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# Topiramate and darbepoetin alpha ameliorate bilirubin-induced neuronal cell damage

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**Abstract. – OBJECTIVE: The aim of this study was to determine whether prophylactic darbepoetin alpha and/or topiramate administration could prevent bilirubin neurotoxicity (BNTx) in experimental model of kernicterus.**

**MATERIALS AND METHODS: A total of 60 Wistar albino rat puppies with experimental kernicterus model were included in the study. The Kernicterus was established administering a bilirubin injection via a cisterna magna puncture 30 minutes after ip drug injection. The puppies were divided into five groups with 12 in each group as shown below: a control group, bilirubin group, darbepoetin alpha group, topiramate group and darbepoetin alpha+ topiramate group. Darbepoetin alpha and/or topiramate were administered on day 5 intraperitoneally (ip). At the 6th and 24th hours, bilirubin induced neurological dysfunction (BIND) score was used to assess behavioral changes. Hearing functions were evaluated on days 10 and 28. On day 30, the Water Maze water tank test was implemented to evaluate spatial memory. The rats were sacrificed on days 6 and 34 and apoptosis in the globus pallidus and hippocampus was examined.**

**RESULTS: The BIND score was improved following darbepoetin alpha treatment. Neither darbepoetin alpha nor topiramate therapy ameliorate spatial memory. There were no significant differences between groups in terms of the auditory brainstem response (ABR). The combined use of darbepoetin alpha and topiramate lead to slight decrease in apoptosis.**

**CONCLUSIONS: Darbepoetin alpha or topiramate administration ameliorates bilirubin induced neurological dysfunction in experimental model of kernicterus.**

*Key Words:*
Kernicterus, Experimental, Rat pup, Darbepoetin alpha, Topiramate.

## Introduction

Unconjugated bilirubin (UCB) is a heme product pigment, and the accumulation of this pigment into the central nervous system during severe neonatal hyperbilirubinemia is the main factor causing bilirubin encephalopathy. The neurological manifestations of UCB toxicity range from transient or definitive auditory and visual impairment to death[1].

The major targets of UCB toxicity are neural and glial cells. The brain regions typically affected in kernicterus include: the globuspallidus, subthalamic nuclei, metabolic sector of the hippocampus, oculomotor nuclei, ventral cochlear nuclei, purkinje cells of the cerebellar cortex and cerebellar dentate nuclei[2]. However, the exact mechanisms of bilirubin encephalopathy remain unclear. The potential molecular mechanisms of bilirubin toxicity are neuro-inflammation, oxidative stress, mitochondrial energy failure and excitotoxicity[3-6].

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Erythropoietin (Epo), which was first described as a humoral mediator that functioned in the maturation and proliferation of erythroid progenitor cells, is one of the most promising neuroprotective agents. Epo may inhibit apoptosis. The other mechanisms of neuroprotection of Epo include the anti-inflammatory, anti-oxidant, angiogenic, anti-epileptic and neurotrophic effects[7]. In addition to the properties of Epo, darbepoetin alpha includes oligosaccharides containing sialic acid and has a longer half-life than darbepoetin alpha, and is more effective in vivo activity[8].

Topiramate (TPM), an anti-epileptic agent, may induce neuroprotection by GABAergic hyperpolarization. TPM has been shown to attenuate AMPA-kainate receptor mediated cell death and calcium influx[9].

Even though there is not any drug treatment to prevent bilirubin encephalopathy available today, the effects of darbepoetin alpha and topiramate have been chosen to investigate in preventing bilirubin encephalopathy due to its affecting the same pathways.

## Materials and Methods

### Animals and Drugs

This study was performed in accordance with the guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care and Use Committee of the DokuzEylül University, School of Medicine. Wistar rats with dated pregnancies were maintained at the same center and housed in individual cages with free access to water and laboratory chow.

