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Effects of Sodium Nitrite on Neural Tube Development in Early-Stage Chick Embryos

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ABSTRACT

AIM: To investigate the effects of sodium nitrite on spinal cord development in early-stage chick embryos.

MATERIAL and METHODS: Sixty specific-pathogen-free fertile Leghorn-type eggs at Day 0 of incubation were used. Group 1 (control) received saline, Group 2 (low-dose) was administered 0.0042 mg of sodium nitrite, and Group 3 (high-dose) received 0.0084 mg. Embryonic disks were microscopically examined after 72 hours of incubation.

RESULTS: In the control group, the surface ectoderm was intact, the neural tube was properly closed, and the neuroepithelium, basement membrane, somites, and notochord exhibited normal morphology. Neural tube defects were identified in seven embryos in the low-dose group and nine embryos in the high-dose group. Statistical analysis revealed that both sodium nitrite–exposed groups exhibited a significantly higher incidence of neural tube defects compared with the control group ($p < 0.05$). However, no statistically significant difference was observed between the low- and high-dose groups.

CONCLUSION: Exposure to low and high doses of sodium nitrite induced neural tube defects in chick embryos, suggesting potential embryotoxic effects.

KEYWORDS: Chick embryo, Embryotoxicity, Neural tube defects, Sodium nitrite

INTRODUCTION

Congenital malformations such as meningocele, myelomeningocele, encephalocele, tethered cord, and diastematomyelia are frequently encountered in neurosurgical practice. Neural tube defects (NTDs), occurring in approximately 1 in 1,000 live births, arise from multifactorial etiologies, including environmental factors, chromosomal abnormalities, maternal folic acid deficiency, and maternal diabetes or elevated glycemic index levels (16). Disruptions at any stage of primary or secondary neurulation can result in these malformations. Additionally, maternal exposure to medications, environmental agents, and dietary additives has been implicated in congenital anomalies.

The clinical spectrum of NTDs ranges from anencephaly to sacral agenesis and is often associated with high morbidity and mortality (8,16). Infants with NTDs have a markedly increased risk of mortality before reaching 5 yr of age.

Sodium nitrite, a common additive in processed meat products, is used primarily as a coloring and antimicrobial agent. Exceeding the recommended maximum daily intake of 0.07 mg/kg/day (4,5) has been linked to carcinogenicity, hypertension, and adverse reproductive outcomes (6,13). Experimental studies also suggest that sodium nitrite may impair brain development and negatively affect learning and memory (9). Although previous research has explored its effects on the nervous system, no study to date has specifically investigated its impact on spinal cord development.

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MATERIAL and METHODS

Experimental Design

A total of 60 specific pathogen-free (SPF), fertile, zero-day Leghorn-type chicken eggs (average weight: 65 ± 5 g) were obtained from the Bornova İzmir Veterinary Experimental Animals Department. The eggs were randomly divided into three groups (n = 20 per group): control, low-dose sodium nitrite, and high-dose sodium nitrite.

Eggs were incubated for 24 hours at 37.8 ± 0.2°C and 60 ± 5% relative humidity, positioned with the pointed end downward. A Cimuka CT60 S incubator (Ankara, Türkiye) was used to ensure uniform temperature, humidity, and automated rotation every 90 minutes to facilitate optimal embryonic development.

Sodium Nitrite Administration

Sodium nitrite was administered at Hamburger–Hamilton (HH) Stage 6, corresponding to approximately 24 hours of incubation. Doses were calculated according to the European Food Safety Authority's recommended human daily limit of 0.07 mg/kg (4,5), using a proportional adjustment for chick embryo mass. Thus, the low-dose group received 0.0042 mg sodium nitrite, whereas the high-dose group received 0.0084 mg (double the low dose).

Group 1 (control) received 0.1 mL of saline. Group 2 received 0.0042 mg/0.1-mL sodium nitrite, and Group 3 received 0.0084-mg/0.1 mL sodium nitrite. A 5-mm hole was aseptically drilled in the eggshell near the blastoderm, and injections were delivered beneath the embryonic disk. The holes were sealed with sterile tape, and the eggs were re-incubated until 72 hours.

