Original Article

Role of Garlic (Allium Sativum) on Lead Induced Delayed Eruption of Incisors in Albino Wistar Rats

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ABSTRACT

Objective: This study planned to evaluate the therapeutic effect of Allium sativum on lead induced delayed eruption of incisors of albino Wistar rats.

Study Design:

Place and Duration of Study: This study was conducted at the Post Graduate Medical Institute, Lahore. Study duration was one year from March 2019 to March 2020.

Materials and Methods: 68 adult albino Wistar rats were divided randomly into four groups (n=17) i.e., control, lead acetate, lead acetate with garlic and garlic alone. Right mandibular incisors were marked 1mm above the level of gingival papillae. The incisors were cut above this mark. The readings were measured by digital Vernier caliper. Incisors length was measured at day 0, 3, 6, 12 and 15 and eruption was calculated. The data was analyzed using SPSS 22.

Results: Eruption of incisors in albino Wistar rats in control was 3.30±0.72mm, in lead 2.43±1.19mm, lead + garlic 3.25±0.71mm and garlic 3.13±.91mm. At day 15, difference between Lead and lead with garlic was statistically significant (p-value 0.049).

Conclusion: The results showed that excessive lead intoxication is also a causative factor of delayed tooth eruption. The use of Allium sativum in routine diet and medicinal formulation could be helpful in patients or residents of lead polluted areas.

Key Words: Garlic, Allium Sativum, Delayed Eruption, Incisors, Albino Wistar Rat.

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INTRODUCTION

The eruption of teeth is a biologic process that has been of considerable interest to humans since early times. Every tooth has a specific time to erupt in the oral cavity. But sometimes deviation is seen clinically in eruption time[1]. The cases of delayed eruption are commonly seen in practice and generally parents are more worried and concerned about it[2,3]. 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[4].

Lead is one of the oldest and toxic heavy metals. It is common environmental and industrial pollutants that affect almost all biological systems [5]. Common sources of lead poisoning are car battery industries, manufacturing of ceramics, lead bearing paints, contaminated food, water and environment[6,7]. Lead can enter body mainly via eating, drinking or inhalation and transport to different tissues like kidney, liver, brain and bones[8]. Lead can pass through blood-brain barrier and placenta barrier[9].

Lead toxicity affects the dental and oral tissues. The presences of lead can interfere with development of enamel called amelogenesis[10]. Lead is found in saliva in children who have excessive lead exposure, which can affect their oral and physical health[11]. Mineralization is delayed during exposure to lead. This lack of mineralization is compensated by relatively longer duration of maturation, reflected in slow eruption[10]. Lead exposure to pregnant rats results in delayed teeth eruption and enamel development of their offspring's. Lead effect on delayed tooth eruption has been proved in hypo-functional incisors of Wistar rats[12].

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Albino Wistar rats are monophyodont mammals. Incisors of albino Wistar rats are open-rooted, which means apical end of root never closes and grow throughout their life with average 1mm per day[13], that can be increased by trimming their incisal edges to make them hypo-functional.

There are many drugs or naturally occurring herbs which can be used as antidote against these heavy metal intoxications. Allium sativum has an important dietary and medicinal role. Garlic has anti-viral, anti-bacterial, antifungal, antioxidant, anti-atherosclerotic and anti-cancer properties[14]. It has been shown to reduce lead toxicity and tissue lead contents in lead exposed humans[15]. Rats suffering from lead toxicity were treated with garlic and vitamin c complex and it was found that they have curative and protective effect[15]. The therapeutic effects of Allium sativum extract on lead toxicity has been proved and published in literature[16, 17]. It contains many chelators which eliminate lead from the body[18] that decreases the accumulation of lead in bone and other tissues of the body due to its antioxidant and chelating ability[18]. However, there is no data available regarding its role in lead induced delayed eruption of teeth.

Keeping in mind the therapeutic and beneficial effects of Allium sativum in lead toxicity on different tissues of the body, this study was designed to investigate the therapeutic role of garlic on lead induced delayed eruption of incisors in albino Wistar rats.

MATERIALS AND METHODS

This study was conducted at Post Graduate Medical Institute, Lahore. Study duration was one year. Adult healthy male albino Wistar rats (n=68) were included in the study. They were equally divided into four groups (n=17).

Sampling Technique: Experimental animals were assigned numbers 1,2,3,4, till 68 using the random number generator and then randomly divided into four groups (n=17) and were assigned as group A, B, C and D. All the rats in each group were housed in an airy cage. Group A was control group, group B was lead group, group C was lead with garlic and group D was garlic group.

