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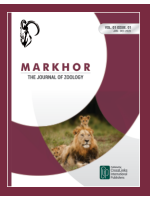

Original Article

Teratogenic Effects of Lead in the Developing Chick Embryos

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ABSTRACT

Lead (heavy metal) can be found in trace levels in the crust of the planet. It may be harmful to both human and animals' health. Nearly all body's organs and systems can be affected by lead and mainly found in smelting and mining areas. **Objective:** To estimate the effect of lead on chick embryos at morphologic, morphometric, and histological levels and to study the toxic effects of lead in developing chick embryos **Methods:** Fertilized eggs were separated into three groups. Two groups were treated with varying concentrations of lead as experimental groups, untreated designated as control group. The dose was administered on the fourth day of incubation, and recovery occurred on the ninth day. **Results:** Significant differences ($p<0.000$) and ($p<0.001$) in CR length, body weight, head size, eye circumference, forelimb and hindlimb were reported. Morphological abnormalities such as hydrocephaly, microcephaly, beak shortening, agenesis, Amelia, micromelia, anophthalmia, microphthalmia, and kyphosis were seen. It also revealed various abnormalities in important organs such as irregular cerebral folia, necrotic intestine, and hemorrhages in bursa fabricious. Lead has been shown in various combinations to cause embryotoxicity and teratological effects in chick embryos. **Conclusions:** Lead is a harmful pollutant and may be responsible for various developmental anomalies in livings beings including animals and humans.

INTRODUCTION

Heavy metals are elements with an out-sized relative atomic mass and are considered basic toxins that have been linked to a variety of organ damage [1]. "Heavy metal" refers to metalloids that can cause toxicity in humans, animals, and the environment. Their accumulation in the food chain, persistence, and biomagnification qualities make their pollution exceedingly careful and represent a serious risk to humans. These are also well-known for their potentially harmful consequences that had carcinogenic and toxicological effects [2]. Lead acts as a teratogen and damage all body organs and systems [3]. It has low melting point [4]. It is silvery but when exposed to air the colour changed into dull gray [5]. The chemical is mainly found in smelting and mining areas [6]. Soil dust and sea salts are the main sources of lead [7]. The main thing that puts lead into the air is gasoline and industrial operations. The soil of urban areas has more concentration of lead in comparison to rural areas [8]. Lead levels in the environment have grown more than 1,000-fold in the last three centuries due to human activities. It usually enters in the environment by burning coal or trash and has very tiny fragments; they can travel a considerable distance once they enter the atmosphere. Rain and other things that fall to the ground or into bodies of water take lead out of the air. This metal is commonly found in baby meals, toys, jewelry, paints, batteries [9] cigarette smoke and whiskey [1]. Eating or

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drinking contaminated food might result in lead exposure [10]. Lead poisoning may be more common in older houses because of the increased risk of decay and deterioration [11]. Inhalation, ingestion, and skin contact are all examples of ways to be exposed [12]. In another study, poisoning by lead is responsible for 0.6% of the global sickness load, with poor nations bearing the brunt of it [13]. It damages neurons in the developing brain via two mechanisms: apoptosis and necrosis [14]. On MRI, the brains of adults who were associated with high levels of lead in children show a reduction in volume, particularly in the prefrontal cortex [15]. It also has a multitude of negative impacts on both men and women's reproductive systems [16]. Absorption, elimination, and dosage response may be affected by dietary deficits or excesses of certain important metals. At low levels, lead may replace calcium and change key neurotransmitters like protein kinase C, which regulates long-term brain excitement and memory storage [17]. The clinical signs and symptoms of lead poisoning are vague and may go unnoticed. Haematological, gastrointestinal, musculoskeletal, and endocrine problems are common in early stages, followed by more serious central and peripheral nervous system disturbances, as well as symptomatic gastrointestinal, musculoskeletal, hematological, and endocrine abnormalities [18]. Gout is a common complication of chronic lead nephropathy [19]. Vitamins (particularly B, C, and E), calcium and iron play an important and competitive role in avoiding lead poisoning symptoms. These vitamins can chelate lead from tissues while also balancing pro/antioxidant levels [16]. The administration of thiamine hydrochloride reduced lead levels in the liver and kidney [20]. Succimer [21] and dimercaprol is used to treat lead poisoning [22]. Lead also causes reductions in birds clutch and egg size, as well as embryo and nestling mortality, growth depression, and behavioral deficiencies that influence survival. Lead reduces migratory activity and makes animals more vulnerable to cold, hunters, and other predators [23]. Anemia, renal, and mental problems are among the characteristic symptoms of lead poisoning [24]. The current investigation was conducted on chick embryos to determine the embryotoxicity of lead.