At HH Stage 20 at 72 hours, embryonic disks were excised and transferred to distilled water maintained at 35°C.

Histological Analysis

Embryos were fixed in 10% neutral buffered formalin at room temperature. Standard tissue-processing procedures were performed, including graded ethanol dehydration, xylene clearing, and paraffin embedding. Sections 4 µm thick were obtained using a rotary microtome (Leica RM2155, Germany) and mounted on glass slides.

Sections were stained with hematoxylin and eosin (H&E) to evaluate morphological alterations in the neural tube, notochord, and neuroepithelium. Images were captured using a light microscope (Olympus BX51, Tokyo, Japan) equipped with a charge-coupled device camera (Olympus DP 72, Tokyo, Japan).

Ethics Statement

In accordance with the European Directive 2010/63/EU, ethical approval was not required for experiments involving chick embryos.

Statistical Analysis

Data were analyzed using MATLAB R2022B (Statistics and Machine Learning Toolbox, Version 12.4). Fisher's exact test was applied to compare the incidence of NTDs among groups. A p value <0.05 was considered statistically significant.

RESULTS

Light microscopic analysis of transverse and sagittal sections from 20 chick embryos in the control group revealed clearly identifiable neural tube and notochord structures. The surface ectoderm was intact, the neural tube was appropriately closed for the developmental stage, and the neuroepithelium, basement membrane, somites, and notochord demonstrated normal morphology (Figure 1A).

In the low-dose sodium nitrite group (0.0042 mg), neural tube and notochord structures were visible in transverse, sagittal, and oblique planes. Three embryos exhibited NTDs characterized by distinct cervical openings (Figure 2A-D). Compared with the intact morphology of the 72-h control group, four embryos displayed various luminal irregularities (Figure 2E-H). In three embryos, the lumen was partially occluded due to neuroepithelial proliferation at the cervical level (Figure 2E-G), whereas one embryo showed abnormal lumen patency at the caudal level (Figure 2H). The remaining 13 embryos had intact neural tubes comparable to those in the control group (Figure 1B).

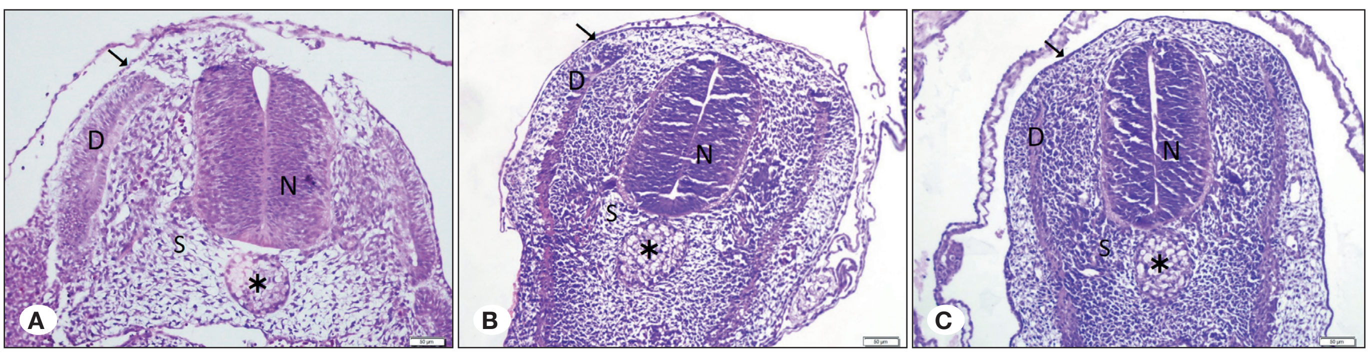

Figure 1: Hematoxylin and eosin–stained photomicrographs showing intact structures in the control **(A)**, low-dose (0.0042 mg; **B)**, and high-dose (0.0084 mg; **C)** groups. ↓: Surface ectoderm; N: Neural tube; ⇦: Notochord; D: Dermomyotome; S: Sclerotome. Scale bars = 50 µm.

