serum malondialdehyde (ng/dl)	0.12±0.037	0.22±0.12	0.000*
Serum glutathione peroxidase (ng/dl)	1.03±0.013	0.79±0.12	0.000*

\* $p < 0.001$  highly significant

## DISCUSSION

In the current study, chloroquine caused highly significant increase in serum malondialdehyde and highly significant decrease in serum glutathione peroxidase. Malondialdehyde is one of the secondary metabolites which are produced in lipid peroxidation process. The increased amount of serum malondialdehyde indicates damage to lipid membrane. While glutathione peroxidase is an antioxidant enzyme which scavenges free radicals. The decreased serum levels of glutathione peroxidase indicate their utilization in scavenging free radicals.

These results are consistent with the study conducted by previous researchers<sup>12</sup>. They had used the same dose of chloroquine of 970 mg/kg of body weight in female wister rats. They found significant elevation in the levels of serum hydroperoxides and thiobarbituric acid reactive species. Serum hydroperoxides and thiobarbituric acid reactive substances were produced in lipid peroxidation process. Reduced level of serum glutathione peroxidase was observed in their study. Reduced serum glutathione peroxidase showed it's increased utilization in scavenging free radicals which were generated in oxidative stress.

Another study which revealed oxidative stress induction by chloroquine<sup>13,14</sup>. They used different doses of chloroquine in their experiment. At the dose of 360 mg/kg, they did not observe any change in the serum levels of malondialdehyde and glutathione peroxidase. But at higher doses of chloroquine i.e at 1000 mg/kg and 2000mg/kg, significant elevation in the serum levels of malondialdehyde was observed. This indicated increased oxidative stress induction. Malondialdehyde was produced as a secondary metabolite in lipid peroxidation process. While significant decrease in the levels of serum glutathione peroxidase at the doses of 1000 mg/kg and 2000 mg/kg were observed due to their increased utilization in scavenging free radicals. While no change in the level of serum glutathione peroxidase observed at the therapeutic dose of 360 mg/kg of body weight.

**Limitations:** Limitations included limited time frame, resources and financial constrains.

## CONCLUSION

It was concluded that chloroquine induces oxidative stress when it is given at the dose of 970 mg/kg of body weight in mice.

**Author's contribution:** SK: Conceptualized the study, analyzed the data, and formulated the initial draft.

HJQ: Contributed to the histomorphological evaluation.

MSA&TL: Contributed to the proofreading the manuscript for intellectual content.

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## REFERENCES

- Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, Gargiulo G, Testa G, Cacciatore F, Bonaduce D, Abete P. Oxidative stress, aging, and diseases. *Clinical interventions in aging*. 2018;13:757.
- Sies H. Oxidative stress: Concept and some practical aspects. *Antioxidants*. 2020 ;9(9):852.
- Singh A, Kukreti R, Saso L, Kukreti S. Oxidative stress: a key modulator in neurodegenerative diseases. *Molecules*. 2019;24(8):1583.
- Kumar Mishra S, Singh P, Rath SK. Protective effect of quercetin on chloroquine-induced oxidative stress and hepatotoxicity in mice. *Malaria research and treatment*. 2013;2013(4).
- Singh D, Cho WC, Upadhyay G. Drug-induced liver toxicity and prevention by herbal antioxidants: an overview. *Frontiers in physiology*. 2016. 26:6:363.
- Asha S, Thirunavukkarasu P, Taju G. Curcumin pretreated hepatoprotectivity against antimalarial drug chloroquine induced hepatotoxicity in albino rats. *EurAsian Journal of BioSciences*. 2019;13(1):41-8.
- Kumar Mishra S, Singh P, Rath SK. Protective effect of quercetin on chloroquine-induced oxidative stress and hepatotoxicity in mice. *Malaria research and treatment*. 2013;2013(4).
- S, Dutz JP. New concepts in antimalarial use and mode of action in dermatology. *Dermatologic therapy*. 2007;20(4):160-74.
- Pari L, Amali DR. Protective role of tetrahydrocurcumin (THC) an active principle of turmeric on chloroquine induced hepatotoxicity in rats. *J Pharm Pharm Sci*. 2005;8(1):115-23.
- Saifi MA, Alyousif MS, Ahmed M. Biochemical investigations on the protective role of curcumin in liver damage by chloroquine. *International Journal of Pharmacology*. 2015;11(7):870-3.
- Ayala A, Munoz MF, Arguelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative medicine and cellular longevity*. 2014.
- Wani S, Basir SF. Analysis of antioxidant activity, total phenolic and total flavonoids contents of allium sativum, mentha arvensis and Murray's Koenigii. *International Journal of Advance Research in Science and Engineering*. 2018; 7(4): 2632-46.
- Tsikakos D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples. *Analytical biochemistry*. 2017; 524: 13-30.
- Gheita TA, Kenawy SA. Measurement of malondialdehyde, glutathione, and glutathione peroxidase in SLE patients. In *Systemic Lupus Erythematosus* 2014;1134:193-99.