ÖZGÜN ARAŞTIRMA / ORIGINAL RESEARCH

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Does the anticholinergic drug Biperiden affect early neural tube development in chick embryos?

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Abstract

Does the anticholinergic drug Biperiden affect early neural tube development in chick embryos?

Objective: Biperiden (BPD) is an anticholinergic agent that acts both centrally and peripherally. It is used to counteract both extrapyramidal side effects of neuroleptic treatment and symptoms of Parkinson's disease in clinical practice. Current study was layout to determine the potential toxic effect of different doses of Biperiden on neural tube closure in 48hr chick embryos.
Method: Sixty fertilized eggs were used in the study. All eggs were placed in the incubator and divided into four groups (15 eggs in each); Control, BPD1, BPD2 and BPD3. At 28hr of incubation, three different doses of Biperiden were administered subblastodermically in all BPD groups. At the end of 48hr of incubation, all eggs were opened and embryos were dissected and evaluated morphologically and histopathologically.
Results: According to these results, the mean crown-rump length and somite number tended to decrease proportionally with the dose. As the dose increases, the number of open neural tube and undeveloped embryos in the experimental groups also increases. There was also a significant difference between the groups in terms of Hamburger-Hamilton stages of embryos evaluated according to the number of somite. Embryos in the Control, BPD1 and BPD2 groups were observed at stage 13, and those in the BPD3 group were observed at stage 12.
Conclusion: These results showed that Biperiden even in the low dose has teratogenicity on neural tube closure in early chick embryos. The somite numbers and crown-rump length were decreased depending on the dose and Biperiden caused developmental retardation in high doses.
Keywords: Biperiden, Chick Embryo, Neural Tube

Öz

Antikolinerjik ilaçlardan Biperiden civciv embriyolarında erken dönemde nöral tüp gelişimini etkiler mi?

Amaç: Biperiden, hem merkezi hem de periferik olarak etki gösteren antikolinerjik bir ajandır. Klinik uygulamada hem nöroleptik tedavinin ekstrapiramidal yan etkilerine hem de Parkinson hastalığının semptomlarına karşı kullanılır. Mevcut çalışmanın amacı farklı dozlarda Biperiden uygulamasının 48 saatlik civciv embriyolarında nöral tüp kapanması üzerindeki potansiyel toksik etkisini belirlemektir.
Yöntem: Çalışmada 60 adet döllenmiş yumurta kullanıldı. Tüm yumurtalar kuluçka makinesine yerleştirildi ve her grupta 15 yumurta olacak şekilde dört gruba ayrıldı: Kontrol, BPD1, BPD2 ve BPD3. Yirmi sekiz saatlik inkübasyonda, Biperiden gruplarına subblastodermik olarak üç farklı Biperiden dozu uygulandı. 48 saatlik inkübasyonun sonunda tüm yumurtalar açılarak embriyolar diseke edildi. Embriyolar morfolojik ve histopatolojik olarak değerlendirildi.
Bulgular: Mevcut çalışmanın sonuçlarına göre ortalama baş-popo uzunluğu ve somit sayısı dozla orantılı olarak azalma eğilimindeydi. Doz arttıkça deney gruplarında açık nöral tüp ve gelişmemiş embriyo sayısı istatistiksel olarak anlamlı şekilde artmıştır. Somit sayısına göre değerlendirilen embriyoların Hamburger-Hamilton evreleri açısından da gruplar arasında anlamlı fark vardı. Kontrol, BPD1 ve BPD2 gruplarındaki embriyolar evre 13'te, BPD3 grubundakiler ise evre 12'de gözlendi.
Sonuç: Çalışmamızda erken civciv embriyolarında Biperiden'in düşük dozda bile nöral tüp kapanmasında teratojeniteye neden olduğunu gösterilmiştir. Doza bağlı olarak somit sayısı ve baş-popo uzunluğu azalmış, yüksek dozda ise gelişim geriliğine neden olmuştur.
Anahtar Kelimeler: Biperiden, Civciv Embriyosu, Nöral Tüp

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INTRODUCTION

Biperiden (BPD) (3-piperidine-1-phenyl-1-bicycloheptenyl-1-propanol) is a muscarinic anticholinergic agent that acts both centrally and peripherally (1). Anticholinergic drugs are used to counteract both extrapyramidal side effects of neuroleptic treatment and symptoms of Parkinson's disease in clinical practice (2). The combination of BPD with Haloperidol (HPD) is commonly used to prevent mental health conditions with psychosis (3). BPD can be also one of the most frequently abused anticholinergic drugs (4). The abuse associated with BPD is explained by its inhibitory effect on anhedonia, potentially due to antipsychotics. Mortality may increase depending on the dose taken and cause a condition that can progress from general condition disorder to coma (5).