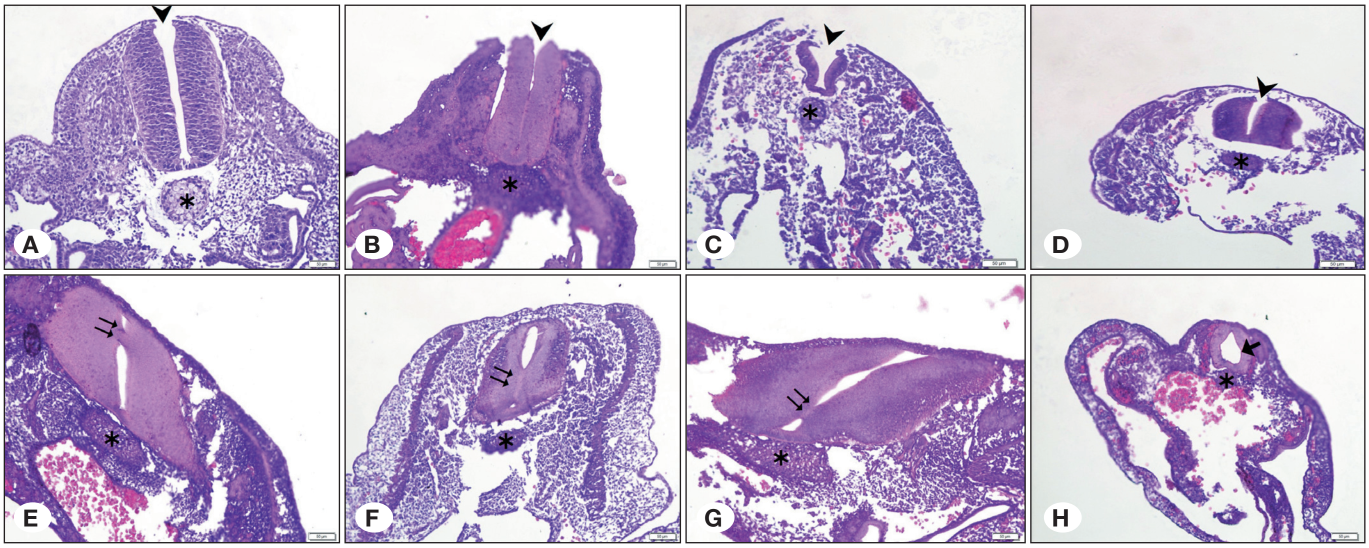

Figure 2: Representative photomicrographs of neural tube defects **(A–D)** and luminal anomalies **(E–H)** in the low-dose (0.0042 mg) group. One embryo with NTD exhibits cervical **(B)** and caudal **(C)** openings. **Arrowhead:** NTD; **Double arrow:** Neuroepithelial proliferation; **Arrow:** Abnormal luminal opening; **Asterisk:** Notochord. Hematoxylin and eosin staining. Scale bars = 50 µm.