METHODS

The experiment was carried out on *Gallus domesticus* fertilized eggs. The eggs were cleaned first with a piece of cotton dipped in alcohol. The eggs were divided into three groups A, B and C. Group A and B were treated with 0.1ml, 0.2ml solution per egg, respectively. Group C were marked as control group. On the fourth day of incubation, the prepared doses were injected into the eggs. A small hole or window was delicately drilled into the eggshell so that the membrane would not be harmed. Using the syringe, 0.1ml of each dose concentration was administered into the yolk sac. Liquid paraffin wax was used to close the opening in the egg and incubate at set temperature 37 ± 0.5^{0}C. At the 9th day of incubation, the embryos were recovered and cracked in a water basin. The embryos were carefully removed from the yolk in an enamel dish. Embryo were separated from the albumin and yolk and rapidly transferred to a vial containing Bouin's fixative. After 48 hours of fixation, it was stored in 70% alcohol. The morphometry study included the evaluation of crown rump length the weighing of body, the diameter of head and eyes, and the size of the limbs. Histological analysis of brain and intestine slides were investigated by using hematoxylin and eosin staining. Graph pad prism software was used to perform one-way Anova test for statistical analysis.

RESULTS

There were two experimental groups i.e., Control group and Treatment group. Control group was not given any treatment whereas experimental groups were treated with lead (0.1ml /Egg), (0.2 ml/Egg). Recovered embryos were observed and the number of malformed, unfertilized, and reabsorbed embryos were observed on the whole examination of embryos (Table 1).

Table 1: Different Lead concentrations have teratogenic effects on 9-day-old chick embryos

Dosage groups (ml/eggs)	eggs treated	embryos recovered	Unfertilized	Malformed%
Control (C)	30	27	3	0.00
0.1ml/egg	30	22	8	48
0.2ml/egg	30	25	5	62.7

Morphometric examination and data analysis indicate significance difference among the embryos of experimental and treatment group. Parameters such as body weight, crown rump lengths, head and eye circumference along with length of forelimb and hind limb decreases with the increase of dose when compared to the control. The significance comparison of the concentration with the control is given in table 2, figures 2 and 3 at ($p< 0.001$).

Table 2: Developing chick embryos treated with various lead concentrations showed differences in morphological features at 9th day

Parameters	Control	Treatment 1 (0.1 ml/egg)	Treatment 2 (0.2 ml/egg)
	Mean ± SD	Mean ± SD	Mean ± SD
C.R Length (mm)	13.70±1.1431	8.55±0.526	7.68±0.3355
Body Weight (mg)	44.18±17.299	12.40±2.6609	2.01±1.2179
Head circumference	5.88±0.4992	3.90±0.6471	2.76±0.1333
Eye Circumference	2.65±0.253	1.28±0.1555	1.15±0.1688
Forelimb	3.15±0.2646	1.39±0.106	1.18±0.577
Hind limb	2.77±0.3988	1.23±0.119	1.06±0.275

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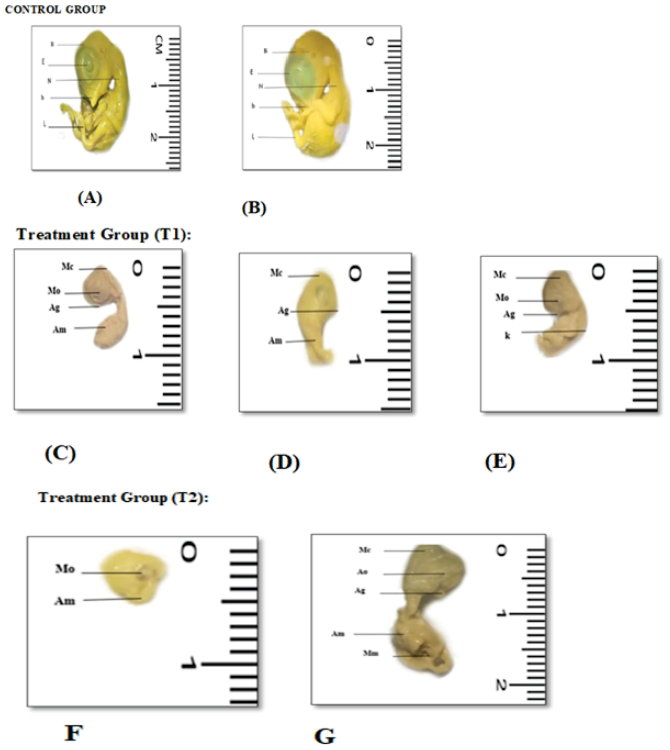

Figure 1: A and B Control group macrograph of a 9-day-old chick embryo showing normal growth: Brain (B), Eye (E), Beak (b), Limb (L) and Neck (N), C, D, E represents 0.1 mg/egg dose group showing abnormal development Microcephaly (Mc), Microphthalmia (Mo), Amelia (Am) and Agenesis (Ag), F, G represents 0.2 mg/egg dose group showing abnormal development Microphthalmia (Mo) and Amelia (Am)

Histological analysis shows lead treated groups have necrosis in intestinal segment, hemorrhages in bursa fabricius along with degenerated internal and external granular layer of cerebellum in figures 2 and 3.