Patients with psychotic or manic symptoms often need physical restraint and antipsychotic (AP) medication. Until the hospitalization period of these patients is initiated, it is essential to treat the patients in order for the patients to spend this period more comfortably. It can relieve aggression and agitation as soon as possible (6). HPD is the most commonly administered drug intramuscularly (IM) at a dose of 5-10mg. Against extrapyramidal system side effects of APs, 5mg ampoule IM or 2mg BPD per oral can be administered in combination with HPD or when side effects occur (7). In Parkinson's syndrome in severe cases, treatment may be started with 10 to 20mg of BPD initially. Single doses of 2.5mg and 5mg BPD can be given to adults for the treatment of drug-induced extrapyramidal symptoms (8).

Meningocele, neural tube (NT) defects, encephalocele and diastematomial anomalies are among the common congenital malformations. Environmental factors, genetic transmission and drugs used in pregnancy are mostly among the causes of these diseases. Among congenital malformations, NT defects have a crucial place in neurosurgical practice (9). NT defects appears in almost 6/10000 newborns and form a heterogeneous group of congenital anomalies. Since drugs used in pregnancy can cause such malformations, preventive medicine comes to the fore at this stage. Nonetheless, the pregnancy category of newly developed drugs and the kinds of malformations they can origin are still unknown (10).

The chicken embryo model has been considered as a good source of pharmacological and toxicological evaluations due to its certain features. The egg embryo is small, easy to handle and inexpensive to obtain; it is a creature with the necessary organs, rich nutrients and strong angiogenesis capacities in an isolated environment. Due to the physiological conditions of the egg embryo, this model is an animal model between in vitro and in vivo. Since they are not considered animals yet, there is less of an animal welfare concern (11,12). Based on our research, there was no study that scrutinize the toxic effects of BPD on NT development by using chick embryo model.

METHOD

The current experimental study was conducted in accordance with the Experimental Animal Principles and Guidelines organized by the National Health and Medical Research Council and the Experimental Animal Care and Use Guidelines (NIH issue no. 85–23, 1985 revised) prepared by the National Institute of Health. The Ethical Animal Research Committee of Afyon Kocatepe University (AKUHADYEK 49533702-64) approved the study.

Study Design

Eggs were divided into four groups (n=15 eggs/each group). Three experimental groups (BPD1, BPD2 and BPD3) and a single control group were used. Control group was administered with 10µL 0.9% NaCl via the subblastodermic route. The groups of BPD1, BPD2 and BPD3 embryos administered with 0.006, 0.012, and 0.025mg/egg Biperiden respectively. Dosages were determined slightly lower than previous animal studies based on egg weight, and no toxic dose group was established (13,14).

Incubation and Injection

The incubator was maintained at a humidity of 60-70% and constant temperature range of 37.8°C ±0.2°C. Before incubation, 60 white fertilized chicken eggs were cleaned (distilled water followed by 70% ethanol) and weighted (65±2g). All eggs were positioned in the incubator to point the sharp points in day 0. BPD (Akineton, 5 mg/mL ampoule, Assos) was diluted in saline and prepared in the selected dosages. The injection procedure was applied according to previous chick embryo studies (15–17). The fertilized eggs were taken out from the incubator at the 28th and 30th hour of the study (Stage 8, according to Hamburger and Hamilton), a small hole of around 0.5cm^2 in diameter was made under sterile conditions. When the embryonic disc became visible, BPD was injected under the embryo disc using a Hamilton injector. After the drug administration was completed, all holes were covered with a sterile cellophane tape and the eggs were located back in the incubator. Incubation was continued until the end of 48th hour and all eggs were removed from the incubator. The vitelline membrane was separated from the embryonic membrane over the yolk. Formalin-fixed, embryo tissue samples were examined to determine any gross developmental abnormalities under the stereomicroscope.

