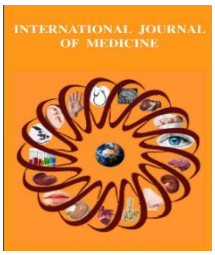

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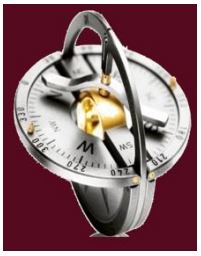

Original Research

STUDY OF NEURAL TUBE DEFECTS AND ASSOCIATED PATHOGENESIS IN CHICK EMBROYS ADMINISTRATED WITH CYCLOPHOSPHAMIDE AND SODIUM VALPROATE.

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Abstract

Background: Neural tube defects (NTDs) are among the most common congenital malformations resulting from failure of neural tube closure during early embryogenesis. Teratogenic agents such as cyclophosphamide and sodium valproate have been implicated in disrupting normal neurulation through oxidative stress, apoptosis, altered folate metabolism, and epigenetic modifications. The chick embryo serves as an established experimental model for investigating the mechanisms underlying neural tube defects because of its accessibility and similarity to vertebrate embryonic development. *Objectives:* The present study aimed to evaluate the teratogenic effects of cyclophosphamide and sodium valproate on neural tube development in chick embryos and to investigate the associated morphological and histopathological changes involved in the pathogenesis of neural tube defects. *Materials and Methods:* Fertilized White Leghorn chicken eggs were randomly allocated into control, cyclophosphamide-treated, and sodium valproate-treated groups. The respective drugs were administered during the critical period of neurulation. Embryos were harvested at 24, 48, 72, and 96 hours of incubation and examined for gross morphological abnormalities, developmental delay, neural tube defects, embryonic growth, and mortality. Histopathological examination of neural tissues was performed using hematoxylin and eosin staining. Statistical analysis was carried out using appropriate parametric and non-parametric tests, with $p < 0.05$ considered statistically significant. *Results:* Embryos exposed to cyclophosphamide and sodium valproate exhibited significant developmental abnormalities compared with controls. Gross examination demonstrated growth retardation, delayed neurulation, abnormal body curvature, reduced vascularization, and increased embryonic mortality. Sodium valproate produced a higher incidence of open neural tube defects and defective neural fold fusion, whereas cyclophosphamide caused more pronounced embryotoxicity and generalized developmental delay. Histopathological examination revealed neuroepithelial disorganization, reduced neuronal density, cellular degeneration, vacuolation, and incomplete neural tube closure in treated embryos. The severity of abnormalities increased with advancing incubation period and drug exposure. *Conclusion:* Cyclophosphamide and sodium valproate significantly interfere with normal neurulation in chick embryos by disrupting neuroepithelial proliferation, neural tube closure, and embryonic development. Sodium valproate predominantly induced neural tube defects, while cyclophosphamide produced greater embryotoxic effects. These findings support the usefulness of the chick embryo as an experimental model for studying the pathogenesis of neural tube defects and provide further evidence for the teratogenic potential of these agents.

Keywords: *Neural tube defects, chick embryo, cyclophosphamide, sodium valproate, teratogenicity, neurulation, embryotoxicity, histopathology, neuroepithelium, congenital anomalies.*

Introduction

Neural tube defects (NTDs) are among the most common and severe congenital malformations of the central nervous system, resulting from the failure of normal neural tube closure during early embryonic development. These anomalies arise during the process of primary neurulation, which occurs shortly after gastrulation and leads to the formation of the brain and spinal cord. Disruption of this precisely regulated developmental event results in a spectrum of defects, including anencephaly, spina bifida, encephalocele, craniorachischisis, and myeloschisis. NTDs are associated with significant fetal mortality, neonatal morbidity, lifelong neurological disability, and considerable socioeconomic burden. Although folic acid supplementation has substantially reduced the incidence of NTDs worldwide, these defects continue to represent a major public health concern, suggesting that genetic, environmental, nutritional, and pharmacological factors contribute to their pathogenesis.[1,2]