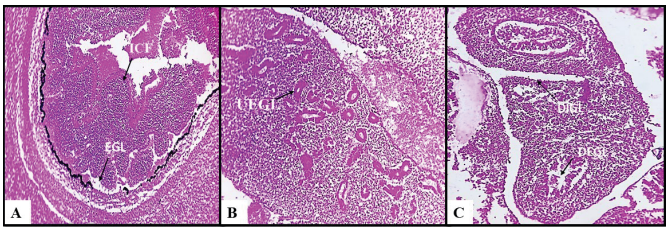

Figure 2: Photomicrographs of histological sections through the developing cerebellum control group fig A showing normal development of ICF: Irregular cerebral folia, EGL: External granular layer, fig B shows lead treated group at 0.1 dose missing external granular layer. 0.2 dose at Fig C Shows Degenerated internal granular layer, Degenerated external granular layer at 10

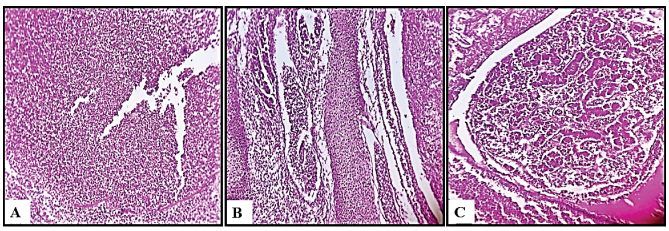

Figure 3: Photomicrographs of histological study showing normal intestine of control group fig A. fig B 0.1 dose showing coccidia macro meronts (CM) and intestinal segment found to have coagulative necrotic (CN) enteritis. fig C 0.2 dose showing haemorrhages in bursa of fabricius.

DISCUSSION

Lead toxicity is now one of the most serious health risks, owing primarily to pollution. The current study sought to assess the embryotoxic and teratogenic effects of lead in chick embryos. According to morphometric analysis significant reductions were made in CR length, weight, and circumference of the skull ($p<0.001$) in the current study. These deformities are supported by the study of 25. da Costa *et al.*, [25] which reported that even modest levels of Pb exposure increased mortality and the prevalence of abnormalities during fetal development, particularly in the cephalic region (CR). SH Gilani, (1973) results support the finding of our results that shows reduced eye circumference and significant reduction in body weight along with Micromelia, Amelia, Microphthalmia and anophthalmia were also common in treated chick embryos [26]. According to Catizone & Gray and Gilani reports cranioschisis, sinuous or ruptured brain, and shortening of the neck observed may be due to the action of lead on an enzyme that directs early brain and cervical spine development, our results indicate at same day lead can cause spine reduction and deform nervous system [26, 27]. De Gennaro, 1978 reported that ultrastructure of the spinal cord reveals that neuroglial astrocytes in the proximity of blood vessels are changed [28]. Vascuolation and disruption of the cell's endoplasmic reticulum were prominent morphological abnormalities. These findings match with the recent studies in which congenital spinal deformities were also observed. Somite growth is retarded in a specific way when exposed to lead. Embryos that grow in contact with lead are smaller than controls with the same number of somites. The leaded embryo not only takes longer to reach normal somite count, but it also fails to reach the typical proportions for the somite stage of development when it does. The same results were seen in recent studies, somites were not formed at the embryonic stage of somitogenesis and the growth of somite's was delay [29]. Lead injected during organogenesis, particularly in situations when there is a calcium deficiency can impede implantation of the embryo, delay its growth during the latter stages of pregnancy, and result is deformities [24]. Our results showed high rate of mortality with genetic deformities in chick embryos. McClain & Becker reported that injection into rats during late pregnancy caused CNS haemorrhage and hydrocephalus, as it did in the chick embryo; these findings are consistent with the histological analysis of the current investigation, which revealed that embryos from groups that were

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exposed to various quantities of lead during treatment showed hydrocephaly and microcephaly [30]. Lead cause exencephaly and spina bifida at the start of central nervous system development in the mouse Kruckenberg whereas in present study hydrocephaly and microcephaly was observed in chick [31]. Evidence is mounting suggesting the loss of spine synapses may be indicative of juvenile learning. In domestic chicks, spine loss affects filial imprinting [32]. These results can be matched with the histological analysis of the present study in which neurochemical changes in CNS were seen in the embryo. Neurodevelopmental defects were also observed.

CONCLUSIONS

Lead was found to be teratogenic in developing chicks in this investigation. On morphological, morphometric, and histological levels, the embryotoxic effects were documented. Several anomalies were discovered throughout the morphological and morphometric analyses. Similarly, histopathological findings revealed that numerous Organs of the chick embryo were rudimentary and malformed. Mostly its effects on the Central nervous system of the brain and induces neurodevelopmental toxicity.

Authors Contribution

Conceptualization: MKAK
Methodology: AS, M, MFB, YF, AH
Formal analysis: MAT, SP
Writing-review and editing: AH, MAT

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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