Histopathological Evaluations

Formalin-fixed embryos were embedded into paraffin. Routine histological processing was used for the light microscopic examination. The embryos were dehydrated using graded alcohol series and incubated in xylene. Serial sections of four micron thickness were taken from the paraffin blocks and stained with the haematoxylin–eosin. All preparations were evaluated using the light microscope by histologist blinded to the groups and photographs were taken.

Table 1: Developmental characteristics of embryos in control and BPD groups after 48 hours

Parameters	Control	BPD1	BPD2	BPD3
Somite Number (n)	19.1±0.99	18.5±1.77	17.0±2.21	14.1±2.14
Stage of Embryo (HH)	13	13	13	12
Open NT (n)	-	3	4	7
Undeveloped (n)	-	-	2	4
Crown-rump Length (µm)	783.10±23.96	713.30±18.9	608.00±87.02	515.00±131.50

Somite numbers and Crown-rump length were presented in mean ± standard deviation. Abbreviations: NT: Neural Tube; BPD: Biperiden; HH: Hamburger-Hamilton.

Statistical Analysis

Analysis of all findings was performed using the Statistical Package for the Social Sciences (SPSS) 22.0 programme. The Shapiro–Wilk test was used to determine the normal distribution of data. The somite number and crown–rump length were analyzed by using non-parametric Kruskal-Wallis tests. Dunn test were employed as post hoc tests and $p<0.05$ were considered significant.

RESULTS

In this study, the possible effects of three different doses of BPD (0.006, 0.012, and 0.025mg/egg biperiden) on NT development were assessed. The crown-rump length, somite numbers, and NT closure were evaluated. All data regarding overall crown-rump length, somite numbers, opened NT, undeveloped embryos, and stage of embryos were shown in Table 1.

Control Group: According to the Hamburger-Hamilton classification, all the characteristics of stage 13 were observed in all untreated control embryos. Their heads were partially turned to the left, the cranial and cervical flexures were formed wide curves, and the telencephalons were markedly enlarged (18). All NTs were closed and no malformation or developmental retardation was observed.

BPD1 (Embryos administered with 0.006mg/egg BPD): Only 3 embryos had opened NT. The NTs of the other embryos were closed and no malformation or developmental retardation was observed.

BPD2 (Embryos administered with 0.012mg/egg BPD): 4 embryos had opened NT. The NTs of the other embryos were closed; however developmental retardation was observed in 2 embryos according to the Hamburger-Hamilton classification with fewer somite numbers than expected.

BPD3 (Embryos administered with 0.025mg/egg BPD): 7 embryos had opened NT. Developmental retardation was observed in 4 embryos.

According to these results, the mean crown-rump length and somite number tended to decrease proportionally with the dose. As the dose increases, the number of open NT and undeveloped embryos in the experimental groups also increases (Table 1). The examination of light microscope findings was consistent with stereomicroscopic examination. It was observed that NT development was negatively affected by BPD (Figure 1 and 2). There was a significant difference between the groups in terms of the stages of the embryos evaluated according to the somite number. Embryos were observed at stage 13 in the control, BPD1, and BPD2 groups, while those were observed at stage 12 in the BPD3 group. A statistically significant decrease was observed in the crown-rump length between control and all BPD groups BPD1 ($p=0.025$), BPD2 ($p=0.000$), and BPD3 ($p=0.000$) respectively. There was a significant decrease also in somite numbers between control and BPD 2 ($p=0.031$); control and BPD3 ($p=0.000$); BPD1 and BPD3 ($p=0.000$).

DISCUSSION

Our knowledge about drug use during pregnancy is unfortunately limited as it is not ethical to conduct prospective studies on the effects of drugs. Most of the drugs and their metabolites pass to the fetus through the placenta (19). When the drug passes the placenta, teratogenicity depends on the fetal development stage. Since the embryo cells have not differentiated during the fertilization and implantation stage, it is possible to regenerate the dead cells, but it is the most sensitive period to teratogens as it is organogenesis after fertilization (20). In recent years, there has been an increment the search for alternative models to animal experiments (21). Most of the available information on NT defects pathogenesis has been provided by animal models rather than human embryos and fetuses. The neuronal development process of the chick embryo is somehow identical to the human embryo development process. Paraaxial mesoderm cells are called somites and are arranged symmetrically around the neural tube during neural tube development. Somites are very important for the formation of vertebral animals in a segmental structure (22,23).

