A Teratological Study of Faulty Neural Tube Closure and Neuro-Protection in Gallus gallus domestics

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***Abstract*—The most severe congenital abnormalities are known as neural tube defects (NTDs), which are characterized by defective neural tube closure during early development. The present study investigates the embryo-toxic effect of serotonin and cadmium chloride and the ability of folic acid and inositol to exert a neuroprotective effect in an in vivo model based on fertilized chick embryos *(Gallus gallus domesticus).* Fourteen eggs were assigned to graded serotonin exposure of 10, 25, and 50 µL, fixed cadmium chloride of 10 µL, and also co-treated with folic acid at 50 µL or inositol at 100 µL. Embryos were incubated for 48–72 hours, harvested, and examined for survival and gross structural changes. The outcome indicated that serotonin and cadmium chloride induced dose-dependent developmental abnormalities, comprising notches in the neural tube, notochord-brain separation, and spina bifida-like development. Combined exposure was embryonic lethal. Folic acid was able to prevent damage in lower dose groups but not with high serotonin. Inositol was not protective and all the inositol-treated embryos failed to develop. The results illustrate the dose sensitivity of embryonic tissues to teratogens and show the preeminent importance of folic acid. Inositol might need co-factors or combination therapy to be effective.**

This study provides experimental evidence for prenatal screening, medically controlled use of drugs, and nutritional supplementations during pregnancy.

***Index Terms*—Anencephaly, Cadmium chloride (heavy metal), Chick embryo model *(Gallus gallus domesticus)*, Embryo-toxicity, Encephalocele, Folic acid, Inositol, Meningocele, Meningomyelocele, Myelocele, Neural tube defects (NTDs), Neurodevelopment, Neuroprotection, Serotonin, Spina Bifida Cystica, and Teratology,**

I. INTRODUCTION

Embryogenesis represents highly regulated and evolutionarily conserved series of changes by which a single fertilized ovum develops into a coherent and organized organism with functional systems. The early stages of embryonic development (e.g. gastrulation, germ layer formation, and neurulation) lay down the first body plan. Specifically, neurulation is the extension and closure of the neural tube, which is an essential characteristic of vertebrate development that ultimately gives rise to the vertebrate central nervous system. In humans, formation of the neural tube commences in the third week of gestation, with closure complete by the end of the fourth week. Neurulation is highly accurate; even very small perturbations during this period can lead to developmental neural tube defects (NTDs), which result in spina bifida, anencephaly, and/or encephalocele, and the defects have the potential to cause lifelong disability and/or death [1].

While some NTDs are linked with genetic syndromes, many are non-syndromic, and due to a multifactorial combination of genetic susceptibility and environmental factors, such as nutrient deficiencies, maternal illness, chemical exposures, or teratogenic drugs. The ethical and legal constraints of human embryo research beyond 14 days post-fertilization means that reliable animal models are needed to study the early developmental processes of human embryos and teratogen effects. The chick embryo *(Gallus gallus domesticus)* has been a successful and powerful model for developmental biology for decades. It is especially suitable for neurulation research because of extra-uterine development, rapid growth, predictable staging of development (Hamburger-Hamilton), and similarity to human embryology. In the chick, neural tube closure occurs in 48–72 hours of incubation, and during this time, the neural plate, neural folds, and neural groove can readily be visualized. In addition, the advantages of accessibility, lower costs, and limited ethical concerns have made the chick embryo a well-established model for examining teratogen effects and neuroprotective interventions to ameliorate perturbations to early neurodevelopment [2]-[4].

In this case, two teratogenic agents, serotonin and cadmium chloride, were selected for study. While serotonin is a critical neurotransmitter in neurodevelopment, higher levels, typically from endogenous or drug-related psychosocial behaviors, have been linked to teratogenicity. For example, congenital anomalies such as exencephaly and hydrocephalus [5]-[7]. Cadmium chloride, a toxicant in the environment, can lead to developmental toxicity by means of oxidative stress, mitochondrial damage, inhibition of DNA repair, and epigenetic adaptations, the neurodevelopment exposure from cadmium chloride has been connected to abnormal neurulation and, growth restriction [8]. Although serotonin and cadmium chloride may have opposing mechanisms of toxicity, it is possible the combination will influence neural tube closure in additive or synergistic outcomes associated with the exposure between the two teratogenic agents.

