

THE ROLE OF LEAD TOXICITY ON ERUPTION RATE OF HYPOFUNCTIONAL INCISORS IN ALBINO WISTAR RATS

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ABSTRACT

OBJECTIVES

This objective of this study was to evaluate the role of a heavy metal- lead acetate in the eruption rate of hypo functional incisors in albino Wistar rats.

METHODOLOGY

An experimental study was done in animal house of Post Graduate Medical Institute, Lahore since March 2019 to March 2020. 34 adult albino Wistar rats were randomly divided into two groups (n=17 for each group) i.e., control and lead acetate group. Right mandibular incisors were selected for this study. Selected incisors were marked 1mm above the level of gingival papillae. The incisors were cut above this mark to make it hypo-functional. The readings were measured by digital Vernier caliper. This was considered as day 0. Incisors length was measured at day 0, 3, 6, 12 and 15 and eruption was calculated. The data was analyzed using SPSS version 22.

RESULTS

Eruption rate was similar throughout the study except last follow up. At the end of this study eruption of incisors in albino Wistar rats in control was 03.30 ± 0.72 mm, in lead 02.43 ± 1.19 mm. At day 15, the difference between control and lead group was statistically significant (p-value 0.033).

CONCLUSION

These results reveal that besides other causes of delayed tooth eruption excessive lead intoxication are also a causative factor of delayed tooth eruption.

KEYWORDS: Delayed Eruption, Lead, Incisors, Albino Wistar Rat.

How to cite this article:

Javaid R, Mirza AJ, Ahmed A, Ibrahim A, Akhtar Q, Sana R, et al. The Role of Lead Toxicity on Eruption Rate of Hypofunctional Incisors in Albino Wistar Rats. J Gandhara Med Dent Sci. 2022;9(4) 42-47
<https://doi.org/10.37762/jgm.9-4.315>

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INTRODUCTION

Every tooth has a specific time to erupt in the oral

cavity, but sometime deviation is seen clinically in eruption time.¹ During tooth eruption, the developing tooth moves through alveolar bone and gingiva to come into the oral cavity. This is active process of eruption. After that the developing tooth moves toward opposing tooth to make occlusion. This is passive tooth eruption, which further continues to compensate for jaw growth, occlusal and interproximal attrition.² The current study is about this passive phase of eruption. Premature tooth eruption is seen very rarely; rather delayed eruption is very common and generally parents are very much worried and concerned about it.³ In normal condition tooth begins to erupt when $\frac{3}{4}$ of total root length has formed.⁴ There are many

factors affecting tooth eruption. There are genetics, nutrition, preterm birth, socioeconomic factors, hormonal factors and systemic diseases like endocrine disorders, vitamin D-resistant rickets, long term chemotherapy, radiation damage, maxillofacial trauma, and one of the most important factors is heavy metal intoxication.⁵ Lead is a toxic heavy metal. It is a group of metal and metalloids with an atomic density higher than the 4000 kg/m³. They are toxic elements which causes serious health illness to human beings and animals, even if they are at very low concentration.⁶ In recent centuries, the human activities have caused 1000 times more increase in the environmental lead level, which has become a global concern nowadays.⁷ Lead is the most common environmental and industrial pollutants that affect almost all biological systems.⁸ Common sources of lead poisoning are car battery industries, manufacturing of ceramics, lead bearing paints, contaminated food, water and environment.⁹ It is also present in petrol and octane. Environmental lead pollution and occupational lead exposure are the common public health problem. Higher risk of lead poisoning is among those children who have the habit of chewing lead containing paint on walls and PVC toys.¹⁰ Lead poisoning affects many tissues that may result in neurological, renal, skeletal, hematopoietic, and reproductive complications. Lead toxicity affects the dental and oral tissues. The presences of heavy metals like lead interfere with development of enamel called amelogenesis.¹¹ Mineralization is delayed during exposure to lead.¹² This lack of mineralization is compensated by relatively longer duration maturation, reflected in slow eruption. In normal occlusion, the Incisor growth in albino Wistar rats is maintained throughout life at an average rate of 0.5 mm/day.¹³ This eruption rate can be increased by trimming their incisal edges to make them hypo functional. This increased eruption rate of the hypo-functional incisor has been reported to be approximately 1 mm/day or 1.5 mm/day.¹⁴ In 2000 Gerlach and his co-researchers documented an experimental study on lead induced delayed eruption of incisors in albino Wistar rats.¹⁵ Therefore keeping in mind, the harmful and toxic effects of lead acetate, this study was conducted to see the effect of daily oral dose of lead acetate in hypo-functional mandibular incisors of albino Wistar rats.