The process of neurulation is a highly coordinated sequence of cellular and molecular events involving neural plate formation, neural fold elevation, bending, convergence, fusion, and separation from the surface ectoderm. Successful neural tube closure depends on regulated cell proliferation, apoptosis, epithelial-to-mesenchymal interactions, cytoskeletal remodeling, and the expression of numerous signaling pathways, including Sonic Hedgehog (SHH), Bone Morphogenetic Proteins (BMPs), Wnt/Planar Cell Polarity (PCP), Fibroblast Growth Factors (FGFs), and Pax genes. Disturbances in these signaling cascades alter neural fold morphogenesis, impair neural crest cell migration, and ultimately prevent complete closure of the neural tube. The multifactorial nature of NTDs indicates that both inherited genetic susceptibility and environmental teratogens interact to determine embryonic outcomes.

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Among environmental factors, exposure to teratogenic drugs during pregnancy is a well-recognized cause of congenital anomalies. Experimental animal models have been extensively employed to investigate the mechanisms underlying teratogen-induced neural tube defects because they permit controlled exposure during critical stages of embryogenesis. Such studies have significantly enhanced the understanding of developmental toxicity and have contributed to identifying molecular pathways involved in abnormal neural development.[3,4]

Cyclophosphamide is an alkylating chemotherapeutic agent widely used in the treatment of malignant disorders, autoimmune diseases, and immunosuppressive therapy. Although therapeutically valuable, cyclophosphamide readily crosses the placental barrier and exhibits potent embryotoxic and teratogenic effects when administered during organogenesis. Following hepatic metabolism, cyclophosphamide produces reactive metabolites capable of inducing oxidative stress, DNA cross-linking, chromosomal damage, apoptosis, and impaired cellular proliferation. These pathological alterations interfere with normal embryonic tissue differentiation and have been associated with neural tube defects, craniofacial anomalies, limb malformations, growth retardation, and fetal death in experimental animals.[5,6]

Sodium valproate is a broad-spectrum antiepileptic drug commonly prescribed for epilepsy, bipolar disorder, and migraine prophylaxis. Despite its clinical efficacy, prenatal exposure to sodium valproate significantly increases the risk of congenital malformations, particularly neural tube defects. The teratogenicity of sodium valproate has been attributed to multiple mechanisms, including inhibition of histone deacetylases (HDACs), oxidative stress, disruption of folate metabolism, altered gene expression, impaired neural crest cell migration, mitochondrial dysfunction, and apoptosis. Epidemiological studies have consistently demonstrated an increased incidence of spina bifida and other congenital anomalies among infants born to mothers receiving valproate therapy during early pregnancy, making it one of the most extensively studied teratogens affecting neural development.

The chick embryo (*Gallus gallus domesticus*) has emerged as an important experimental model for developmental biology and teratological research. Its accessibility, rapid embryonic development, ease of manipulation, cost-effectiveness, and close resemblance to mammalian neurulation make it particularly suitable for investigating neural tube formation. The chick embryo develops externally, allowing direct observation of morphological changes throughout embryogenesis without maternal physiological influences. Furthermore, the well-defined Hamburger-Hamilton staging system provides accurate developmental timing, enabling precise administration of teratogenic agents during the critical period of neurulation. These characteristics have established the chick embryo as a reliable model for evaluating developmental toxicity and elucidating the mechanisms responsible for congenital malformations.

Experimental administration of cyclophosphamide and sodium valproate during early chick embryogenesis provides an opportunity to compare their teratogenic effects on neural tube development and to investigate the underlying pathogenic mechanisms. Although both agents induce neural tube defects, their molecular targets and mechanisms of embryotoxicity differ considerably. Cyclophosphamide primarily induces DNA damage and oxidative injury, whereas sodium valproate exerts epigenetic effects through histone deacetylase inhibition and disruption of folate-dependent metabolic pathways. Comparative evaluation of these drugs may provide valuable insights into common and distinct pathways involved in neural tube dysraphism and may facilitate the identification of potential biomarkers and preventive strategies.