Two neuroprotective agents, folic acid and inositol, were included in the study to investigate potential neuroprotective strategies. Folic acid is most well recognized as an essential coenzyme in one-carbon metabolism associated with protection against NTDs, yet it is documented that approximately 30% of NTDs occur post-folic supplementation (category of NTDs) indicate the need for additional treatments. Inositol, a sugar alcohol involved in phospholipid metabolism and is involved in intracellular signaling showed promise in reducing the incidence of NTDs in folate resistant models, but such mechanisms are still under investigation [10],[11].

This study will use the chick embryo model to (1) investigate the individual and combined teratogenic effect of serotonin and cadmium chloride on neural tube closure, and (2) evaluate the neuroprotective effect of folic acid and inositol in the presence of teratogens. This approach acknowledges developmentally relevant insults are multifactorial, which simulation may be useful in preventative strategy development for NTDs in higher risk pregnancies.

II. MATERIALS AND METHODS

✓ Materials Used

a. Fertilized Eggs: 12-hour-old, *Gallus gallus domesticus* (White Leghorn), uniform size and age
b. Chemicals & Reagents: Serotonin Hydrochloride (5-HT), Cadmium Chloride, Folic Acid, Myo-inositol, Formaldehyde, Eosin & Hematoxylin, 70%, 80%, 90% Alcohol, Ethanol (for sterilization), Distilled Water
c. Equipment & Tools: Manual Egg Incubator (humidity control), Candling Lamp, 1 mL Sterile Syringe (fine-gauge needle), Forceps, Scissors, Scalpel, Stereozoom Microscope, Disposable Gloves

✓ Experimental Design

a. Divide 14 fertilized eggs into treatment groups

✓ Egg Preparation

a. Wipe gently with 70% ethanol
b. Label according to treatment group

✓ Candling & Windowing

a. Locate air cell by candling
b. Create a small opening at the blunt end using sterile needle or forceps

✓ Injection

a. Draw reagent into sterile syringe
b. Inject slowly into air sac (avoid disturbing embryo)
c. Seal opening with sterile tape

✓ Incubation

a. Place eggs horizontally in incubator
b. Maintain 35–37 °C and 60–70% humidity
c. Incubate for 48–72 hours

✓ Embryo Retrieval

a. Remove shell near air cell using sterile forceps
b. Separate embryo from yolk, place in glass slide with saline
c. Fix embryo in formaldehyde (~1 minute)
d. Stain with eosin & hematoxylin (15–20 minutes)
e. Wash through graded alcohol series (70–90%)

✓ Observation under Stereozoom Microscope

a. Check neural tube closure
b. Assess brain and eye formation (development, symmetry)
c. Count somites and check symmetry
d. Examine mesoderm separation, heart tube

structure, gross deformities, and survival

Table II.I: Treatment groups with dosage details of serotonin, cadmium chloride, folic acid, and inositol used in Fertilized chicken eggs.

Group	Treatment		
1	Control		
	Teratogen	Heavy Metal	Supplements
2	serotonin 10 µL	-	-
3	-	cadmium chloride 10 µL	-
4	serotonin 10 µL	cadmium chloride 10 µL	-
5	serotonin 10 µL	cadmium chloride 10 µL	Folic acid 50 µL
6	serotonin 25 µL	-	-
7	serotonin 25 µL	cadmium chloride 10 µL	-
8	serotonin 25 µL	cadmium chloride 10 µL	Folic acid 50 µL
9	serotonin 50 µL	-	-
10	serotonin 50 µL	cadmium chloride 10 µL	-
11	serotonin 50 µL	cadmium chloride 10 µL	Folic acid 50 µL
12	serotonin 10 µL	cadmium chloride 10 µL	Inositol 100 µL
13	serotonin 25 µL	cadmium chloride 10 µL	Inositol 100 µL
14	serotonin 50 µL	cadmium chloride 10 µL	Inositol 100 µL

Note- cadmium, chloride concentration was kept constant(10µL) due to its height toxicity. Folic acid(50µL) was used based on its standard supplement dose. Inositol was tested as an exploratory agent.

