

*Original Investigation*

# The Effect of Phenyramidol on Neural Development in Early Chicken Embryo Model

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## ABSTRACT

**AIM:** To investigate the effects of Phenyramidol (Phe) on neural development in an early chicken embryo model.

**MATERIAL and METHODS:** Sixty fertile non-pathogenic Super Nick eggs were incubated for 24 hours (h) and divided into four groups of 15 eggs each. Phe was administrated through the sub-blastoderm, and the eggs were incubated for another 24 h. All eggs were opened after 48 h of incubation, and the embryos were evaluated morphologically and histopathologically.

**RESULTS:** In Group 1 (control group), none exhibited neural tube defects (NTDs) (0%), 1 (6.6%) was undeveloped; in Group 2 (low dosages), 1 did not develop (6.6%); in Group 3 (normal dosages), 2 (13.4%) had NTDs, 1 (6.6%) was undeveloped; in Group 4 (high dosages), 5 (33.3%) had NTDs, 2 (13.3%) were undeveloped.

**CONCLUSION:** In light of the results, it was determined that the use of increasing doses of Phe led to defects in midline closure in early chicken embryos. This is the first report in the literature on Phe used in an early chicken embryo model.

**KEYWORDS:** Neural tube defect, Growth retardation, Myorelaxant drug, Phenyramidol, Undeveloped embryo, Chicken embryo

**ABBREVIATIONS:** NTDs: Neural tube defects, Phe: Phenyramidol

## INTRODUCTION

Congenital malformations are physical deformities that affect one or more organs. The worldwide incidence of central nervous system malformations is second only to that of cardiovascular congenital malformations. Neural tube defects (NTDs) constitute an important part of these congenital malformations (14,19), with an incidence of approximately 6/10000 pregnancies (26).

Pain that originates in the musculoskeletal system is a frequently encountered disorder that leads to loss of work, especially in industrialization countries; therefore, many drugs, such as simple analgesics, non-steroidal anti-

inflammatory drugs, opioids and myorelaxants are often used to relieve the pain. Phenyramidol HCl [(Phe) (Cabral®, Recordati Ilac, Tekirdag, Türkiye)] is a potent, non-narcotic central myorelaxant that affects the nervous system. The drug belongs to pregnancy category C, which indicates that no studies have been conducted on the drug in humans (17). In our study, we determined the effects of Phe on the neural development in an early chick embryo model.

## MATERIAL and METHODS

The study was conducted at Ankara University, in the Departments of Neurosurgery and Neuroembryology

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Laboratories. Sixty fertile non-pathogenic Super Nick eggs were obtained from the Institute of Akyurt Poultry Husbandry. All eggs weighed (overall weight =  $65 \pm 2$  g) and incubated for 24 hours (h) at a temperature of  $37.8 \pm 2^\circ\text{C}$  and 65–75% humidity in an incubator that rotated the eggs every 2 h after which the eggs were opened using the window procedure (Figure 1), and were divided into four groups of 15 eggs each. Under sterile conditions, selected dosages (low, normal, and high) of Phe were prepared and 12.5  $\mu\text{L}$  was administered through the sub-blastoderm in Groups 2, 3, and 4 at concentrations of 10, 60, and 280 mg/mL, respectively, using a 0.12-, 0.75-, or 3.5-mg Hamilton microinjector (Figure 2). Group 1 served as the control group and 12.5  $\mu\text{L}$  distilled water was administered through the sub-blastoderm. The eggs were covered with sterile drapes after injection, rotated  $180^\circ$ , and incubated for another 24 h. All eggs were evaluated morphologically and histopathologically. The eggs were opened using of new method and evaluated under the Nikon ZMS-20 light microscope in terms of neural development. The embryos were classified according to defect, normal or undeveloped criteria.

Analyses of all findings were conducted using SPSS v 17.0 (SPSS Inc., Chicago, IL, USA). The results are expressed as number and percentage. Fischer's Chi-Squared test was performed to determine any differences among the groups.

## RESULTS

Of the embryos in Group 1, none exhibited NTDs (0%), 1 (6.6%) was undeveloped, and 14 (93.3%) were intact. Of the embryos in Group 2 (low dosages), 1 did not develop (6.6%) and 14 (93.3%) were intact. Of the embryos in Group 3 (normal dosages), 2 (13.4%) had NTDs, 1 (6.6%) was undeveloped, and 12 (80%) were intact. In Group 4 (high dosages), 5 (33.3%) had NTDs, 2 (13.3%) were undeveloped, and 8 (53.4%) were intact (Figures 3A, B; 4A, B). Drug dosages in the groups are shown in Table I.

There was no significant difference in NTDs between Groups 1 and 2 ( $p > 0.05$ ). There was a significant difference in NTDs between Groups 1 and 4 ( $p < 0.05$ ) and between Groups 2 and 4 ( $p < 0.05$ ). Based on our findings, Phe may cause NTDs and abnormality in early chick embryos in a dose-dependent manner.

