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Teratogenic effect of silver nitrate ($AgNO_3$) on chick embryo development

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Abstract
Study was conducted to examine the teratogenic effect of silver nitrate ($AgNO_3$) on chick embryo, *Gallus gallus domesticus*. Chick embryos of 48 hrs age were treated with different concentrations (0.5mg/ml, 1mg/ml and 1.5mg/ml) of $AgNO_3$ and examined after 72 hr of age. Results showed noticeable developmental abnormalities in the embryos exposed to 1.5 mg/ml concentration of $AgNO_3$ such as improper somitogenesis, reduced size of brain and heart with retarded brain structure. Wing buds and limb buds were lacking. No remarkable defects in embryos exposed to 0.5 mg/ml concentration of $AgNO_3$. Treatment with 1.0 mg/ml concentration of $AgNO_3$ showed little decrease in the size of brain with no development of wing and limb buds. The severity of defects in embryo was more with increasing concentration of $AgNO_3$, demonstrating that silver nitrate has teratogenic effect on chick embryos, potentially disrupting normal process of organogenesis.

Keywords: Chick embryo. heavy metals, silver nitrate, teratogenic effect

Introduction

Heavy metals are the metallic elements that exhibit toxicity, naturally occurring in the soil because of paedogenetic processes, which include weathering of parent materials, and from anthropogenic sources, including traffic emissions, industrial processes, vehicular emission, and the combustion of coal and other fuels. Rising level of heavy metals in the body of living systems can be harmful due to its disruptive effect on metabolism of vital organs and glands (Timothy & Tagui Williams, 2019) [12]. Gilani and Alibhai (1990) [5] studied teratogenicity of metals, cadmium, arsenic, cobalt, copper, indium, iron, manganese, and molybdenum to chick embryos and found malformation in different organs of the body.

Certain metals are toxic to embryonic and foetal tissues and they can induce teratogenicity in mammals (Domingo 1994) [2]. Yamamoto *et al*., (2012) [4] examined effects of cadmium on embryos of *Gallus gallus* after the treatment of $CdCl_2$ to eggs and reported critical morphological abnormalities. Amini *et al*., (2019) [15] studies effect of lead on the development of somites in chick embryos and concluded that lead has disturbing effects in the development of somites in chick embryo which can result in serious mortal threat to the life. Silver (Ag) is one of the heavy metals widely used in industries such as photography, imaging, electronics, and electrical applications. Aquatic and terrestrial organisms are exposed to silver when it is released in the environment through industrial discharge.

The toxicity of silver varies significantly depending on its form. Ionic silver (Ag^+) is toxic to various aquatic organisms, but other forms of silver are considerably less toxic (Urcell & Eters, 1998) [13]. In the studies on embryos of zebrafish, $AgNO_3$ induces toxicity showing deposition of Ag^+ on the embryonic chorion (Mosselhy 2016) [9]. Hussein and Singh (2016) [10] studied effect of neonicotinoid insecticide imidacloprid on development of chick embryo and reported developmental defects such as, growth retardation, limb defects, head enlargement, ectopia viscerale and decreased weight. In view of all these embryotoxicity of various toxicants and to take the study further, present investigation work was undertaken to assess the toxicological effects of $AgNO_3$ on early developmental stages of chick embryo, *Gallus gallus domesticus*.

Material and Methods

Fertilized eggs of White Leghorn chickens (*Gallus gallus domesticus*) incubated for 48 hrs were procured from the hatcheries centre. Eggs were washed with distilled water and 70 % ethanol and divided into control and experimental groups. Solutions of AgNO3 of different concentrations *viz*., 0.5mg/ml, 1mg/ml and 1.5mg/ml were prepared. Experimental group was further divided into three subgroups designated as E1, E2, and E3 containing equal number of eggs. Eggs from E1, E2, and E3 groups were microinjected in their air sacs with 0.5 mg/ml, 1mg/ml and 1.5 mg/ml solutions of AgNO3 respectively. The hole was then sealed with the cello tape. The control group of eggs was left untreated. All the eggs were then incubated for 24 hr at 37.5°C with 70-80% humidity. Subsequently, the 72-hour-old embryos were used for morphological analysis. Eggs were monitored periodically during incubation period for any abnormality or irregularity. After 72 hrs of incubation, eggs from both control and experimental groups were used for mounting of embryos on a slide. Embryos then fixed with 70 % alcohol and stained with alcoholic eosin and observed under light microscope for further morphological analysis.