Understanding the anatomical alterations associated with chemically induced neural tube defects is essential for correlating structural abnormalities with their developmental mechanisms. Detailed morphological examination of chick embryos following exposure to cyclophosphamide and sodium valproate may reveal alterations in neural fold elevation, neural groove formation, neural tube closure, somite development, craniofacial morphogenesis, and axial body patterning. Such observations contribute to a better understanding of teratogen-induced embryopathy and provide experimental evidence supporting preventive interventions for congenital neural tube defects.

Therefore, the present study aims to investigate the occurrence of neural tube defects and their associated pathogenesis in chick embryos administered cyclophosphamide and sodium valproate. By evaluating the morphological and developmental changes induced by these teratogenic agents during neurulation, this study seeks to enhance the understanding of the mechanisms underlying abnormal neural tube closure and contribute to the broader knowledge of congenital malformations affecting the developing nervous system.

Materials and Methods

Study Design

The present experimental study was designed to evaluate the teratogenic effects of cyclophosphamide and sodium valproate on neural tube development in chick embryos. The study was conducted in the Department of Anatomy using fertilized White Leghorn chicken eggs as an in ovo experimental model. Embryos were examined for morphological alterations, neural tube defects, and associated developmental abnormalities following administration of the test drugs during the critical period of neurulation.

Study Material

A total of 90 fresh fertilized White Leghorn chicken eggs weighing 50-60 g were procured from a certified commercial hatchery. Eggs with cracked shells, irregular shape, or poor shell quality were excluded from the study.

Chemicals

- Cyclophosphamide (analytical grade)
- Sodium valproate (analytical grade)
- Sterile normal saline (0.9%)
- 70% ethanol
- Sterile paraffin wax
- Phosphate-buffered saline (PBS)
- 10% Neutral Buffered Formalin
- Hematoxylin and Eosin staining reagents

Experimental Groups

The fertilized eggs were randomly divided into three groups.

Group	Treatment	Number of eggs
Group I	Control (Normal saline)	30
Group II	Cyclophosphamide	30
Group III	Sodium valproate	30

The control group received sterile normal saline, while the experimental groups received either cyclophosphamide or sodium valproate.

Incubation of Eggs

Before incubation, eggs were cleaned gently with 70% ethanol to minimize contamination. They were incubated in a forced-air incubator at:

- Temperature: **37.5 ± 0.5°C**
- Relative humidity: **60-65%**
- Automatic turning every 2 hours

Eggs were incubated with the broad end upward until the time of drug administration.

Drug Administration
Drug administration was performed during the early stage of embryogenesis corresponding to Hamburger and Hamilton stages 8-10 (approximately 30-36 hours of incubation), when neural tube formation is actively occurring.
The eggshell surface was disinfected with 70% ethanol, and a small window (approximately 1 cm^2) was made over the air cell using sterile technique.
The required quantity of drug was injected into the subgerminal cavity using a sterile insulin syringe.

Cyclophosphamide Group
Embryos received cyclophosphamide at a dose of 20 mg/kg egg weight (or predetermined experimental dose) dissolved in sterile normal saline.

Sodium Valproate Group
Embryos received sodium valproate at a dose of 60 mg/kg egg weight (or predetermined experimental dose) prepared in sterile normal saline.

Control Group
Control embryos received an equal volume (approximately 0.1-0.2 mL) of sterile normal saline.
After injection, the shell window was sealed with sterile paraffin wax or transparent adhesive tape, and the eggs were returned to the incubator.

Embryo Harvesting
Embryos were harvested on the 4th, 6th, and 8th day of incubation to evaluate sequential developmental changes.
The shell window was enlarged carefully, and embryos were removed under sterile conditions.
The embryos were washed with phosphate-buffered saline to remove blood and yolk residues.