## DISCUSSION

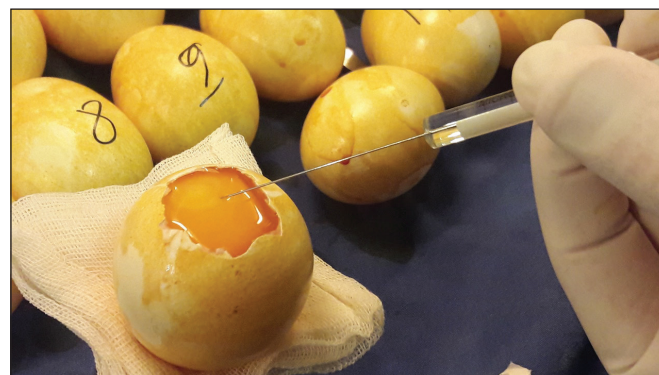
Although NTDs are the most common congenital malformation of the central nervous system, the cause has not been clearly determined. It is believed to be multifactorial with the effects stemming from both genetic and environmental factors (4,12). Some environmental factors, such as maternal infections, trauma, folic acid deficiency, and drug use, can affect neural development and cause neural developmental disorders in the early stages of pregnancy (2,8). There have been studies on early chicken embryo models, such as the effects of magnetic resonance imaging and cell phone use during pregnancy (9,21); however, too many studies have been with drugs. The drugs studied are antiepileptics, such as

valproic acid, levetiracetam, lacosamide; analgesics, such as meloxicam, flurbiprofen, metamizole; folic acid antagonists, such as methotrexate; and myeline protector proteins such as glatiramer (3,6,7,13,15,16,18,20,22). One of the most common features of these studies is that they were conducted on early chicken embryo models. This study timeframes correspond to the first trimester in pregnancy, during which neural development begins (5).

Between 20% and 90% of pregnant women worldwide experience low back pain (11,23,24). The etiology is explained as being from a combination of hormonal changes, postural changes, and reduced stability from increasing strains on core



**Figure 1:** Chicken egg opened using the window technique after incubating for 24 hours.



**Figure 2:** Phenyramidol injected through the sub-blastoderm under sterile conditions.

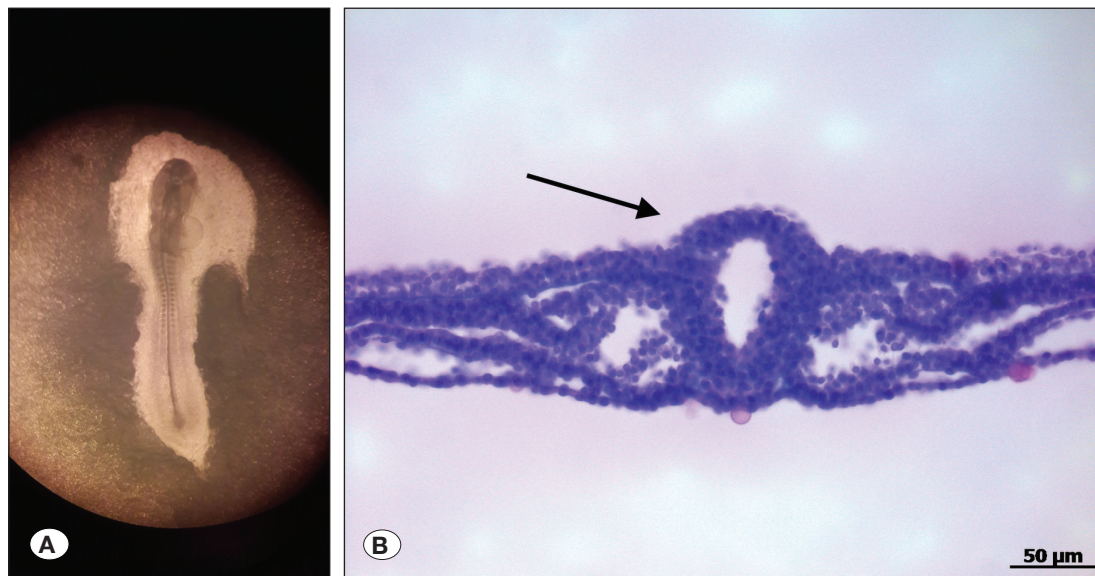
muscles, metabolic factors, genetic factors, and increased pregnancy (24). Low back pain results in increased sick leave, higher rates of functional disability, and increased access to healthcare for symptom management (10); therefore, pain elimination or reduction is important for a healthy pregnancy.