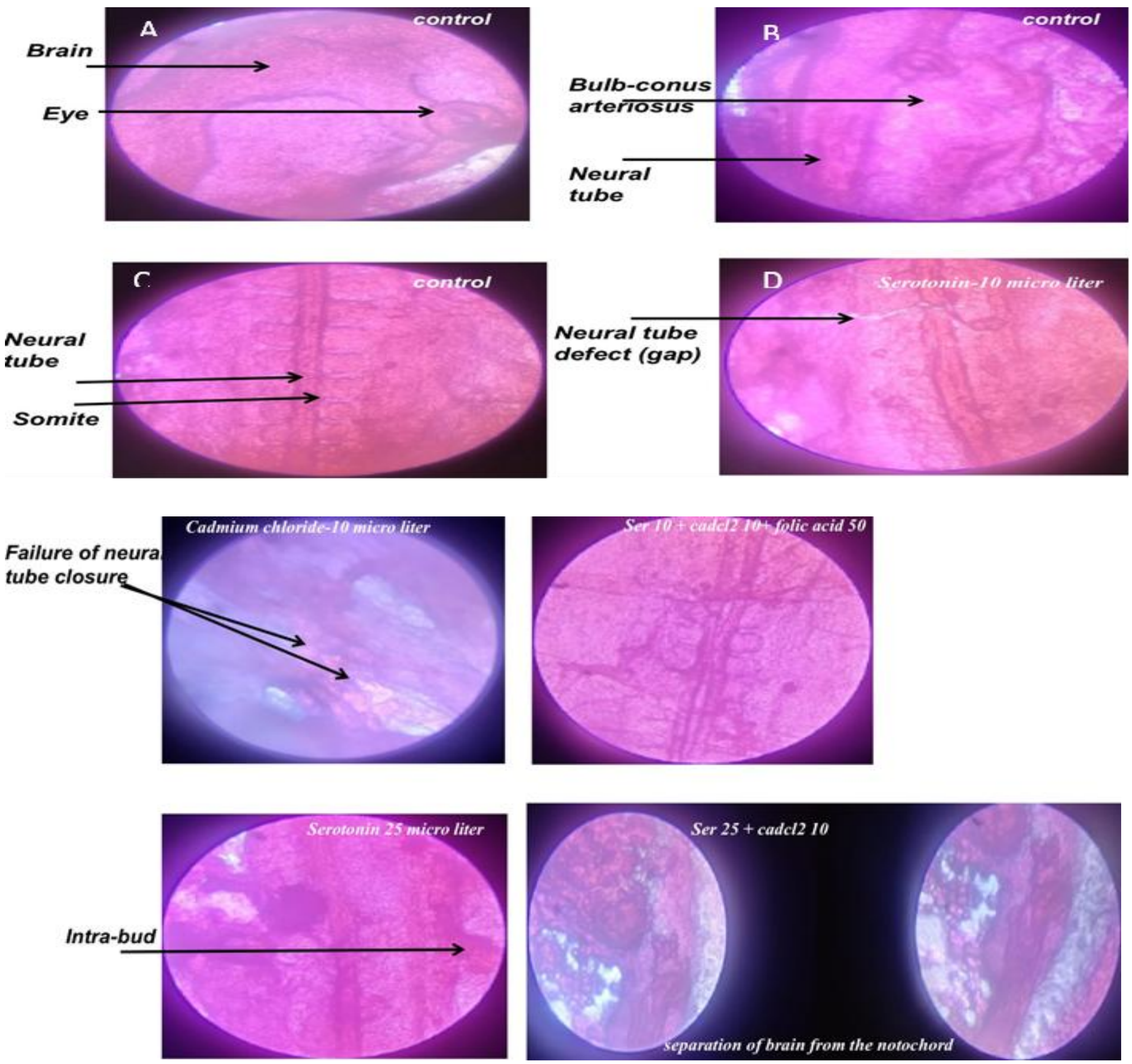
III. RESULTS AND DISCUSSION

14 fertilized *Gallus gallus domesticus* eggs were treated with various treatments consisting of serotonin, cadmium chloride, folic acid, and inositol. Embryos were cultured and examined between 48–72 hours of development for structural effects, mainly on neural tube closure and early organogenesis.

