Protective Effect of Pyridoxine on Lead Nitrate Induced Histomorphological Changes in Rat Liver

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ABSTRACT

Objective: To study the effects of Pyridoxine on lead nitrate generated histological and morphological changes in rat liver.

Study Design: It was an experimental study.

Place and Duration of Study: The study was carried out at Anatomy Department of College of Physicians and Surgeons, Islamabad, Pakistan from 03rd September, 2019 to 31st December, 2020.

Materials and Methods: In this study 90 male rats were used and divided into three groups. Control group A was kept on normal diet. Experimental group B1 was given lead nitrate and B2 was given lead nitrate and pyridoxine by oral gavage for a period of 2 weeks. All rats were sacrificed after 2 weeks. Results of quantitative variables such as inflammatory and Kupffer cells were expressed in mean ± S.D. To detect significant difference among the groups, ANOVA accompanied by Post Hoc Tukey test was applied and qualitative data such as central vein congestion, steatosis, and necrosis were expressed in percentages and Chi Square test.

Results: Exposure to pyridoxine reduces the degenerative changes caused by lead nitrate such as increase in the number of Kupffer and inflammatory cells. Central vein congestion, steatosis and necrotic foci were also reduced with a significant p-value.

Conclusion: An ameliorative effect of pyridoxine on the degenerative changes in the liver induced by lead nitrate was proven through a decrease in the number of kupffer and inflammatory cells. Central vein congestion, steatosis and necrotic foci were also reduced significantly.

Keywords: Congestion, Free Radicals, Lead Nitrate, Necrosis, Oxidative Stress, Pyridoxine and Steatosis.

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Introduction

Environmental pollution is defined as, presence of a substance in the surroundings which may be toxic and will produce harmful effects to living things. Pollutants usually enter the atmosphere by the production of tobacco smoke, oil, steel, industrial exhaust, coal and iron.1,2 Expeditious urbanization and industrialization constitute major public health crises because of the existence of lead in atmosphere.3 Lead usually enters the body either through inhalation or ingestion.3,4 Its toxicity depends upon different factors; age, administration route, frequency, duration, chemical form and proficient route of excretion.5 According to the World Health Organization (WHO) lead poisoning has dreadful health consequences that account for 0.6 percent of the global ailments.6 As stated by the American Centre for Disease Control (CDC), lead is one of the chief environmental toxins which results in different diseases of environmental origin among children.4 Extensive studies have revealed that cells which are exposed to adverse environmental conditions (toxic and harmful substances) can cause over production of reactive oxygen species (ROS) leading to lipid peroxidation and increase in the...
content of unsaturated fatty acids in cell membranes which results in oxidative stress.\(^5\)\(^7\)

For the control and treatment of different ailments, trend has been changed by the use of antioxidants because they are cost effective and easily present in both natural and man-made compounds. One of the antioxidants is pyridoxine which is also known as vitamin B6 and its active form is known as pyridoxal phosphate.\(^8\)

It is a water-soluble vitamin that your body needs for several functions. It acts as a coenzyme and involved in numerous metabolic reactions of the body which are concerned with the metabolism of different nutrients mainly protein.\(^9\)\(^10\)

Vitamin B6 supplementation reduces oxidative stress associated complications such as depression, degenerative diseases of the nervous system and diabetes to some extent.\(^5\)\(^11\)

It plays an important role in the conservation of glutathione (GSH) and also helps in the regeneration of hepatic tissue with improvement in the blood levels of alanine transaminase, aspartate transaminase, alkaline phosphatase by inhibiting the production of ROS and increasing GSH levels.\(^12\)\(^13\)

Mechanism behind hepatic damage caused by lead occurs through the depletion of antioxidants mainly GSH and overproduction of ROS. ROS in turn cause damage to membrane proteins, lipids and DNA by altering their composition which leads to cellular injury. Pyridoxine plays a vital role in maintenance of GSH by converting homocysteine into cysteine which participates in the synthesis of GSH.\(^14\)

Over the past few decades, light has been shed independently on the effects of lead and pyridoxine on different parts of human body. In the current study, effects of pyridoxine and lead nitrate have been studied together on the histology of liver.

**Materials and Methods**

The present study was done at the Anatomy Department, College of Physicians and Surgeons, Islamabad, Pakistan. The period of study was 03\(^{rd}\) September, 2019 to 31\(^{st}\) December 2020. Ninety adult male Sprague Dawley rats variety were purchased from National Institute of Health Sciences (NIH). The rats were split into three groups (control group A and experimental group B1 and B2) each group comprised of 30 rats. Experimental group B1 received 50mg/kg body weight of lead nitrate and 100mg/kg body weight of pyridoxine solvated in 10ml of distilled water and given to rats by oral gavage day-to-day for a duration of 14 days.\(^11\) Rats were fed with a pellet diet and water ad libitum.