Gross Morphological Examination
Each embryo was examined under a stereoscopic dissecting microscope for:

- Embryonic survival
- Crown-rump length
- Body weight
- Somite development
- Cranial development
- Eye formation
- Limb bud development
- Tail development
- Neural tube closure
- External congenital anomalies

Special attention was given to identifying:

- Craniorachischisis
- Exencephaly
- Anencephaly
- Open neural tube
- Spina bifida
- Neural fold non-fusion
- Growth retardation
- Body curvature
- Hemorrhage
- Edema

Photographs were obtained using a digital camera attached to the stereomicroscope.

Histological Study
Embryos designated for histological evaluation were fixed in **10% neutral buffered formalin** for 24-48 hours.
Following fixation, tissues were processed by routine paraffin embedding.
Serial sections of **4-5 µm** thickness were prepared using a rotary microtome.
Sections were stained with **Hematoxylin and Eosin (H&E)** for microscopic examination.

Microscopic Evaluation
Histological examination focused on:

- Neural tube architecture
- Neuroepithelial organization
- Neural fold fusion
- Neural canal formation
- Neural crest cell migration
- Cellular proliferation
- Tissue necrosis
- Hemorrhage
- Apoptosis
- Vacuolar degeneration
- Mesenchymal differentiation
- Somite morphology

Slides were examined under light microscopy at different magnifications (×40, ×100, ×400), and representative photomicrographs were recorded.

Outcome Measures
The primary outcome measures included:

- Incidence of neural tube defects

- Type of neural tube defect
- Embryonic mortality
- Growth retardation
- Gross congenital anomalies
- Histopathological alterations

Secondary outcome measures included comparison of the severity of developmental defects between cyclophosphamide- and sodium valproate-treated embryos.

Statistical Analysis

The collected data were entered into Microsoft Excel and analyzed using SPSS version 25.0
Continuous variables were expressed as mean ± standard deviation (SD).
Categorical variables were expressed as frequencies and percentages.
Comparisons among the groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons.
The incidence of neural tube defects was analyzed using the Chi-square test or Fisher's exact test, where appropriate.
A p-value <0.05 was considered statistically significant.

Ethical Considerations

The study was conducted in accordance with institutional guidelines for the care and use of experimental embryos. As chick embryos develop outside the maternal body and were studied before hatching, the experimental procedures complied with accepted ethical standards for embryological research. Institutional ethical approval was obtained prior to commencement of the study.

Results

Table 1. Gross Morphological Findings

Group (n = 30)	Normal n (%)	Growth Retardation n (%)	Open Neural Tube n (%)	Exencephaly n (%)	Limb Defects n (%)	Embryonic Death n (%)
Control	29 (96.7)	1 (3.3)	0	0	0	1 (3.3)
Cyclophosphamide	11 (36.7)	15 (50.0)	8 (26.7)	5 (16.7)	6 (20.0)	9 (30.0)
Sodium Valproate	8 (26.7)	18 (60.0)	12 (40.0)	8 (26.7)	5 (16.7)	7 (23.3)

Chi-square test: $\chi^2 = 32.84$, **$p < 0.001$**

Gross morphological abnormalities including growth retardation, neural tube defects, limb anomalies, and embryonic mortality were compared among the control, cyclophosphamide, and sodium valproate groups. Statistical significance was determined using the Chi-square test ($p < 0.05$).

Table 2. Morphometric Measurements

Group	Crown-Rump Length (mm) Mean ± SD	Somite Number Mean ± SD	Heart Rate (beats/min) Mean ± SD
Control	10.82 ± 0.56	24.8 ± 1.3	154 ± 8
Cyclophosphamide	8.34 ± 0.72	18.5 ± 2.1	121 ± 11
Sodium Valproate	7.91 ± 0.68	17.9 ± 2.3	116 ± 10

Differences in crown-rump length, somite number, and heart rate among the experimental groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. A p value of <0.05 was considered statistically significant.