Simple analgesics, nonsteroidal anti-inflammatory drugs, opioids, and myorelaxants are used for pain relief. Guvenc et al. found that metamizole sodium, an analgesic, causes NTDs in the chicken embryo model. In another study with 36 chicken embryos (7), Ozeren et al. showed that even the

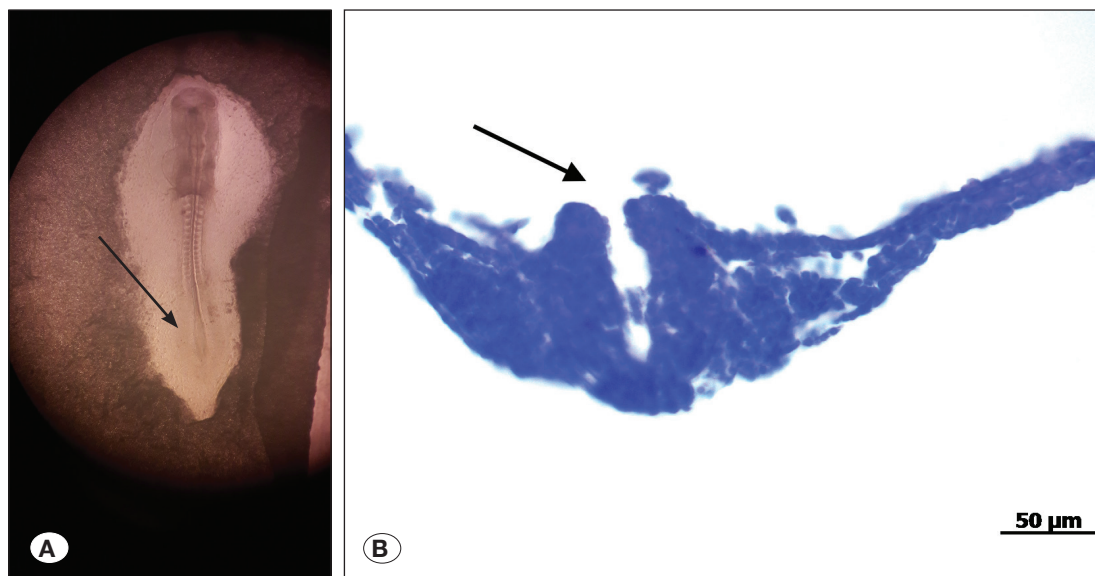
**Table I:** Variable Dosages and Effects in Different Groups of Phenyramidol

Groups	Control n (%)	Low (10 mg/ml) n (%)	Normal (60mg/ml) n (%)	High (280mg/ml) n (%)
Undeveloped	1 (6.6)	1 (6.6)	1 (6.6)	2 (13.3)
NTD	- (0)	- (0)	2 (13.4)	5 (33.3)
Intact	14 (93.3)	14 (93.3)	12 (80)	8 (53.4)

**NTD:** Neural tube defect.



**Figure 3:** Neural tube of chicken embryo. **A)** Light microscopic (x40), and **B)** histopathological (hematoxylin and eosin staining) views.



**Figure 4:** Neural tube defect in a chicken embryo (black arrows). **A)** Light microscopic (x40), and **B)** histopathological (hematoxylin and eosin staining) views.



normal therapeutic doses of flurbiprofen increase the risk of NTDs and anencephaly (15). Cetinkal et al. administered various dosages of meloxicam, a COX-2 inhibitor, to 100 non-pathogenic chicken embryos (3). Their results showed NTDs in the embryos after having received suprathreshold dosages of the drug. All of these studies showed that nonsteroidal anti-inflammatory drugs can affect neural development, even at normal dosages.

Briner administered muscimol and bicuculline, which are  $\gamma$ -aminobutyric acid (GABA)<sub>A</sub> agonists, and baclofen and hydroxyl-baclofen, which are GABA<sub>B</sub> agonists, to pregnant rats. The results of their research indicated that GABA<sub>A</sub> agonist and antagonist and GABA<sub>B</sub> agonist could lead to the enlargement of the vertebral arc and create NTDs (1).

We choose to study Phe because its effects on pregnancy were not studied and were unknown. Phe is an efficacious and well-tolerated analgesic used in the treatment of the acute conditions of lumbago, acute musculoskeletal pain, and integumental pain. It works by interrupting the interneuronal reflexes in the spinal cord and brain stem and has been found to be beneficial for musculoskeletal disorders and integumental pain in both oral and injectable forms (17). The results of our study showed that Phe, especially in increasing dosages, affects neural development to and leads to NTDs.

There are many steps in neurulation, such as the development of neural plaque by thickening ectoderm, remodeling and bending of neural plaque, closure of the neural cleft, and closure of the caudal eminence, and ionic changes also play important roles in this development (25). We believe that it is not known exactly which step in this process is affected by Phe, but the drug can block ion exchanges and thus lead to defects by creating stress on neural development.

## ■ CONCLUSION

High doses of Phe might have an effect on neural development and cause NTDs in early chicken embryo models; therefore, those in the first trimester of pregnancy should avoid using this drug for pain relief.

This is the first experimental study on Phe involving neural development with an early chicken embryo model. Additional studies are needed to show the mechanisms by which embryos are damaged and the mechanisms surrounding Phe's teratogenous effects that are associated with genetic and environmental factors and to minimize the rate of congenital defects.

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## ■ DECLARATION of INTEREST STATEMENT

The authors have no ethical conflicts to disclose. The authors have no conflicts of interest to declare.

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