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Toxicity of copper sulfate ($CUSO_4$) on early development of chick embryo

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Abstract
Copper sulfate ($CuSO_4$) is been extensively used in agricultural, industrial, domestic and technological sectors. It is a potentially toxic element causing environmental pollution which poses hazardous effects on the survival of animals. Present article delas with the effect of various concentrations (0.5 mg/ml, 1.0 mg/ml, and 1.5 mg/ml) of Copper sulfate on early embryonic development of Chick embryo (*Gallus domesticus*). Copper sulfate ($CuSO_4$) found to impair the process of organogenesis in chick embryo resulting in underdevelopment of vital organs like brain, heart, limb and wing buds. The severity of impairment is high with increasing concentration of copper sulfate. It indicates the risk of interference with essential developmental processes due to excessive exposure to heavy metal pollutants like copper sulfate.

Keywords: Chicken, copper sulfate, embryonic development, heavy metals

Introduction

Heavy metals are naturally occurring metallic elements which are extensively used in various sectors such as industries, agriculture, domestic, technology and are prevalent in the environment (Tchounwou *et al*., 2012) [22]. Heavy metals such as cadmium (Cd), copper (Cu), and lead (Pb), mercury (Hg), silver (Ag), arsenic (As), zinc (Zn), iron (Fe), etc. are tremendously released in the environment through many natural and anthropogenic activities. They contaminate the soil, groundwater, surface water, food resulting in significant exposure risks for wildlife, particularly avian species (Szabó *et al*., 2024) [21]. Heavy metals, in low concentration are crucial for metabolic processes but same in high concentration are hazardous. Such potentially toxic elements enter the body of birds through oral route or absorbed from surrounding environment and bioaccumulated in various tissues due to continuous exposure to heavy metals (Aljohani, 2023) [3]. Jinsong *et al*., (2006) [11] reported the amount of trace copper, lead, cadmium and iron in environmental and biological samples. Copper (Cu) is one of the trace elements needed by body which is involved in enzymatic activities, mitochondrial respiration, and antioxidant defense mechanisms (Gaetke & Chow, 2003) [8]. However, when in excess, it causes toxic effect, disturb cellular homeostasis resulting in oxidative stress. Copper sulfate ($CuSO_4$) is one of the most common causes of copper contamination in the environment (Wu *et al*., 2008) [29]. Copper sulfate serves as fungicide, herbicide and pesticide. Its blend with lime known as Bordeaux mixture is used to control fungus growth on grapes, melons, and other berries (Richardson 1997; Conway and Pretty 2013; Wightwick *et al*., 2013) [18, 6, 28]. Survival of many birds, aquatic organisms and animals is under the risk due to harmful pesticides (Mahmood *et al*. 2016) [15]. Many workers reported the teratogenic effect of copper sulfate ($CuSO_4$) on embryonic development in various species (Tavakkoli *et al*., 2015; Scott *et al*., 2016; Tendulkar and Kamble, 2020; Arafat *et al*., 2020; Unigwe *et al*., 2022) [10, 1, 24, 4, 25]. Chick embryos have been extensively used to assess the toxicity of various environmental pollutants, including heavy metals such as cadmium, lead, and copper (Surai, 2002) [20] because of their easy availability, convenient egg size, developmental and physiological similarities with reptiles, mammals and even man. Previous investigation suggest that heavy metals can cross the embryonic membranes and interfere with cell proliferation, differentiation, and organogenesis (Yuan *et al*., 2011) [30]. Studies on toxic effect of $CuSO_4$ on embryonic development demonstrated adverse impact such as, growth retardation, morphological abnormalities, and increased embryonic mortality (Tchounwou *et al*., 2008) [23]. In this study attempts are made to evaluate the effects of different concentrations of $CuSO_4$ on early chick embryo development by assessing morphological changes, survival rates, and potential teratogenic effects. Understanding the embryotoxic potential of $CuSO_4$ can provide insights into environmental risks posed by copper pollution and its implications for both ecological and human health.