METHODOLOGY

34 Adult healthy male albino Wistar rats weighing

180-200 grams were selected from Animal House at Post Graduate Medical Institute, Lahore. The duration of this study was 12 months and study were conducted at Post Graduate Medical Institute, Lahore. Experimental animals were assigned number 1,2,3,4, till 34 using random number generator. All the rats were kept in experimental research laboratory at controlled room temperature (22-24°C) and humidity (45-65 %) under 12/12 hours natural light and dark cycle. All animals were fed on rodent chow and distilled water *ad libitum*. Animals are randomly divided into two groups (n=17). Control group was assigned as A, and leaded water group was assigned as B. Sample size was calculated by the following formula keeping the power of study equal to 80% and level of significance equal to 5%.

$$n = \frac{\left(Z_{1-\alpha/2} \sqrt{2p(1-p)} + Z_{1-\beta} \sqrt{p_1(1-p_1)p_2(1-p_2)} \right)^2}{(p_1 - p_2)^2}$$

(Sample Size determination in health studies version 2.0.21 WHO) (Israel, 1992). The exclusion criteria were gingival and periodontal infections in this study. For the preparation of drug, 1500 ppm leaded water, 30 g lead acetate, 8 ml 1N HCl (to ensure solubility) and 10 g glucose (for favorite taste) were dissolved in 20 liters of distilled water.¹⁶ Group-A rats were given diet with rat chow and water. In Group-B, rats received leaded water (1500ppm). Right mandibular incisors were selected for the study. All the albino Wistar rats were weighed on digital weighing machine and noted. The rats were anaesthetized with intraperitoneal ketamine injection (100 mg/kg body weight) using 1c.c disposable syringe. Reference point was marked 1.0 mm above the level of gingival papilla by rotary diamond bur TF-12 EF. Incisor was cut above that reference mark (Figure 1). The readings were taken between upper boarder of gingival papillae and the marked reference point at day 0, 3, 6, 9, 12 and 15 (Figure 2). The procedure described by Gerlach in 2000 with some modifications. The actual eruption in three days was measured by subtracting the 1st reading from the 2nd reading. In the same way readings were taken on day 6, 9, 12 and 15 and eruption rate was calculated respectively (Figure 3). Blood level Count (BLC), the blood samples of 0.5 ml were taken from cardiac puncture on day 0, 3, 6, 9, 12, 15. The anesthetized albino Wistar rats were placed on a board and were handled by a well-trained assistant. Heart was palpated and a disposable syringe was used to draw the blood specimen. The BLC was determined by atomic absorption spectroscopy with perkin-Elmer HGA (Heat Graphite atomizer) according to the method

described by Parsons and Slavin.¹⁷

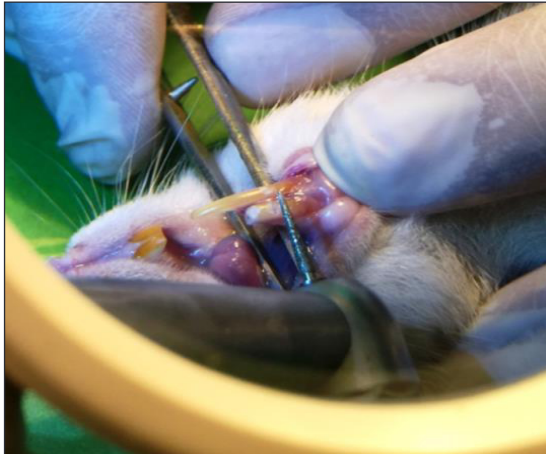


Figure 1: Marking the Reference Point and Cutting Right Mandibular Incisor By High-Speed Turbine and Tapering Fissure Diamond Bur



Figure 2: Measuring Between Gingival Papillae and Reference Point by Vernier Caliper



Figure 3: Measuring Between Reference Point and Gingival Papillae Level At After Every Day 3 By Digital Vernier Caliper

RESULTS

Table 1: Comparison of Eruption (Mm) Between Control and Ledged Group

	Control	Lead	P-Value
Day 3	03.17±1.43	03.22±1.55	0.999
Day 6	02.94±1.45	03.01±1.52	0.998
Day 9	03.23±1.0	02.73±1.14	0.649
Day 12	03.15±1.33	02.57±1.55	0.553
Day 15	03.30±0.72	02.43±1.19	0.033*

Post Hoc Tukey's test, p-value significant at 0.05

Since day 9 a gradual reduction in eruption rate was seen in leaded group, but no statistically significant difference was observed between control and lead group at different times of study except at day 15(p-value 0.033).

Table 2: Comparison of Blood Lead Concentrations In µg/Dl Among Groups

	Control	Lead	P-Value
Day 3	09.40±0.66	09.27±1.49	0.996
Day 6	11.91±0.95	56.79±8.89	<0.001
Day 9	13.67±1.98	80.17±9.38	<0.001
Day 12	13.14±1.38	90.6±10.09	<0.001
Day 15	13.33±1.09	121.71±12.16	<0.001

Post Hoc Tukey's test, p-value significant at 0.05

At day 3, in blood lead count there was no statistically significant difference was observed between control and lead group (p-value 0.996). Since day 6 to day 15, statistically significant difference was observed between control and lead group (p-value <0.001).