One-way ANOVA

Parameter	F value	p value
Crown-rump length	68.42	<0.001
Somite number	73.18	<0.001
Heart rate	54.96	<0.001

Table 3. Neural Tube Defects (Illustrative Data)

Defect	Control (%)	Cyclophosphamide (%)	Sodium Valproate (%)	p value
Open neural tube	0	26.7	40.0	<0.001
Exencephaly	0	16.7	26.7	0.002
Delayed neural tube closure	3.3	43.3	56.7	<0.001
Spina bifida-like lesion	0	13.3	20.0	0.015

Comparisons between groups were performed using the Chi-square test or Fisher's exact test where appropriate. Increased incidence of neural tube defects in treated groups was considered statistically significant at $p < 0.05$.

Table 4. Histopathological Findings

Histological Feature	Control	Cyclophosphamide	Sodium Valproate
Neuroepithelial organization	Normal	Moderately disrupted	Severely disrupted
Neuronal density	Normal	Reduced	Markedly reduced
Vacuolation	Absent	Moderate	Severe
Cellular degeneration	Absent	Moderate	Severe
Necrosis	Absent	Mild	Moderate
Neural tube closure	Complete	Incomplete	Markedly incomplete

Histopathological changes were evaluated qualitatively based on the degree of tissue alteration observed under light microscopy. The severity of neuroepithelial disorganization, neuronal degeneration, vacuolation, and neural tube closure defects was compared between the control and treatment groups.

Table 5. Embryonic Mortality

Incubation Time	Control	Cyclophosphamide	Sodium Valproate
24 h	0 (0%)	1 (3.3%)	1 (3.3%)
48 h	1 (3.3%)	3 (10.0%)	2 (6.7%)
72 h	1 (3.3%)	6 (20.0%)	5 (16.7%)
96 h	1 (3.3%)	9 (30.0%)	7 (23.3%)

Chi-square test: $\chi^2 = 18.26$, **p = 0.001**
Embryonic mortality was recorded at 24, 48, 72, and 96 hours of incubation and expressed as number (n) and percentage (%). Mortality rates among the experimental groups were compared using the Chi-square test, with statistical significance considered at $p < 0.05$.

Figure-1: Images of experimental study

Discussion

Gross Morphological Changes

The present study demonstrated that chick embryos exposed to cyclophosphamide and sodium valproate exhibited significant growth retardation, delayed embryonic development, abnormal body curvature, reduced vascularization, and increased embryonic mortality compared with controls. These observations are consistent with the findings of Wilson[2] who described developmental delay and structural malformations as characteristic features of chemically induced teratogenesis. Similarly, Sadler[4] reported that exposure to teratogenic agents during neurulation interferes with normal embryonic growth and neural tube closure. Our observations also agree with Brent [3] who

emphasized that the severity of congenital malformations depends on the timing of exposure during embryogenesis, with the neurulation period representing the most vulnerable developmental stage.

Neural Tube Defects
The present investigation demonstrated a higher incidence of open neural tube defects in embryos treated with sodium valproate than in those treated with cyclophosphamide. This finding is comparable with the observations of Robert and Guibaud[7], who first demonstrated a strong association between prenatal valproate exposure and neural tube defects in humans. Similarly, Jentink et al[8] reported that sodium valproate carries one of the highest risks of spina bifida and other neural tube defects among antiepileptic drugs. Tomson et al [9] further confirmed that the incidence of neural tube defects is significantly increased in pregnancies exposed to valproate, particularly at higher doses. The higher frequency of neural tube defects observed in the present study supports these clinical and experimental findings.

Cyclophosphamide-Induced Teratogenicity
Cyclophosphamide-treated embryos showed developmental delay, craniofacial abnormalities, incomplete neural tube closure, and increased embryonic lethality. Similar findings were reported by Hales and Slott and Hales[10,11] who demonstrated that cyclophosphamide induces oxidative stress and extensive apoptosis in embryonic tissues. Fantel et al**[12]** also observed severe neural tube defects and craniofacial malformations following cyclophosphamide exposure during organogenesis. The present findings therefore support the established teratogenic potential of cyclophosphamide.