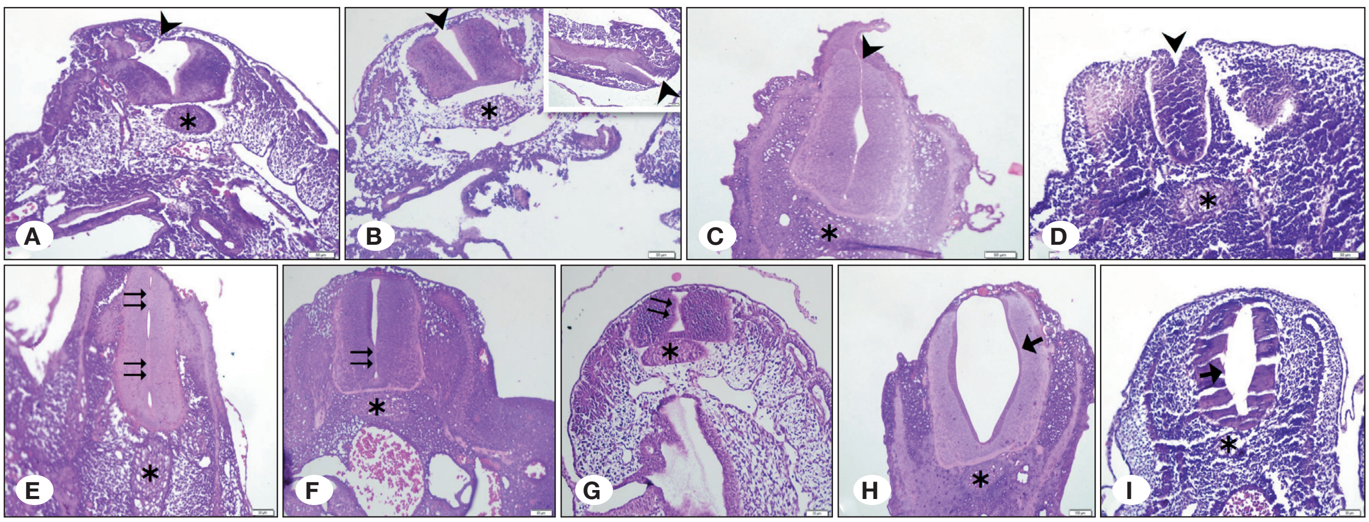

Figure 3: Representative photomicrographs of neural tube defects **(A–D)** and luminal anomalies **(E–I)** in the high-dose (0.0084 mg) group. **Arrowhead:** NTD; **Double arrow:** Neuroepithelial proliferation; **Arrow:** Abnormal luminal opening; **Asterisk:** Notochord. Hematoxylin and eosin staining. Scale bars = 50 µm.

In the high-dose group (0.0084 mg), four embryos exhibited NTDs (Figure 3A-D), and five embryos presented with luminal anomalies (Figure 3E-I). Neuroepithelial proliferation (Figure 3E-G) and abnormal lumen patency (Figure 3H,I) were evident among these cases. Eleven embryos maintained normal neural tube morphology similar to the control group (Figure 1C).

Statistical Results

A significance level of $p < 0.05$ was considered statistically meaningful. Compared with the control group, the incidence of NTDs was significantly higher in the low-dose sodium nitrite group ($p = 0.0083$) and in the high-dose group ($p = 0.0012$). However, no statistically significant difference was observed between the low- and high-dose groups ($p = 0.7475$) (Table I-III).

DISCUSSION

Neural tube defects represent a group of congenital malformations ranging from mild vertebral closure defects, such as spina bifida occulta, to severe abnormalities involving the spinal cord, including meningocele and myelomeningocele. These defects often result in vertebral anomalies, neural tissue damage, and are frequently accompanied by cardiac, skeletal, urogenital, or distal bowel abnormalities. Multiple environmental and maternal factors contribute to their development, including folate deficiency, maternal smoking, air pollution, exposure to specific medications such as anticonvulsants, and exposure to food additives (3,12,15). Substances such as colorants, preservatives, and contaminants in soil or wa-

Table I: Comparison Between the Control Group (daily dose) and the Low-Dose Sodium Nitrite Group

	Daily Dose	Control	p-value
Normal	13	20	**0.0083**
Defect	7	0	

Table II: Comparison Between the Control Group and the High-Dose Sodium Nitrite Group

	High Dose	Control	p-value
Normal	11	20	**0.0012**
Defect	9	0	

Table III: Comparison Between the Low-Dose Group and the High-Dose Sodium Nitrite Group

	Daily Dose	High Dose	p-value
Normal	13	11	0.7475
Defect	7	9	

ter may increase the risk of congenital spinal malformations during early gestation (15).

The chick embryo is a well-established model for investigating developmental anomalies, particularly of the spinal cord, due to its rapid embryonic growth, the similarity of its neurulation process to that of humans, and the feasibility of *in ovo* manipulation. Therefore, this model was selected to examine the potential teratogenic effects of sodium nitrite on neural tube closure.