Table 3: Correlation between Eruption Rate and BLC among groups

	Control	Lead
Day 3	0.723	0.379
Day 6	0.634	0.602
Day 9	-0.596	-0.689
Day 12	-0.697	-0.296
Day 15	-0.054	-0.528

In the final follow up, the relationship between eruption rate and blood lead count in control group was weak and inverse (r-0.054) while in lead group it was strong and inverse (r-0.528).

DISCUSSION

Heavy metals contamination and toxicities are highly documented in the literature. Lead is a toxic heavy metal which is being intake by humans by inhalation, water, fruits, and vegetables growing on sewage or contaminated water.¹⁷ This causes increase in blood lead concentration of living organisms that produces many toxic effects on their health.¹⁸ There are many harmful effects of lead toxicity in oral cavity like, it delays enamel formation, increase incidence of dental carries,

smaller sized molars of rats etc. The current study was conducted to see the effects of daily oral dose of lead acetate on eruption rate of incisors in albino Wistar rats. Gerlach et al. conducted an experimental study on albino Wistar rats and found that lead causes delayed eruption.¹⁵ A single intraperitoneal injection of lead acetate 40mg/kg was administered that results in elevated blood lead count (BLC) in the beginning and resulted in transient delay in eruption. These affects were reduced in later follow up. I used daily oral dose of 1500 ppm leaded water according to the preparation by Sadeghi in 2012.¹⁶ This resulted in gradual increase in BLC and reduction in eruption rate which become significant at 5th follow up. A mark was made 1mm above the gingival papilla and then incisor was cut above this mark. This change in methodology was made to avoid gingival and pulpal inflammation due to trauma by rotary diamond bur if cutting was done at gingival papilla level. This protective management was incorporated in this study, which had not been observed in the previous studies.¹⁹ Bideskan et al conducted a study.²⁰ He used 1500 ppm lead acetate drinking water and found that it resulted in increased blood lead count (BLC). In control group, BLC was 8µg/dl and in leaded group it became 124µg/dl. Same comparable elevated BLC was seen in this study. Minor difference might be due to increase weight of rats and duration of study. In 2018, Saleh and his co-researchers conducted a study.²¹ They used 160 and 360 ppm leaded water in an animal experimental study on female rats. They noticed the same change in BLC among control and experimental group, as seen in current study. In adults, 90-95% of total body lead is stored in trabecular and cortical bone, whereas, in children, 70 to 95% accumulates in trabecular bone because of its high turnover rate. Therefore, the concentration of lead in blood and soft tissue organs can vary throughout life according to the metabolic activity of bone tissue. In vivo studies had shown that Pb poisoning decreased in bone mineral content and hence one of the reasons of delayed tooth eruption.²² Borany Tort and his colleagues in 2018 found that elevated blood lead count causes damage to gingiva, periodontal ligament, and alveolar bone tissues. It may interfere with calcium metabolism. As health of all these structures are important in the normal process of tooth eruption. This factor might be one of the reasons of tooth eruption, found in current study.²³ Khalid M. and Abdollahi M. in 2020 found that high blood lead level (BLL) is related to iron, magnesium, and zinc deficiency. Lead can cause metabolic disorder of various essential

elements even at low BLL. It affects biological processes related to bone growth and development. The deficiency of these tooth tissue elements may affect other essential elements that may cause teeth to be more susceptible to different pathologies. In the current study there was not any significant increase in the BLL initially but later it was significantly monitored in the test results, this finding of increase BLL also confirms the more significant increase delay in the eruption rate of incisors on day 5 as shown in table 1. This finding however suggests that more studies with a bigger sample size are required to check the statistically significant results. Cofactors and other elements are essential for the enzyme system, promoting the normal growth and development of hard tissues, and are also crucial as nonorganic material in bone and teeth. Insufficient essential elements in the body may lead to hypo mineralization, reduced bone strength, reduced abrasion resistance, and tooth formation defect. This may lead to enamel degeneration, delay dentin formation, inhibit alveolar bone growth, and delay tooth eruption.²⁴

LIMITATION

This is an animal study; the results of this study may be varied from Human study. In this study, hypo-functional mandibular incisors were used, that results may differ from normal occlusion and hyper functional occlusion. The results may differ for teeth other than mandibular central incisor. The results may differ for different concentration of lead preparation.

CONCLUSION

Heavy metal-lead is an established environmental pollutant which effects tooth eruption and should be considered along with other local factors. Lead exposure should be avoided by living and working in Pb free environment. Precautions should be adopted in lead polluted environment to reduce the hazards of lead toxicity which help in minimizing the risk of dental diseases including delayed eruption of teeth.

CONFLICT OF INTEREST: None

FUNDING SOURCES: None

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