Table III.I: Summary of teratological effects observed in *Gallus gallus domesticus* embryo

Group	Treatment			Results
1	Control			Normal development, no defects
	Teratogen	Heavy Metal	Supplements	
2	serotonin 10 µL	-	-	Neural tube gap
3	-	cadmium chloride 10 µL	-	Neural tube closure failure
4	serotonin 10 µL	cadmium chloride 10 µL	-	Embryo did not survive
5	serotonin 10 µL	cadmium chloride 10 µL	Folic acid 50 µL	partial rescue, minor defects
6	serotonin 25 µL	-	-	Intra-bud formation, disrupted vasculature
7	serotonin 25 µL	cadmium chloride 10 µL	-	Detachment between brain and notochord

8	serotonin 25 µL	cadmium chloride 10 µL	Folic acid 50 µL	Notochord preserved, mesodermal development inadequate
9	serotonin 50 µL	-	-	No development, rotten egg
10	serotonin 50 µL	cadmium chloride 10 µL	-	No development, rotten egg
11	serotonin 50 µL	cadmium chloride 10 µL	Folic acid 50 µL	No development, folic acid inefficient at high dose
12	serotonin 10 µL	cadmium chloride 10 µL	Inositol 100 µL	No development
13	serotonin 25 µL	cadmium chloride 10 µL	Inositol 100 µL	No development
14	serotonin 50 µL	cadmium chloride 10 µL	Inositol 100 µL	No development