Sodium nitrite and nitrate are water-soluble compounds widely used in processed meats—such as sausages, bacon, jerky, and deli products—as curing and preservative agents to enhance color, stabilize flavor, and extend shelf life (16,19). Sodium nitrite reacts with myoglobin to form nitrosomyoglobin, producing the characteristic reddish-pink color of cured meats (16,19). However, once ingested, nitrite can react with amines to form nitrosamines, compounds known to increase carcinogenic risk (6,18). The World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) have classified processed meats as "probably carcinogenic to humans," largely due to these nitrosamine-mediated effects (11,14).

Nitrites also induce oxidative stress, contributing to genotoxicity, mutagenesis, and reproductive and embryotoxic effects (6,16,18,19). Additionally, dietary exposure to nitrites and nitrates has been linked to hypertension and adverse reproductive outcomes (3,4). The European Food Safety Authority (EFSA) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) have established acceptable daily intakes (ADIs) of 0.07 mg/kg and 0.06 mg/kg body weight, respectively (4,5). Sodium nitrite is naturally found in vegetables such as lettuce and beets but is primarily ingested through processed meats, where consumption may exceed the recommended limits.

In this study, a dose equivalent to the EFSA-defined ADI (0.07 mg/kg) was designated as the low-dose group, whereas the high-dose group received twice that concentration. Notably, developmental abnormalities of the spinal cord were detected even at doses corresponding to the accepted daily limit. While spinal dysraphisms typically occur in the lumbosacral region, three cases in this study exhibited rare cervical localization.

Acute toxicity of sodium and potassium nitrite has been associated with smooth muscle relaxation, vasodilation, hypotension, and methemoglobinemia. Prior studies indicate that sodium nitrite exerts embryotoxic and teratogenic effects at high concentrations (1,10,15). Prolonged maternal exposure may impair fetal growth, disrupt neural and skeletal development, reduce maternal and fetal weight gain, and cause congenital anomalies (17). Chronic or high-dose exposure has also been linked to infertility, placental dysfunction, and fetal loss (7). Furthermore, impaired dendritic development associated with nitrite exposure may contribute to cognitive impairment (2).

Similarly, sodium nitrite exposure in zebrafish embryos has been shown to produce mild anxiogenic behaviors and altered brain metabolic profiles, including decreased levels of GABA, glutamine, and certain fatty acids, indicating disruption of neurochemical balance (6).

In our study, NTDs and spinal cord closure defects occurred even at exposure levels equivalent to the upper threshold of the acceptable daily intake. Although the frequency of NTDs was greater in the high-dose group, the difference between dose levels was not statistically significant. These findings underscore the potential developmental risks associated with sodium nitrite exposure, even within current safety margins.

This study is the first, to the best of our knowledge, to demonstrate the adverse effects of sodium nitrite on embryonic spinal cord development. As a preliminary investigation, our results emphasize the need to re-evaluate the safety thresholds of sodium nitrite exposure during pregnancy and encourage further research using larger sample sizes and additional vertebrate models.

CONCLUSION

The findings of this study indicate that sodium nitrite exerts a direct teratogenic effect on neural tube development in chick embryos. Statistically significant neural tube closure defects were observed in low- and high-dose groups. Notably, cervical spinal dysraphism—a rare presentation—was detected in three cases. These results suggest that sodium nitrite may induce embryotoxic effects even at low exposure levels. However, as the chick embryo model does not fully replicate human developmental conditions, these findings cannot be directly extrapolated to humans. Nevertheless, the results highlight the importance of regulating sodium nitrite intake and warrant further studies to elucidate its teratogenic potential.

Declarations

Funding: This study did not receive any funding or financial support.

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Disclosure: We don't have any conflict of interest and any financial support.

Ethical approval: In accordance with the European Directive 2010/63/EU, ethical approval was not required for experiments involving chick embryos.

AUTHORSHIP CONTRIBUTION

Study conception and design: EA, ST

Data collection: EA, EBKO, DSA, DA, ST

Analysis and interpretation of results: EA, DSA, ST

Draft manuscript preparation: EA

Critical revision of the article: EA, ST

Other (study supervision, fundings, materials, etc...): EA

All authors (EA, EBKO, DSA, DA, ST) reviewed the results and approved the final version of the manuscript.

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