Results and discussion

The embryos from control group (Photo plate 1) showed typical normal development at 72 hrs age such as somitogenesis, proper development of amnion, neural tube, wing buds, limb buds, optic vesicle, and caudal tip. The heart and the brain with all its regions *viz*. diencephalon, forebrain, midbrain, and hindbrain exhibited normal developmental patterns. After exposure to 0.5 mg/ml concentration of $AgNO_3$ (Photo plate 2), no remarkable effects were observed but shrinkage in different regions of the brain. 1.0 mg/ml $AgNO_3$ treatment (Photo plate 3) revealed normal development of optic vesicle, heart, notochord, somites, and caudal tip. No neural tube defect was found. The brain size was little reduced along with lack of wing buds and limb bud's development. Embryos exposed to 1.5 mg/ml concentration of $AgNO_3$ (Photo plate 4) displayed noticeably undeveloped and retarded brain structures with marked diminution in the size of both the brain and the heart. Somitogenesis was found to be abnormal. The embryo size was highly reduced, no development of wing buds and limb buds was noticed. These results point to a dose-dependent detrimental impact of silver nitrate on the development of organs and embryonic growth.

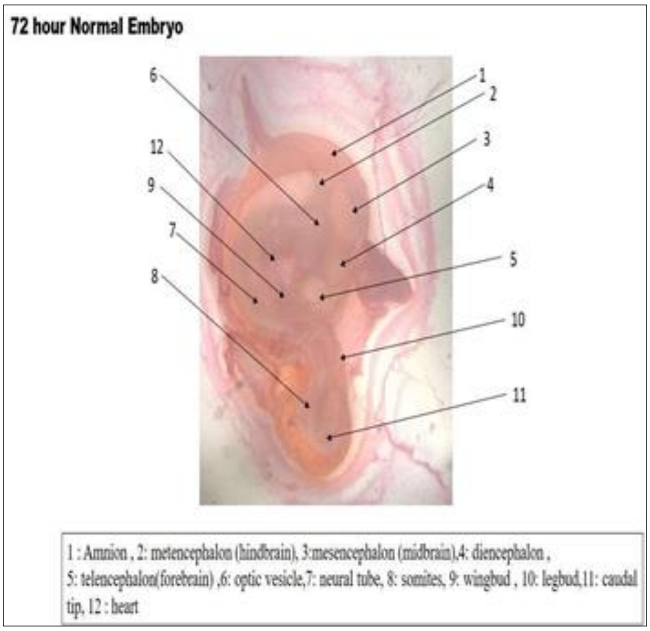

Fig 1: 72hr Normal Chick Embryo

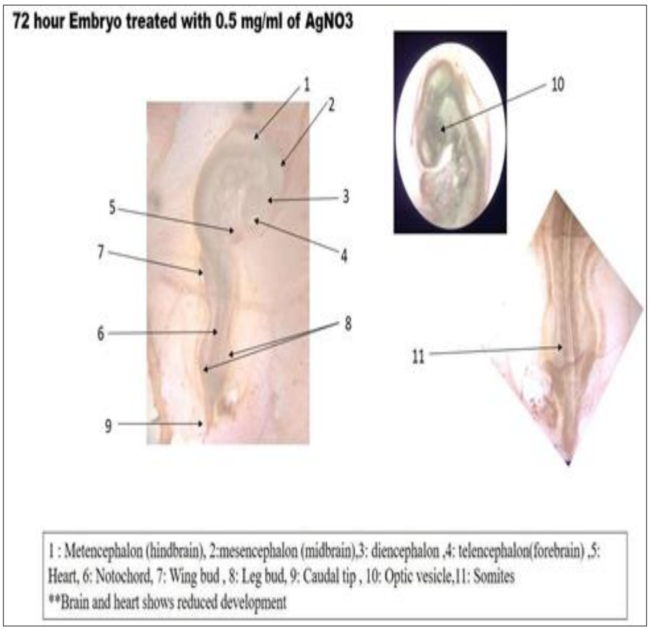

Fig 2: 72 hr chick embryo treated with 0.5 mg/ml concentration of $AgNO_3$

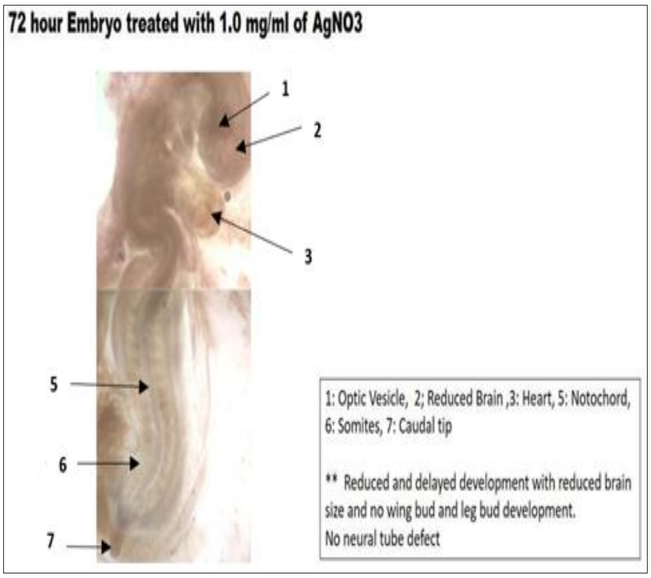

Fig 3: 72 hr chick embryo treated with 1.0 mg/ml concentration of $AgNO_3$

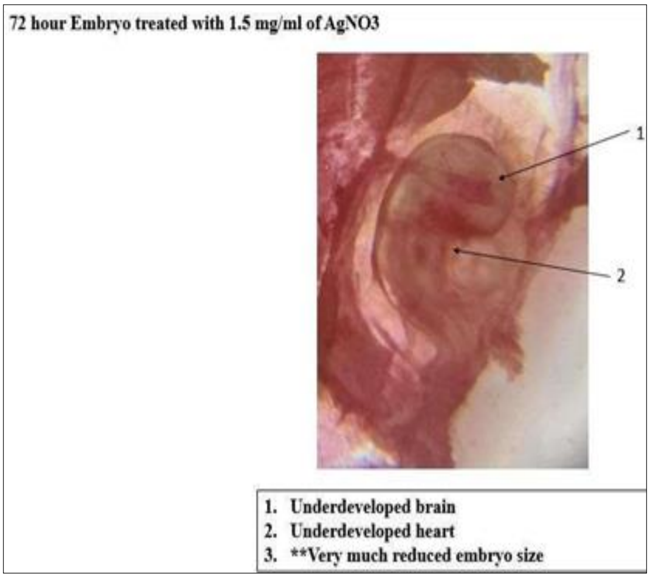

Fig 4: 72 hr chick embryo treated with 1.5 mg/ml concentration of

On exposure to LD50 values of heavy metals, deformities like reduced body size, micromelia, twisted neck, hemorrhage, everted viscera, and microphthalmia were reported in chick embryo indicating that the heavy metals are toxic and teratogenic to chick embryogenesis (Gilani and Alibhai 1990) [5]. Similar results were observed by Gilani and Marano (1980) [6] in chick embryo after exposure to Nickel chloride. Yamamoto *et al*., (2012) [4] reported powerful teratogenic