ABSTRACT: Lead is heavy metal, found in water, air, paints, food etc. Lead poisoning affects the entire human body particularly the nervous system, gastrointestinal tract, and the blood-forming tissues. The present study aims to see effects of lead acetate on developing chick embryo. Fertilized eggs were procured for the study. The experiment was divided into four groups, with two eggs in each group having different concentrations of lead acetate i.e. 12.5µg to 100µg. The results clearly showed that as the concentration of lead acetate increases, the normal growth of embryo gets disrupted and leads to abnormal chick embryo development especially at concentration of 100µg, the development of embryo was totally deformed.

KEYWORDS: Lead acetate, chick, development, fertilized eggs.

INTRODUCTION AND REVIEW OF LITERATURE:

Sources of lead are many. Lead is a transition metal that is mainly found in drinking water, dust, air and even food, fruits and vegetables through soil. Lead acetate (Pb(CH$_3$COO)$_2$), also used as sweetener, can cause serious health problem. Lead acetate is used in many hair products. Consumption and use of such products can cause problems like stomach cramps, nausea, nervous system damage and irritation on the skin. Lead interferes with genes that affect the pathways of cell signaling during neural or nervous system development, such as Pax transcription factors and FGF-8, a paracrine factor. These factors are critically important in establishing the boundaries of the fore-brain, mid-brain as well as hind-brain (Gilbert, 2000). Anwer et al. (1988) worked on effects of heavy metals on the development of chick embryo wherein fertilized eggs were injected with heavy metals and then the embryos were taken out from the eggshell on the twenty-first day post incubation. They found the chicks to be deformed in beak, legs, and body. Moreover, the incidents of hydrocephalus, microphthalmia, and anophthalmia, also have been found.
Researchers have performed assessment of risk for behavioral and physical effects of lead on adult birds (Burger, 1995). Studies also have shown that there is a relationship between lead exposure and body weight of embryos. They demonstrated that increasing levels of lead caused a decrease in the body weight of chick embryos. Burger in 1995 also found many deformities in birds in preliminary studies. Our objective was to see the effect of lead on overall development of chick embryo. The significant difference in brain weight of barn swallows exposed to lead compared to those not exposed to lead. The effect of sodium selenite and lead nitrate and on damaging the DNA has also been seen earlier (Das et al. 1995). The deleterious effect of lead on neural system was quite a lot (Huo et al., 2013). For humans, neurological and cognitive behavioral defects have been seen due to lead exposure (Needleman et al. 1990, Huo et al.; 2013). It has been seen that the children with lead poisoning often seen to have severe brain damage that leads to blindness, deafness, coma, convulsions and then death. This is because of the fact that lead is very easily absorbed in blood and affects various systems (Bergeson, 2008). The chick can be easily and successfully used in the experiments to determine the level of lead exposure which leads to severe damage and defects (Wani et al.; 2015).)

Lead acetate (\(\text{Pb(CH}_3\text{COO)}_2\)), probably first sweetener which has been used since long. The aim of present study was to observe the development of chick embryos, injected with different concentrations of lead acetate. Main objective was to see overall development of chick by physical or microscopic examination.

MATERIALS AND METHODS

**Procurement of Eggs:** Fertilized eggs were obtained from Department of Animal Husbandary, fisheries Central Poultry Development, Industrial area, Chandigarh.

- Chemicals and other materials like :–lead acetate, sodium chloride, distilled water, microscopes, petri dish, autoclavable bottles, needles, test tubes were arranged.
- An egg incubator set at 37°C.

**Experimental Design:** Two-day old chick embryos *i.e.* 48 hour-old fertilized eggs were used for the experiment. The experiment was divided into five groups, with two eggs in each group. One group was injected with autoclaved distilled water only and it served as control or standard group. The chick embryos of other four groups were injected with different concentrations of
lead acetate \( i.e. 12.5\text{µg} , 25\text{µg} , 50\text{µg} \) and 100µg. Various dilutions of lead acetate were filtered and sterilized before injecting.

Fig.1: Lead acetate (different dilutions)

The injected chick embryos were sealed with paraffin wax and then incubated at 37°C for two days. After incubation, the chick embryos were four-days old. The embryos were taken out by breaking the egg shell gently by window preparation method and the embryos were observed under a dissecting microscope. We staged the embryos and observed for any morphological changes.

Fig.2: 48 hour–old chick embryos (fertilized eggs) injected with different concentrations of lead acetate and ready for further incubation.

RESULTS AND DISCUSSIONS

Standardization of concentrations of lead acetate: we injected four different concentration of lead acetate in our experiments \( i.e. \) from concentrations of lead acetate (12.5 µg) to high concentration of lead acetate (100µg).
Fig. 3 (A) Embryo with distilled water.  
Fig. 3 (B) Embryo with 12.5µg of Lead acetate.

Fig. 3 (C) Embryo with 25µg of Lead acetate.  
Fig. 3 (D) Embryo with 50µg of Lead acetate.

Fig. 3 (E) Embryo with 100µg of Lead acetate.
Figure 3 clearly shows the morphological changes due to lead acetate injection. Figure 3 (A) injected with distilled water shows normal development of chick embryo, whereas Figure 3(B),3(C),3(D) & 3(E) are chick embryos injected with increasing concentrations of lead acetate (12.5 µg to 100µg). It is clearly visible from Figure 3(E) that at 100µg concentration, the embryo completely died with totally disrupted morphology. Whereas, the chick embryo with 50 µg concentration of lead acetate Figure 3(D) shows severe underdevelopment of brain with undistinguishable regions, when compared with normal growth i.e. chick embryo injected with distilled water showing three distinct regions, i.e. forebrain, midbrain and hindbrain regions of brain clearly. The chick embryo injected with 25µg of lead acetate Figure 3(c) showed swelling in the brain region whereas figure 3(B) with embryo having 12.5µg of lead acetate shows blood pooling in the vertebral and tail region. So it was visible that when the chick embryo was injected with higher concentrations of lead acetate i.e. 50µg &100µg, embryos of both the groups died. We concluded that an injection of 50µg of lead or more at an early embryonic age is lethal.

The present study shows that when the two days old chick embryo is exposed to the heavy metal like lead, the normal growth of embryo gets disrupted and leads to abnormal chick embryo development like changes in morphology of brain, blood pooling and total death of embryo at concentration of 50 and 100µg.

This type of study on lead exposure can be performed on other organisms too in the embryonic stage. This study can be very helpful in drawing parallel lines to human lead poisoning, i.e. up to what concentration lead is safe to consume.

CONCLUSION

Major sources of Lead acetate are drinking water, dust, air. It is also seen in fruits and vegetables, where it comes through soil. Lead acetate (Pb(CH₃COO)₂), which is also used as sweeteners. The study clearly shows that the lead which is considered to be a heavy metal, if enters body through water intake or other means up to 25 micrograms did not show any considerable effect on the development of chick but little more amount has adverse effect on chick development, hence concluded that the lead acetate can severely affect the development of chick embryo.
REFERENCES