**Parameters**

Quantitative parameters include number of inflammatory and Kupffer cells. Before observing the slides, ocular of the microscope was fitted with graticule to facilitate the counting of cells. To avoid overlapping, four fields of each specimen were examined, two on x axis and two on y axis under the objective of 40X in an ocular grid and mean of each sample was taken.

Qualitative parameters include central vein congestion, steatosis and necrosis. Central vein congestion and steatosis were examined under 40X. Foci of necrosis were counted at 10X and confirmed at 40X by using Modified Histological Activity Index Grading.\(^15\)

**Statistical Analysis**

The variables were investigated statistically using SPSS version 22. Quantitative variables were expressed as means ± SD. ANOVA with post hoc Tukey test was also applied to quantitative variables to perceive notable differences between control and experimental groups. Qualitative variables were demonstrated in percentages and Chi square test. The \(p\)-value of ≤ 0.05 was considered as significant.

**Results**

Congested central vein, steatosis and necrosis measured in all groups are given in Table 1 and Table 2 as percentages respectively. Parameters like number of Inflammatory and Kupffer cells in all groups are given in Table 3 and Table respectively as mean ± SD with their \(p\)-values.

| Groups     | Central vein Congestion | Steatosis |
|------------|-------------------------|-----------|
| Group A    | 0.0%                    | 0.0%      |
| Group B1   | 86.2%                   | 67.1%     |
| Group B2   | 47.0%                   | 40.0%     |

| Groups     | Absent | 1 or less foci | 1-4 foci | 5-10 foci | \(p\)-value |
|------------|--------|----------------|----------|-----------|-------------|
| Group A    | 100%   | 0%             | 0%       | 0%        | 0.001       |
| Group B1   | 3%     | 43%            | 37%      | 17%       |             |
| Group B2   | 40%    | 37%            | 23%      | 0%        |             |
Discussion

Lead is identified as a toxic agent for both humans and animals. Results of this study are in line with a former study in which lead acetate was used.\(^\text{16}\) Results of the current study are also in accordance with a study in which lead was given for a duration of six weeks on renal tissue.\(^\text{17}\) Lead is responsible to have a negative role on the immune system of the body by releasing various chemical mediators like leukotrienes and cytokines such as IL-1, IL-6 and TNF-\(\alpha\) which in turn produce ROS; responsible for damaging physiological and morphological changes in a cell.\(^\text{18}\)

The outcome of our study is in agreement with the previous studies done in 2010 and in 1991 in which pyridoxine supplementation was shown to decrease cytokine production.\(^\text{19, 20}\) The number of inflammatory cells were reduced in group B2 because pyridoxine counteracts against the process of inflammation by suppressing pro-inflammatory cytokines such as IL-6 and TNF-\(\alpha\).\(^\text{20}\) The probable mechanism in suppressing the inflammatory response is the inhibition of lipid peroxidation with the help of the hydroxyl and amine group present in the pyridoxine pyridine ring.\(^\text{21}\)

In comparison with the group A, number of Kupffer cells per unit area in group B1 was highly increased. Outcome of the current study is in concordance with the study done in 2009 and 2012.\(^\text{16, 22, 23}\) However, the number of Kupffer cells were significantly less as compared to group B2. Mechanism behind increased number of Kupffer cells in group B1 is that they participate in the development of hepatic injury by releasing various cytokines. Hepatic damage can be treated by controlling the process of oxidative stress that leads to inflammatory responses.\(^\text{24}\)

Group A did not present any vein congestion in the present study, while in group B1, 86.2% showed central vein congestion. In group B2, 47% showed central vein congestion. This observation supports the results of previous studies.\(^\text{22, 25, 26}\) Central vein congestion was ameliorated in experimental group B2, because pyridoxine protects the capillary endothelium from oxidative damage by increasing the synthesis of nitric oxide and endothelial cell alignment.\(^\text{7, 8}\)

Group A did not show any foci of necrosis, on the other hand in group B1 43% showed one or less foci,
37% showed 1-4 foci and 17% showed 5-10 foci. In group B2, 37% showed one or less foci and 23% showed 1-4 foci. Observations of the current study is in harmony with the earlier studies done on liver and on a renal tissue. Necrosis is reduced in group B2 which can be attributed due to the vitamin B6’s role in the kynurenine pathway and by limiting the accumulation of toxic metabolites which in turn preserves the status of cellular energy. None of the rats in group A showed hepatic steatosis, whereas group B1 and B2 showed 67.1% and 40% steatosis respectively. Result of the present study is in agreement with the previous work. Though exact mechanism is not known.

Conclusion
We demonstrated an ameliorative effect of pyridoxine on the degenerative changes in the liver induced by lead nitrate througha decrease in the number of Kupffer and inflammatory cells. Central vein congestion, steatosis and necrotic foci were also reduced significantly.

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