effect of cadmium on chick embryo in the form of abnormalities in their cephalic region with no development of critical structures after exposure to the highest concentration. High concentration of lead acetate resulted into reduction in the size of nucleus and synthesis of collagen, inhibition of the formation of cartilage matrix in somite in chick embryo (Amini *et al*., 2019). Copper sulfate (CuSO4) impair the process of organogenesis in chick embryo leading to underdevelopment of crucial parts like heart, brain, limb and wing buds and the intensity of impact is dose dependent, directing to the possible threats to developmental processes (Kanchan and Pramod, 2025) [7].

Silver nanoparticles (AgNPs) cause developmental impairments in varied biological models and their (AgNPs) toxic effects are determined by their particle size, charge, and surface chemistry (Wang 2024) [14]. On the contrary, Sawosz (2009) [3] reported that there is no negative effect of nanoparticles on survival, growth, development and morphology of 48 h and 20 days old chicken embryos. Johnson *et al*., (2022) [8], Tanguay (2022) [11] stated that the toxic effects of Ag^+ on the development of embryo differ remarkably from those of Ag NPs and are moderate than that of silver nanoparticles. Toxicities of silver ions in developing embryos are concentration and time dependent. Studies on zebra fish embryo exposed to Ag^+ concentrations as low as 0.05 µM resulted in decrease in normal development and an increase in mortality and developmental abnormalities, including fin fold defects, tail and spinal cord malformations, and eye and head deformities. These effects found to be more prominent with increasing dose concentration and the duration of exposure (Johnson *et al*., 2022) [8]. All such studies give insights into the embryotoxicity of silver compounds on diverse embryos and highlight the necessity of caring towards form and concentration of silver compounds in developmental research. However, more detailed and extensive research work in this subject need to be carried out further.

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