Selection of Experimental Animals: Adult healthy male albino Wistar rats were selected from Animal House at Post Graduate Medical Institute, Lahore. The animals 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 experimental animals were fed on rodent chow and distilled water ad libitum.

Preparation of Drug (1500 ppm leaded water): For the preparation of 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 [19, 20].

Selection of Plant Material: Fresh bulb of Allium sativum were collected from the Botanical Garden of The Punjab University Lahore and the samples were verified by its Department of Botany.

Preparation of garlic juice: Fresh Allium sativum bulbs were collected, cloves were peeled, washed with distilled water and dried under shed. The clean bulbs were crushed with an electric grinder and the extract was decanted through muslin cloth[19.] . The fresh extract was made daily from 700-800g garlic.

Procedure: The experimental rats were housed in a climate-controlled environment in accordance with the international principles for the use of laboratory animals. Following acclimatization for one week, procedure was started. Animals were randomly assigned a group (n=17). Group A rats were given diet with rat chow and water. In Group-B, rats received leaded water (1500ppm). In Group-C, rats received leaded water and fresh garlic juice (1ml / 100g body weight) by gavages once a day [19] and group-D received just garlic juice.

All the rats were weighed on digital weighing machine and noted. The rats were anaesthetized with intraperitoneal ketamine injection (100 mg/kg body weight). Right mandibular incisors were marked 1.0 mm above the level of gingival papilla by rotary diamond bur TF-12 EF to make a reference point. Rest of the incisor above that reference mark was cut off by the diamond bur to make it hypo-function [12].

The readings were taken between upper boarder of gingival papillae and the marked reference point on right mandibular central incisors and were considered as day 0 [12].

After three days the distance was measured between the marked reference point and the upper margin of gingival papillae by digital Vernier caliper. This was considered second reading at day 3. The actual eruption was measured by subtracting the 1st reading from the 2nd reading on day 3. In the same way readings were taken on day 6, 9, 12 and 15 and eruption rate was calculated respectively.

Blood Lead Level: The blood samples of 0.5 ml were taken from cardiac puncture on day 0, 3, 6, 9, 12, 15. Blood Lead Count (BLC) was determined by atomic absorption spectroscopy with perkin-Elmer HGA (Heat Graphite atomizer)[21,12] in the department of environmental sciences, University of veterinary and animal sciences, Lahore.

RESULTS

In this study 68 rats were studied, among them 17 were studied as control group, 17 were studied as experimental group 1(leaded group) and 17 were studied as experimental group 2 (lead and garlic group) and 17 rats were studied in experimental group 3 (garlic group).

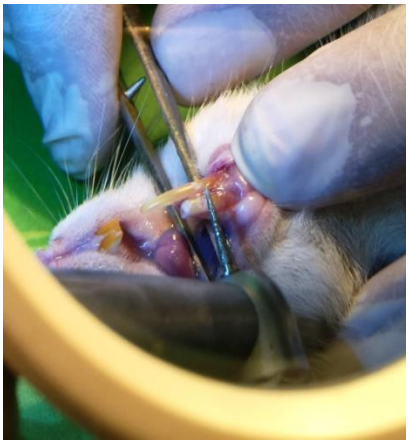

Figure No.1: Marking the reference point and cutting right mandibular

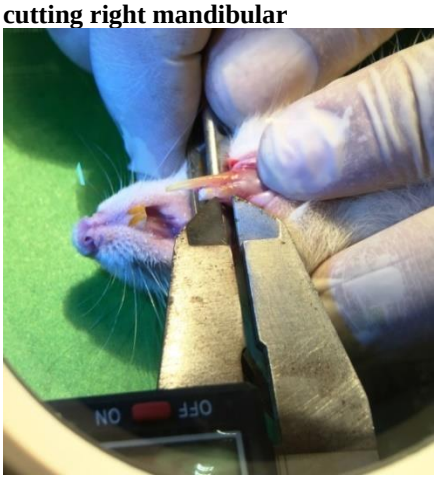

Figure No.2: Measuring between gingival papillae and reference point

Table No.1: Comparison of average difference in eruption (mm) among groups

	Control	Lead	Lead + Garlic	Garlic	p-value
Day 3	3.17±1.43	3.22±1.55	3.30±1.24	3.29±1.48	0.992
Day 6	2.94±1.45	3.01±1.52	2.85±1.27	3.03±1.24	0.981
Day 9	3.23±1.0	2.73±1.14	3.14±1.45	3.11±1.35	0.661
Day 12	3.15±1.33	2.57±1.55	3.03±1.12	3.24±1.06	0.436
Day 15	3.30±0.72	2.43±1.19	3.25±0.71	3.13±.91	0.022*

One way ANOVA, *P-value significant at 0.05

At day 15 as compared to day 12, there was statistically significant difference between mean eruptions of different groups (p-value 0.022).