Materials and methods

Fertilised eggs of *Gallus domesticus* (White Leghorn Strain) of 48 hr. stage were obtained from Venketeshwara Hatcheries Pvt. Ltd. Eggs were washed with distilled water and wiped with 70% ethanol. For experimentation, Avian saline solution (0.75% of sodium chloride) was used to prepare different concentrations of copper sulfate ($CuSO_4$) solution (0.5 mg/ml, 1mg/ml, and 1.5mg/ml). A small hole was drilled on the blunt side of the egg with the help of a pointed needle to inject copper sulfate ($CuSO_4$) solution with the help of a micro-injection. Each group of 12 eggs were treated with 0.5 mg/ml, 1mg/ml, and 1.5mg/ml copper sulfate ($CuSO_4$) solution and designated as experimental groups. The hole was then sealed with the help of a cello tape. The eggs were rotated side to side for uniform spreading of $CuSO_4$ solution over the airspace membrane. One of the groups of same number of eggs was untreated and maintained as a control. Both, treated and untreated eggs were kept in the BOD incubator at 37.5 °C with a relative humidity of 70 – 80% for 24 hrs. Eggs were periodically observed throughout the incubation period to monitor embryonic development and ensure the well-being of the embryos. Any abnormalities or irregularities observed during the incubation period were recorded for analysis. After incubation, eggs with 72 hrs. of development from control and experimental groups were cleaned with a disinfectant solution to remove any external contaminants. The eggshell was gently cracked open, keeping the underlying membranes or the embryo intact. The embryo was located and gently separated from the surrounding tissues using sterile forceps and scissors. Care was taken to avoid damaging the embryo during extraction. Once extracted, the embryo was transferred into a sterile avian solution. Any excess tissues or debris surrounding the embryo were carefully removed using sterile forceps and scissors. Throughout the process, sterile techniques were strictly followed to prevent contamination and maintain the integrity of the samples. The embryo then mounted on a slide and studied microscopically for further morphological analysis.

Results and discussion

After 72 hrs incubation, embryo from control group (Photo plate 1) showed normal growth with significant development such as leftward torsion of body with 90 degrees to rest left side on the yolk, cranial and cervical flexures in the head region, distinct enlargement of telencephalon, formation of extraembryonic membranes and yolk sac, hind limb and fore limb buds appeared, well developed optic cup, 'S' shaped heart, 35 pairs of somites, amnion covering the embryo completely. In experimental groups (Photo plate 2 - 4) an increase in the concentration of copper sulfate was associated with a decrease in somitogenesis, although no neural tube defects were identified. Nevertheless, notable alterations in brain development were observed compared to that in embryos from control group. Conversely, embryos subjected to 0.5 mg/ml and 1 mg/ml of $CuSO_4$ (Photo plate 2 & 3) demonstrated a reduction in the size of brain regions [metencephalon (hindbrain), mesencephalon (midbrain), diencephalon, and telencephalon (forebrain)], while those exposed to 1.5 mg/ml (Photo plate 4) exhibited significantly retarded or underdeveloped brain structures. Furthermore, embryos treated with 1 mg/ml of $CuSO_4$ (Photo plate 3) showed impairment in the formation of wing and leg buds with their diminished sizes, whereas those exposed to 1.5 mg/ml (Photo plate 4) displayed markedly underdeveloped wing and leg buds. Exposure to copper sulfate resulted in to impaired looping of heart tube and a progressive decrease in heart size with increasing concentrations of copper sulfate, suggesting a dose-dependent detrimental effect on embryonic growth and organ development.

The developing embryos of birds, such as chickens, are susceptible to pollutants like heavy metals, which can disrupt normal developmental processes. Copper has fungicide and bactericide properties and hence used in agriculture for over a century (La Torre *et al*. 2018) [14]. Earlier developmental stages showed greater susceptibility to copper toxicity, supporting the critical window hypothesis proposed by Hamburger and Hamilton (1951). Gamakaranage (2018) [9] states that, "toxicity of copper sulphate depends on its significant entity, bioaccumulation and bioreactivity with doses of ingestion." Embryos in the eggs are vulnerable, because these chemicals can infiltrate through the porous eggshell and increase the mortality or developmental abnormalities of embryos (Kertész 2001) [12]. Developmental abnormalities included neural tube defects, limb malformations, and compromised vascular development, increasing in both frequency and severity with higher CuSO4 concentrations, consistent with the dose-response relationship (Chen and Wong 2019) [5].