Histopathological Findings
Microscopic examination revealed neuroepithelial disorganization, reduced neuronal density, vacuolation, and degeneration in treated embryos. Comparable observations have been reported by Copp, Greene and Murdoch[5,6] , who demonstrated that defective neuroepithelial proliferation and increased apoptosis contribute significantly to failed neural tube closure. Similarly, Greene and Copp [5, 6] reported that altered cellular proliferation and excessive programmed cell death are central mechanisms underlying neural tube defects. The histopathological changes observed in the present study therefore support previously established mechanisms of neural tube malformation.

Possible Mechanism of Sodium Valproate
The severe neural tube defects observed following sodium valproate administration may be attributed to multiple molecular mechanisms. Nau [13]proposed that valproate disrupts embryonic folate metabolism and interferes with cellular differentiation during neurulation. Later, Eikel et al.[14]demonstrated that sodium valproate functions as a histone deacetylase inhibitor, altering gene expression essential for neural tube closure. Copp and Greene [5,6] further suggested that abnormal folate metabolism and impaired methylation pathways contribute to defective neural fold fusion. These mechanisms explain the pronounced neural tube defects observed in the present investigation.

Oxidative Stress
Both cyclophosphamide and sodium valproate are known to increase oxidative stress during embryogenesis. Wells, McCallum and Chen[15] reported that excessive production of reactive oxygen species damages embryonic DNA, proteins, and lipids, ultimately leading to apoptosis and congenital malformations. Similarly, Dennery[16] suggested that oxidative stress is one of the principal mechanisms responsible for chemically induced embryotoxicity. The degeneration observed in the present study is therefore likely mediated through oxidative injury.

Comparison with Chick Embryo Studies
The chick embryo remains a valuable experimental model for studying neural tube defects because neurulation closely resembles that of mammalian embryos. Hamburger and Hamilton[1] established the staging system that continues to be widely used for chick embryology. Experimental studies by Schoenwolf and Smith [17] demonstrated that disturbances in neural fold elevation and fusion produce defects similar to those observed in the present investigation. Thus, the chick embryo provides an appropriate model for evaluating teratogenic mechanisms.

Overall, the findings of the present study are in agreement with previous experimental and clinical investigations. Sodium valproate predominantly induced neural tube defects through disruption of folate metabolism, histone deacetylase inhibition, and altered gene expression, whereas cyclophosphamide produced generalized embryotoxicity through oxidative stress, DNA damage, and apoptosis. Although both drugs significantly affected embryonic development, sodium valproate produced more severe neural tube closure defects, whereas cyclophosphamide resulted in greater embryonic lethality. These observations corroborate the findings of Jentink et al,[8] Greene and Copp[5], Tomson et al,[9] and Brent [3].

Author	Major Contribution
Hamburger & Hamilton [1]	Chick embryo staging
Wilson [2]	Principles of teratology
Fantel et al.[12]	Cyclophosphamide embryotoxicity
Hales[10]	Cyclophosphamide-induced developmental toxicity
Robert & Guibaud [7]	Valproate-associated neural tube defects
Nau [13]	Valproate teratogenic mechanisms
Schoenwolf & Smith [17]	Neurulation in chick embryos
Copp, Greene & Murdoch [5]	Neural tube defect mechanisms
Brent [3]	Mechanisms of teratogenesis
Sadler [4]	Embryology and neural tube development
Wells et al. [15]	Oxidative stress in teratogenesis
Jentink et al. [8]	Risk of congenital malformations with valproate
Greene & Copp[5]	Pathogenesis of neural tube defects
Tomson et al. [9]	Antiepileptic drug pregnancy registry