Table No.2: Comparison of eruption (mm) between leaded and lead + garlic group

	Lead	Lead + Garlic	p-value
Day 3	3.22±1.55	3.30±1.24	0.998
Day 6	3.01±1.52	2.85±1.27	0.986
Day 9	2.73±1.14	3.14±1.45	0.773
Day 12	2.57±1.55	3.03±1.12	0.204
Day 15	2.43±1.19	3.25±0.71	0.049*

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

No statistically significant difference was observed between lead and lead & garlic group at different time of study except at day 15 (p-value 0.049).

Figure No.3: Showing the refrence point mark level after 3 days

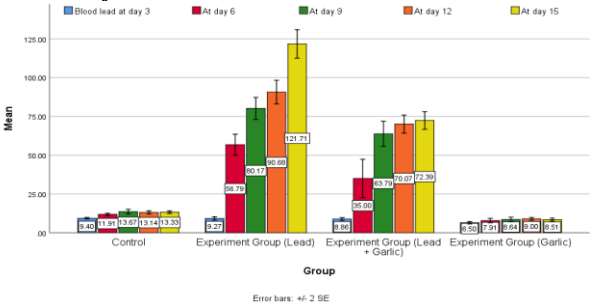

Figure No.4: Graphic comparison of BLC among different groups.

DISCUSSION

This study was conducted to determine the therapeutic effect of Allium sativum on the delayed eruption of incisors in albino Wistar rats due to lead toxicity. The role of Lead toxicity has been proved and documented in literature [12]. Multiple studied have been conducted to see the therapeutic effect of garlic on heavy metal toxicity in different tissues, but no study was available that explained the role of garlic on delayed eruption

Gerlach et al. documented the role of lead toxicity on delayed tooth eruption in albino Wistar rats [12]. Methodology of both studies was different; however results of both studies proved delayed effect of lead toxicity on eruption (Table 1). The protective

management was incorporated in current study to avoid trauma to the pulp and gingiva, which has not been observed in the previous studies [12, 22].

Sadeghi et al. studied the therapeutic role of garlic to reduce lead toxicity in hippocampus of albino rats [23]. In both studies it was proved that garlic reduces the blood lead count (BLC), which showed the significant effect of Allium sativum in lead induced toxicity.

Bideskan et al. studied the effect of garlic in lead exposed pregnant rats [24]. In both studies Same dose of lead and garlic was used and comparable reduction in BLC was noticed by administration of garlic juice. Minor variation was seen that might be due to difference in weight of animals.

Saleh et al. conducted a study to see the protective effect of allium sativum on lead exposed pregnant rats. In lead group BLC was elevated but in lead with garlic group, it was reduced. The same beneficial effect was seen in the current study [25].

Mumtaz et al., documented the beneficial effects of garlic in lead induced toxicity in various organs of animal as well as human model. He reported that lead toxicity caused harmful effects in reproductive organs, kidney, CNS, liver, lungs, blood and bone. The garlic reversed these harmful effects by decreasing lead absorption in bones and soft tissues [15].

The associations between blood and lead were statistically significant. In the current study, the blood lead concentration was used as an important indicator of lead intoxication and its effect on tooth eruption. The figure-4 shows significant information regarding increased blood lead concentration in lead group but this intoxication was tremendously decreased in the lead with garlic group due to the neutralizing effect of garlic.

From this study, it has been found that garlic has therapeutic effect on lead induced detoxification and thus is effective in reducing delayed eruption of teeth. Excessive dose of garlic has its own adverse effects [26]. However, more studies are required to evaluate the detoxifying effect of garlic in lead induced delayed eruption of teeth.

CONCLUSION

Lead is an established environmental pollutant which effects tooth eruption and should be considered along with other local factors. However the use of garlic in routine diet should be suggested to such patients or residents of lead polluted localities so that toxic effects of lead could be neutralized. Community based human trials can be very much beneficial.

Author's Contribution:

Concept & Design of Study:	Rashid Javaid
Drafting:	Daud Anthoney, Maruf Christopher
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Revisiting Critically:	Rashid Javaid, Daud Anthoney
Final Approval of version:	Rashid Javaid

Conflict of Interest: The study has no conflict of interest to declare by any author.

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