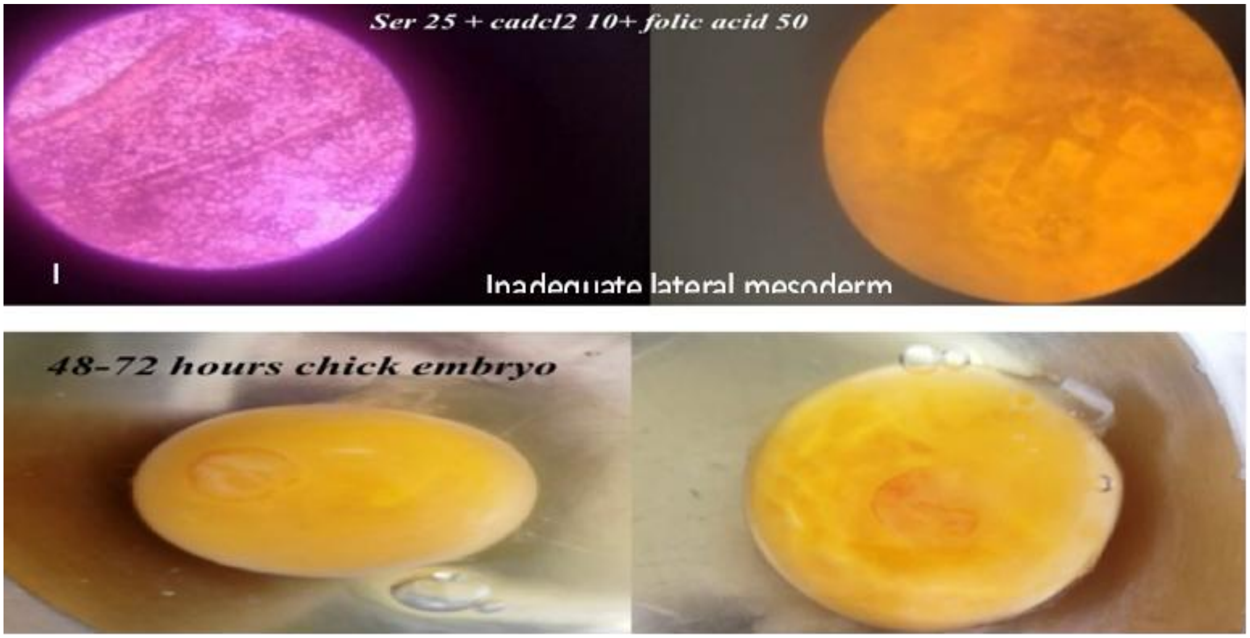


Fig 3.1: Morphological changes in chick embryos (48–72 h incubation) after serotonin, cadmium chloride, and folic acid treatment. Control embryos (A–C) developed normally with normally formed brain, eye, and intact neural tube, and with well-segmented somites and bulb-conus arteriosus visible. (D) Serotonin treatment (10 µL), and not (A–C), produced a focal disruption in the neural tube, which signifies failure of closure at this location. (E) Cadmium chloride (10 µL) produced gross neurulationmabnormalities, including failure of neural tube closure. (F) Folic acid treatment (50 µL) with a combination of serotonin (10 µL) and cadmium chloride (10 µL) partially restored the midline continuity, indicating folic acid's protective role against toxin- induced malformation. (G) Increased concentration of serotonin (25 µL) perturbed axial organization, resulting in abnormally developed intra-bud. (H) Simultaneous treatment with high-dose serotonin (25 µL) and cadmium chloride (10 µL) caused over-separation of brain from notochord, which is a sign of general disruption in neural and axial patterning. (I) Supplementing the serotonin (25 µL) + cadmium chloride (10 µL) treatment with folic acid (50 µL) resulted in better neural alignment and fewer gross malformations.

The investigation evaluated the teratogenic impacts of serotonin and cadmium chloride on early embryogenesis in chicks, with a focus on neural tube closure. The study found clear serotonin dose-dependence toxicity, substantial independent cadmium chloride effect, and anticonvulsant impact for folic acid (with ambiguous impacts for inositol). The study demonstrated that serotonin induced mild neural tube defects with lower doses; however, cadmium chloride caused complete failure of neural tube closure. When serotonin and cadmium chloride were used together, only folic acid was shown to have significant preventative effects in some cases. It should be noted that while folic acid was used at a high dose, it did not completely block the teratogenic effects, as some embryos continued to display laterality mesoderm defects or partial 'rescue' from gastrulation. At the highest doses of serotonin and cadmium chloride folic had no meaningful ability to block embryo death, which suggests that the folic acid therapeutic window was dose mediated. Inositol, when given as a stand- alone supplement did not impact early embryonic viability or formative development. These findings bring attention to the fact that very small amounts of gestational exposure to some teratogens can cause some significant issues such as miscarriage; structural abnormalities/or learning disabilities at the fetal stage. This work supports the teratogenic effect of serotonin and cadmium chloride, protective effect of folic acid and that there is no potential for inositol to be an independent agent of preventative care for neural tube defects.

IV. FUTURE PROSPECT

Conduct gene expression experiments on neural tube markers such as Pax3 and Shh to confirm microscopic findings. Experiment with the combination of inositol and folic acid, to see if their action is of increased strength when combined. Increase the 72- hour incubation period to look at organogenesis for a longer duration. Discuss the therapeutic uses of some neuroprotective agents (e.g., antioxidants, zinc) for the treatment of chemical-induced birth defects. Disseminate information on the fetal health by making aware of unrecognized teratogens.

V. CONCLUSION

This study aimed to clarify the teratogenic effects of serotonin and cadmium chloride on the development of embryonic chicks. More specifically, we were interested to understand the adverse effects on the closure of the neural tube. We also considered the public health development factors of folic acid and inositol, in addition to the potential teratogenic interference of serotonin and cadmium chloride. The data clearly show that doses affect the degree of cadmium chloride poisoning interference on the outcomes – with greater doses resulting in more severe abnormalities and additional embryonic mortality. Interestingly, at a consistent and low level of cadmium chloride with no other confounding factors, there could still be successful interference and failure to close the neural tube. This could also have amplified the mortality rate associated with the deafness that the cadmium chloride saturated poisoning phenomenon posed for the serotonin exposure.

There was also only partial protection implicit with regard to folic acid and lower levels of serotonin concentrations. This new insight is entirely consistent with its intended prevention of neural tube defects - but it was not as effective when exposure to the teratogenic dose was at the highest degree. It would appear that inositol was not able to demonstrate ability as a neuroprotective agent on its own during the embryogenesis phase, as there was no hard evidence demonstrated in the experiments that it performed this function in any of the experimental groups.

In a general sense, the study attacks the sensitive biochemical mechanisms embedded in embryogenesis, reinforced the importance of proper nutrition and pharmaceutical safety during the early stages of pregnancy, as well as opened up a number of new studies in the area of co-therapy, dose thresholds, and mechanisms of neuroprotection.

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