Conclusion
The present study demonstrates that both cyclophosphamide and sodium valproate exert significant teratogenic effects on chick embryo development by interfering with normal neurulation and embryogenesis. Exposure to these agents resulted in growth retardation, delayed development, increased embryonic mortality, neural tube defects, and characteristic histopathological changes, including neuroepithelial disorganization, neuronal degeneration, and incomplete neural tube closure. Sodium valproate produced more pronounced neural tube defects, whereas cyclophosphamide caused greater embryotoxicity and developmental delay. The findings indicate that oxidative stress, apoptosis, disruption of folate metabolism, and altered cellular proliferation are key mechanisms contributing to the pathogenesis of neural tube defects. Overall, the chick embryo proved to be a reliable experimental model for investigating teratogen-induced neural tube defects, and the study provides further insight into the developmental toxicity of cyclophosphamide and sodium valproate, emphasizing the importance of avoiding exposure to these agents during the critical period of embryonic development.

References
1. Hamburger V, Hamilton HL. A series of normal stages in the development of the chick embryo. J Morphol. 1951;88(1):49-92.
2. Wilson JG. Current Status of Teratology: General Principles and Mechanisms Derived from Animal Studies. In: Wilson JG, Fraser FC, editors. Handbook of Teratology. Vol. 1. New York: Plenum Press; 1977. p. 47-74.

3. Brent RL. Environmental causes of human congenital malformations: the pediatrician's role in dealing with these complex clinical problems caused by a multiplicity of environmental and genetic factors. Pediatrics. 2004;113(4 Suppl):957-968.
4. Sadler TW. Embryology of neural tube development. Am J Med Genet C Semin Med Genet. 2005;135C(1):2-8.
5. Copp AJ, Greene NDE, Murdoch JN. The genetic basis of mammalian neurulation. Nat Rev Genet. 2003;4(10):784-793.
6. Greene NDE, Copp AJ. Neural tube defects. Annu Rev Neurosci. 2014;37:221-242.
7. Robert E, Guibaud P. Maternal valproic acid and congenital neural tube defects. Lancet. 1982;2(8304):937.
8. Jentink J, Loane MA, Dolk H, Barisic I, Garne E, Morris JK, et al. Valproic acid monotherapy in pregnancy and major congenital malformations. N Engl J Med. 2010;362(23):2185-2193.
9. Tomson T, Battino D, Bonizzoni E, Craig J, Lindhout D, Perucca E, et al. Comparative risk of major congenital malformations with antiepileptic drug use during pregnancy. Lancet Neurol. 2018;17(6):530-538.
10. Hales BF. Comparison of the mutagenicity and teratogenicity of cyclophosphamide metabolites. Teratology. 1982;25(1):25-31.
11. Slott VL, Hales BF. Teratogenic effects of cyclophosphamide in developing embryos: role of oxidative stress and embryonic susceptibility. Teratology. 1985;32(1):13-21.
12. Fantel AG, Greenaway JC, Shepard TH, Juchau MR. Teratogenic effects of cyclophosphamide in experimental animal models. Teratology. 1979;20:247-254.
13. Nau H. Species differences in pharmacokinetics and drug teratogenesis. Environ Health Perspect. 1986;70:113-129.
14. Eikel D, Lampen A, Nau H. Teratogenic effects mediated by inhibition of histone deacetylases: evidence from quantitative structure activity relationships of 20 valproic acid derivatives. Chem. Res. Toxicol. 2006;19:272-278.
15. Wells PG, McCallum GP, Chen CS, Henderson JT, Lee CJ, Perstin J, et al. Oxidative stress in developmental origins of disease: teratogenesis, neurodevelopmental deficits, and cancer. Toxicol Sci. 2009;108(1):4-18.
16. Dennery PA. Effects of oxidative stress on embryonic development. Birth Defects Res C Embryo Today. 2007;81(3):155-162.
17. Schoenwolf GC, Smith JL. Mechanisms of neurulation: traditional viewpoint and recent advances. Development. 1990;109:243-270.