Copper profoundly bioaccumulates in the tissues (Aaseth and Norseth 1986) [2] and can activate the synthesis of free radicals leading to impairment of proteins and DNA (Ogórek *et al*. 2017) [16]. The embryotoxicity of CuSO4 possibly results from oxidative stress, with copper ions generating reactive oxygen species (ROS) that damage cellular components essential for morphogenesis (Valko *et al*., 2016) [26]. Tendulkar and Kambale (2020) [24] reported copper sulfate contamination and bio accumulation in the embryological tissues of experimental model *Gallus gallus* and observed prominent alterations in vascularisation and angiogenesis of developing embryo. Copper sulfate induce various pathologies across different organ systems during developmental stages. Oğuz and Enli (2014) [17] assessed morphological and biochemical parameters in term Hubbard broiler chicks exposed to toxic levels of copper sulfate exhibiting significant reduction in mean brain volume in the copper-treated group (8079 μm^3) compared to the control group (10075 μm^3). The embryotoxic potential of copper sulfate is dose dependent and may cause developmental anomalies (Várnagy and Budai 1995) [27]. These findings have environmental implications regarding copper contamination from agricultural and industrial sources (EPA, 2023).

The results obtained from present investigation work indicate significant dose-dependent effects of $CuSO_4$ on various developmental parameters, including cardiac and brain development, somite count, and neural tube formation, limb and wing buds formation during the critical organogenesis period at 48 hours of incubation. The results revealed that higher concentrations of $CuSO_4$, particularly at 1.5 mg/ml, resulted in considerable developmental impairments, such as underdevelopment of the brain, and heart, angiogenesis, limb and wing buds, and somitogenesis. Although no neural tube defects were identified, the significant impact on brain development suggests that excessive copper exposure during embryonic development can interfere with essential developmental processes.