BPD has been assigned to pregnancy category C by the FDA. There was no adequate information from the use of BPD during pregnancy. There was no indication that BPD poses a particular teratogenic risk; however, it is recommended to be careful as there is insufficient data about its use in the first trimester (24).

BPD is used to treat both symptoms of Parkinson's disease and antipsychotic-induced extrapyramidal symptoms in clinical practice (2). Akathisia is the most common extrapyramidal side effect of antipsychotics; thus anticholinergics can also be used in the treatment of

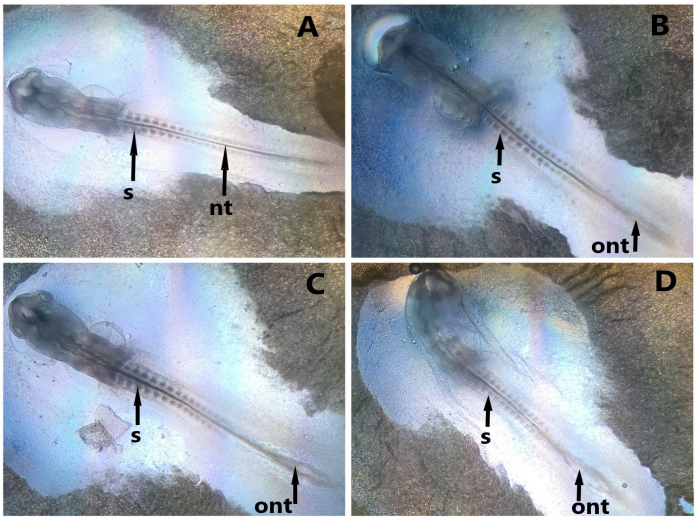

Figure 1. Light microscope images of chick embryos (A) Control, (B) BPD1, (C) BPD2, (D) BPD3. (nt: neural tube, ont: open neural tube, s: somite) (the magnification ratio 2×).

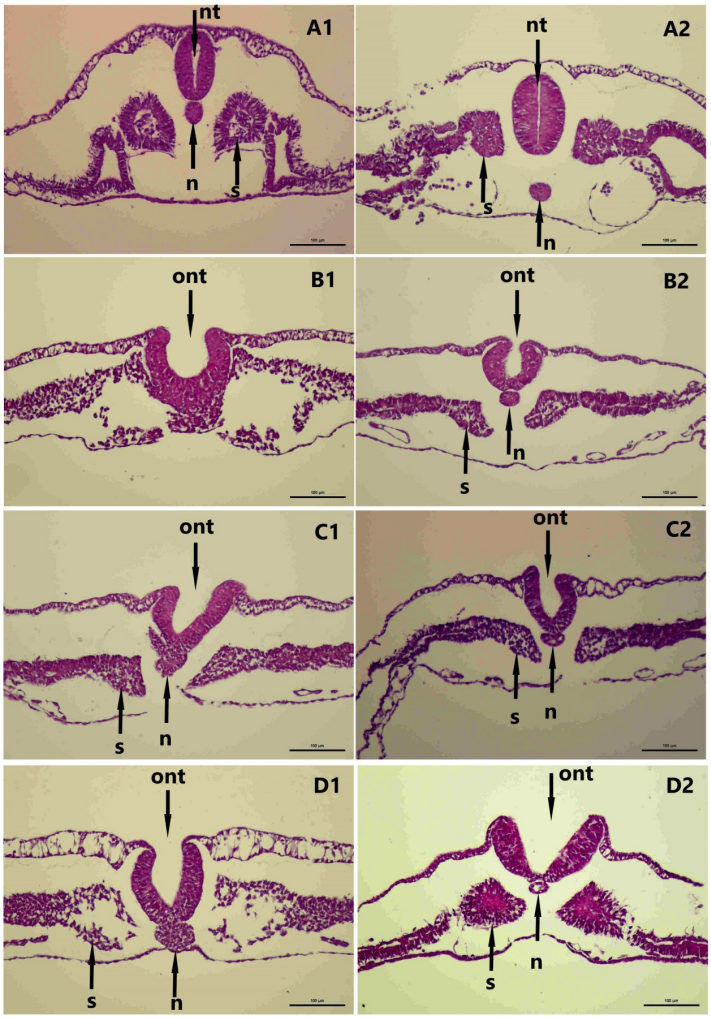

Figure 2. Histopathological images of chick embryos under light microscope. A1-A2: Control, B1-B2: BPD1, C1-C2: BPD2 and D1-D2: BPD3. (nt: neural tube, ont: open neural tube, s: somite, n: notocord) (the magnification ratio 20×).