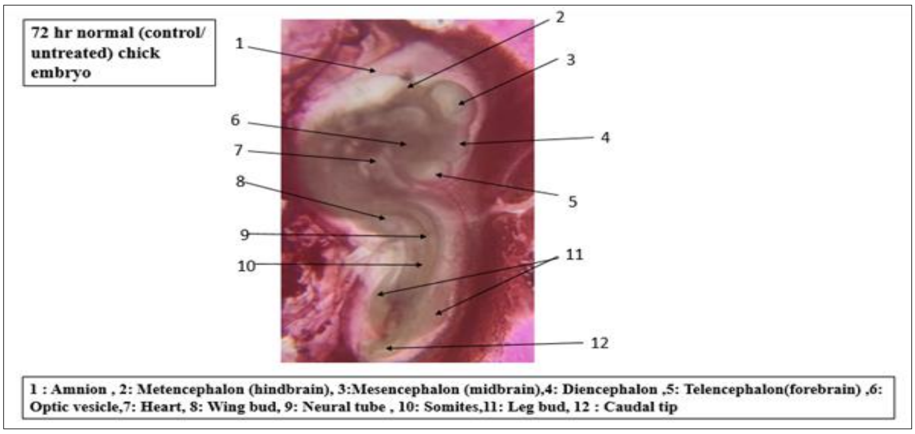

Fig 1: 72 hrs Normal (Cantrol/ untreated) chick embryo

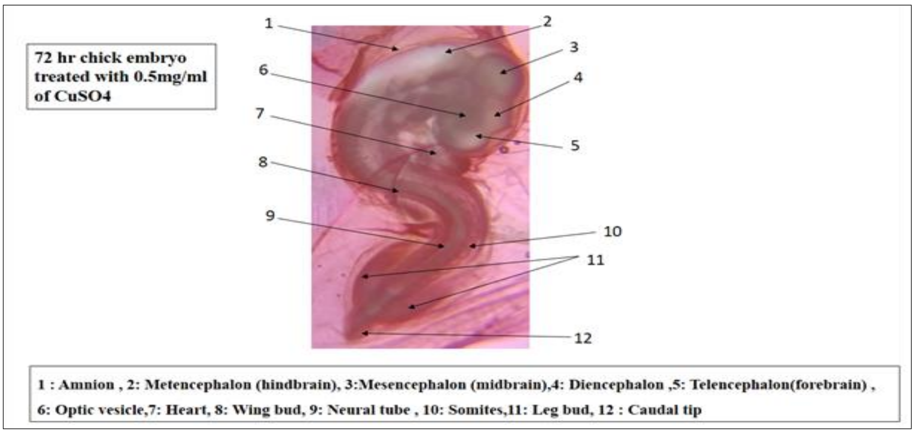

Fig 2: 72 hrs chick embryo treated with 0.5 mg/ml concentration of $CuSO_4$

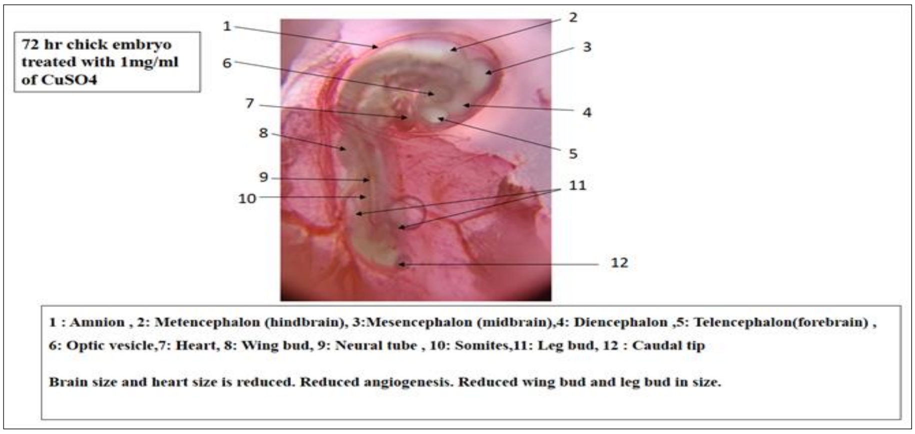

Fig 3: 72 hrs Chick embryo treated with 1 mg/ml concentration of $CuSO_4$

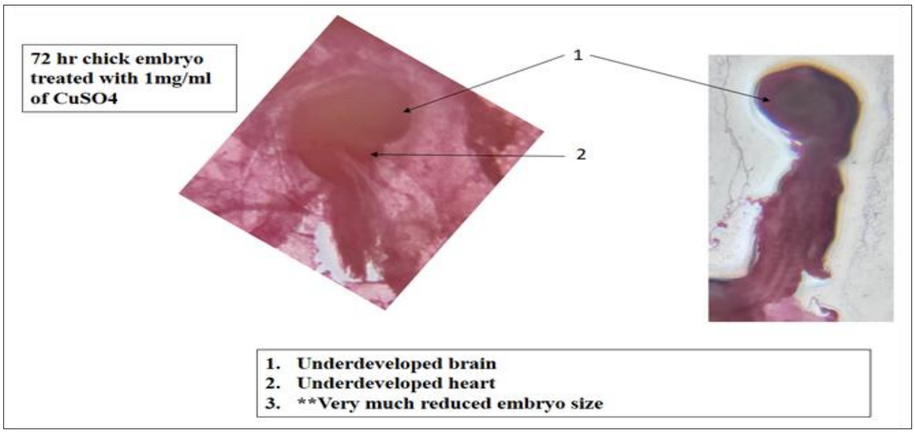

Fig 4: 72 hrs Chick embryo treated with 1.5 mg/ml concentration of $CuSO_4$

Conclusion
The results obtained in this investigation work pinpointed threats associated with heavy metal pollution, particularly of copper and underlined the obligation for environmental regulation to minimize the exposure during sensitive developmental stages. Illustration of dose dependent effects of $CuSO_4$ offers understanding about the mechanism developmental toxicity and probable long-term consequences for wildlife and human health. the results further emphasize the need for strong control measures for environmental pollution to alleviate hazards caused due to pollutants and their potential to disrupt normal developmental processes.

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