neuroleptic-induced acute akathisia (25). The prevalence of prescribing anticholinergic drugs in psychiatry is particularly high in patients with schizophrenia (26). Centrally acting anticholinergic drugs like BPD are also likely to be abused (4). It has been noted that patients with schizophrenia take more than the recommended dose (27). Non-medical use has also been detected in drug-addicted patients (28). One of the most preferred drugs is BPD (2,29). It may be important to investigate the use of BPD during pregnancy as it is prescribed in many different areas. In addition, since anticholinergic therapy can significantly reduce the need for unnecessary invasive ventilation, studies on the effect of BPD have been conducted. Rapid administration of anticholinergics is important in the early detection and rapid treatment of Acute Laryngeal Dystonia (30-31).

This study was layout both to fill this knowledge gap and determine the potential toxic effect of different doses of BPD on NT closure**.** The adverse effects of BPD on the NT closure in early chick embryos were correlated with BPD dose. Dosages were determined slightly lower than previous animal studies based on egg weight, and no toxic dose group was established. For mice (35-45g), 1.0, 5.0, or 10.0mg/kg BPD doses were used (13). In another animal study, two different doses of BPD (3 and 10mg kg^{-1}) were given to rats (14). Use of higher-dose BPD led to decreased crown-rump length and somite number; moreover, the number of open NT and undeveloped embryos was higher. The embryonic stage was 13 in the control, BPD1 and BPD2 group and 12 in BPD3. While there was open NT in both the tail and neck region in the high dose group, NT was open only in the tail region in the lower dose groups. We chose for a dose of 0.006, 0.012 and 0.025mg/egg as this lies well within the range of the recommended doses for BPD (1–4mg) (32).

The most well-known side effects of BPD on the central nervous system are drowsiness, dizziness and headache (32). According to placebo-controlled study of BPD in patients with akathisia, response rates were similar in the BPD and placebo groups (25). BPD did not show a significant effect on learning in rats; however, it was concluded that there may be a potential side effect of BPD at 10mg kg^{-1}, as it takes more time for mice to start the task (14). In the study examining the effect of BPD on conditioned place preference acquisition, behavioral effects that could be related to the abuse potential of BPD were not established (2).

There are studies showing how different drugs affect NT and embryo development in chick embryos. In a study investigating the effect of non-steroidal anti-inflammatory drug, diclofenac sodium, on the development of NT, crown–rump length and somite number decreased significantly as the dose of the drug increased (15). It has also been shown that Bisphenol A, endocrine disrupting chemical, causes a statistically significant decrease in somite number and crown–rump length in chick embryo (16). In another study, a dose-dependent teratogenic effect of Pethidine hydrochloride, fast and effective analgesic, on NT in chick embryos was demonstrated (17). High dose Metamizole sodium, non-opioid analgesic, was shown to cause a neural tube defect and developmental retardation (33). Another study demonstrated that rizatriptan, a serotonin (5 hydroxytryptamine) 1B/1D receptor, has a negative effect on NT closure (22). Unlike other studies, Quetiapine, a widely used antipsychotic, has been shown to not cause NT defect formation in chick embryos (23).

CONCLUSION

Selection of drug for pregnant women is a complex decision because the potential risks and benefits both for the women and fetus should be assessed. Current results showed that BPD even in the low dose has teratogenicity on NT closure. The average somite numbers and crown-rump length were decreased depending on the dose. We believe that when BPD is used in the first months of pregnancy when organogenesis continues, it may trigger the development of NT defects in the newborn and have negative effects on neurulation. However, further studies on different embryo models are needed to fully reveal the effect of BPD on neurulation before reaching a definite conclusion.

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Peer-Review

Both externally and internally peer reviewed.

Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article.

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Ethical Declaration

Ethical permission was obtained from the Afyon Kocatepe University, the Ethical Animal Research Committee (AKUHADYEK) for this study with 18.05.2021 and number 49533702-64, and Helsinki Declaration rules were followed to conduct this study.

Authorship Contributions

Concept: HG, Design: HG, Supervising: HG, EA, Financing and equipment: HG, EA, Data collection and entry: HG, EA, Analysis and interpretation: HG, EA, Literature search: HG, Writing: HG, EA, Critical review: HG, EA

REFERENCES

1. Pehl C, Wendl B, Kaess H, Pfeiffer A. Effects of two anticholinergic drugs, Trospium Chloride and Biperiden, on motility and evoked potentials of the oesophagus. Aliment Pharmacol Ther. 1998;12(10):979-84. https://doi.org/10.1046/j.1365-2036.1998.00398.x.
2. Allahverdiyev O, Nurten A, Enginar N. Assessment of rewarding and reinforcing properties of Biperiden in conditioned place preference in rats. Behav Brain Res. 2011;225(2):642-5. https://doi.org/10.1016/j.bbr.2011.07.050.
3. Coura CP de M, Fragoso VM da S, Valdez ECN, Paulino ET, Silva D, Cortez CM. Study on the interaction of three classical drugs used in psychiatry in albumin through spectrofluorimetric modeling. Spectrochim Acta - Part A Mol Biomol Spectrosc. 2021;255: 119638. https://doi.org/10.1016/j.saa.2021.119638.
4. Gjerden P, Bramness JG, Slørdal L. The use and potential abuse of anticholinergic antiparkinson drugs in Norway: A Pharmacoepidemiological Study. Br J Clin Pharmacol. 2009;67(2):228-33. https://doi.org/10.1111/j.1365-2125.2008.03342.x.
5. Katsanoulas K, Papaioannou A, Fraidakis O, Michaloudis D. Undiagnosed Central Anticholinergic Syndrome may lead to dangerous complications. Eur J Anaesthesiol. 1999;16(11):803-9. https://doi.org/10.1046/j.1365-2346.1999.00583.x.
6. Yap HL. Early psychosis intervention. Singapore Med J 2010;51(9):689-93.
7. Örüm MH. Biperiden dependence and differential diagnosis with Neuroleptic Malignant Syndrome: A Case Report. Istanbul Bilim Univ Florence Nightingale J Med. 2019;4: 194-6. https://doi.org/10.5606/fng.btd.2018.033.
8. Bakker C, Esdonk MJ, Stuurman R (F). E, Borghans LGJM, Kam ML, Gerven JMA et al. Biperiden challenge model in healthy elderly as proof-of-pharmacology tool: A Randomized, Placebo-Controlled Trial. J Clin Pharmacol. 2021; May 21. https://doi.org/10.1002/jcph.1913.
9. Song G, Cui Y, Han ZJ, Xia HF, Ma X. Effects of Choline on Sodium Arsenite-induced neural tube defects in chick embryos. Food Chem Toxicol. 2012;50(12):4364-74. https://doi.org/10.1016/j.fct.2012.08.023.
10. Tureci E, Asan Z, Eser M, Tanriverdi T, Alkan F, Erdincler P. The effects of Valproic Acid and Levetiracetam on chicken embryos. J Clin Neurosci. 2011;18(6):816-20. https://doi.org/10.1016/j.jocn.2010.11.005.
11. Chen L, Wang S, Feng Y, Zhang J, Du Y, Zhang J et al. Utilisation of chick embryo chorioallantoic membrane as a model platform for imaging-navigated biomedical research. Cells. 2021;10(2):463. https://doi.org/10.3390/cells10020463.
12. Victorelli FD, Cardoso VM de O, Ferreira NN, Calixto GMF, Fontana CR, Baltazar F et al. Chick embryo chorioallantoic membrane as a suitable in vivo model to evaluate drug delivery systems for cancer treatment: A review. Eur J Pharm

Biopharm. 2020;153:273-84. https://doi.org/10.1016/j.ejpb.2020.06.010.

13. Palombo P, Engi SA, Yokoyama TS, Bezerra AG, Curado DF, Anésio A et al. Effects of Biperiden (cholinergic muscarinic m1/m4 receptor antagonist) on Ethanol conditioned place preference in mice. Neurosci Lett. 2021;6(745):135551745. https://doi.org/10.1016/j.neulet.2020.135551.

14. Szczodry O, van der Staay FJ, Arndt SS. Modelling Alzheimer-like cognitive deficits in rats using biperiden as putative cognition impairer. Behav Brain Res. 2014;1(274):307-11. https://doi.org/10.1016/j.bbr.2014.08.036.

15. Ertekin T, Bilir A, Aslan E, Koca B, Turamanlar O, Ertekin A, et al. The effect of Diclofenac Sodium on neural tube development in the early stage of chick embryos. Folia Morphol. 2019;78(2):307-13. https://doi.org/10.5603/FM.a2018.0080.

16. Atay E, Ertekin A, Bozkurt E, Aslan E. Impact of Bisphenol A on neural tube development in 48-hr chicken embryos. Birth Defects Res. 2020;112(17):1386-96. https://doi.org/10.1002/bdr2.1791.

17. Rakip U, Bilir A, Arikan ES. Effect of Pethidine Hydrochloride on the development of neural tube: A genetic analysis study in a chick embryo model. World Neurosurg. 2021;150: e613-e620. https://doi.org/10.1016/j.wneu.2021.03.065.

18. Hamburger V, Hamilton HL. A Series of normal stages in the development of the chick embryo. J Morphol. 1951;88(1): 49-92. https://doi.org/10.1002/jmor.1050880104.

19. Wunsch MJ, Stanard V, Schnoll SH. Treatment of pain in pregnancy. Clin J Pain. 2003;19(3): 148-55. https://doi.org/10.1097/00002508-200305000-00002.

20. Van De Velde M, De Buck F. Anesthesia for non-obstetric surgery in the pregnant patient. Minerva Anestesiol 2007;73(4):235-40.

21. de Carvalho FB, de Gomes MG, Savall ASP, Fidelis EM, Pinton S, Ribeiro ACF, Munieweg FR et al. Evaluation of curcumin-loaded polymeric nanocapsules with different coatings in chick embryo model: Influence on angiogenesis, teratogenesis and oxidative stress. Pharmacol Reports. 2021;73(2):563-73. https://doi.org/10.1007/s43440-021-00218-2

22. Sayin M, Gurgen GS, Sayin SS, Guvenc G, Yuceer N. Does the anti-migraine drug rizatriptan affect early neural tube development in chick embryos? Turk Neurosurg. 2019;29(1):106-9. https://doi.org/10.5137/1019-5149.JTN.23431-18.1.

23. Ovalioglu TC, Canaz G, Arslan S, Ovalioglu AO, Sar M, Aydin AE et al. Effects of Quetiapine on neural tube development in the early stage of chicken embryos. Turk Neurosurg. 2021;31(1): 137-41. https://doi.org/10.5137/1019-5149.JTN.32887-20.1.

24. Stamilio, David M. "Drugs for Pregnant and Lactating Women." (2004): 408. https://doi.org/10.7326/0003-4819-140-5-200403020-00030

25. Baskak B, Atbasoglu EC, Ozguven HD, Saka MC, Gogus AK. The effectiveness of intramuscular Biperiden in acute akathisia: A double-blind, randomized, placebo-controlled study. J Clin Psychopharmacol. 2007;27(3):289-94. https://doi.org/10.1097/jcp.0b013e3180582439.

26. Toto S, Hefner G, Hahn M, Hiemke C, Roll SC, Wolff J et al. Current use of anticholinergic medications in a large naturalistic sample of psychiatric patients. J Neural Transm. 2021;128(2): 263-72. https://doi.org/10.1007/s00702-020-02298-5.

27. Hirose S. Insomnia related to Biperiden withdrawal in two schizophrenic patients. Int Clin Psychopharmacol. 2000;15(6):357-9. https://doi.org/10.1097/00004850-200015060-00007.

28. Wang HE. Street drug toxicity resulting from opiates combined with anticholinergics. Prehospital Emerg Care. 2002;6 (3):351-4. https://doi.org/10.1080/10903120290938463.

29. Espi Martinez F, Espi Forcen F, Shapov A, Martinez Moya A. Biperiden dependence: Case report and literature review. Case Rep Psychiatry. 2012;2012: 949256. https://doi.org/10.1155/2012/949256.

30. Sambeth A, Riedel WJ, Klinkenberg I, Kähkönen S, Blokland A. Biperiden selectively induces memory impairment in healthy volunteers: No interaction with citalopram. Psychopharmacology (Berl). 2015;232(11):1887-97. https://doi.org/10.1007/s00213-014-3822-9.

31. Guvenc Y, Dalgic A, Billur D, Karaoglu D, Aydin S, Daglioglu E et al. The Effects of Levetiracetam on neural tube development in the early stage of chick embryos. Turk Neurosurg. 2013;23(5):617-22. https://doi.org/10.5137/1019-5149.JTN.7471-13.0.