Female rats, whose vaginal smear tests indicated they were in oestrus, were left to coitus. The day of coitus was considered the 0'th day of pregnancy. The animals inhabited an environment with a 12-hours dark, 12-hours daylight cycle, regulated at 20-25°C and 50% humidity, and were fed standard food.

The pups were kept with their mothers following birth. Five groups were established as the control group, bilirubin group, darbepoetin alpha group, topiramate group and darbepoetin alpha+topiramate group. Each group consisted of 12 Wistar rat pups.

Darbepoetin alpha (Aranesp) was stored as an injectable solution at a concentration of 25 $\mu g$/ml. Topiramate (Sigma-Aldrich, St. Louis, MO, USA) was stored as a solution at a concentration of 40 mg/mL. Bilirubin (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 0.5 M NaOH solution (100 mg/ml) and diluted in dd$H_2O$ to a concentration of 10 mg/ml, and the pH was adjusted to 8.5 with HCl (0.5 M); the solution was stored at 22°C, in the dark. Darbepoetin alpha was administered at10 $\mu g$/kg[10] and topiramate 50 mg/kg[11] on day 5 intraperitoneally (ip). The Kernicterus model was established by administering a 10 $\mu g$/g bilirubin injection via a cisterna magna puncture 30 minutes after ip drug injection[12]. An equal volume of CSF specimen was drained before bilirubin was applied. No attempt was made to the control group. The BIND score was used at the 6th and 24th hours after drug injection to assess behavioral changes. On days 10 and 24, hearing functions were evaluated by auditory evoked brain stem potency tests (ABR). When the rats were 30 days old, they were subjected to the Water Maze water tank test to evaluate spatial memory. Rats were sacrificed on days 6 and 34; apoptosis in the globuspallidus and hippocampus was examined by caspase-3 and TUNEL methods.

### Bilirubin Induced Neurological Dysfunction (BIND) Score

Pubs (m) were evaluated at the 6th and 24th hours after drug injection at 5th days to assess behavioral changes and were assigned BIND score using a previously described numeric rating scale that quantifies gait abnormalities and dystonia on a scale of 0-5 based on the following findings: 0 = normal; 1 = mildly abnormal with slight hindlimb ataxia, dystonia, and gait abnormality; 2 = mild hindlimb ataxia, dystonia, and gait abnormality with impaired righting reflex; 3 = abnormal as in 2, but with a more severe movement disorder and prolonged righting reflex; 4 = severe failure of locomotion, general lack of spontaneous movement with occasional bursts of hyperactivity and no righting reflex; and 5 = moribund including seizures and/or agonal respirations[13].

### Audiological Tests

Wistar rats were assessed by auditory evoked brain stem potency tests on days 10 and 24. Prior to the test, intraperitoneal ketamine hydrochloride (40 mg/kg) and xylazine hydrochloride (5 mg/kg) were anesthetized. The two-channel ICS Medical Charter Compact Diagnostic Systems device (MCU 80), (ICS Medical Corporation, M-USA) was used for recordings via insert earphones and headphones in silent room[14]. The insert earphones were placed directly into the rat's external auditory canals. A neonatal probe tip

was used to seal the external auditory canal. Subdermal needle electrodes were placed over the vertex (active), the right and left retro auricular regions (reference) and on the dorsum (ground). The stimuli used were alternating clicks (pulse duration 0.1 ms) at a rate of 31.1/s. EEGactivity was preamplified (100,000×) and band-pass analog-filtered from 100 to 3000 Hz for click stimuli. The number of averaged responses was 1024 and each averaged response was replicated. Each series of stimuli began from supra-threshold level with subsequent measures using lower intensity levels. Stimulus level was decreased progressively and intensities that appeared to be near threshold were replicated. Threshold was defined as the lowest intensity to elicit repeatable components of ABR in at least two trials. Electrode resistances were kept below 3 k-ohm. The stimulus was initially delivered at 85 dB HL, and the intensity level was reduced by 20 dB steps up to the threshold. Closer to threshold levels, the threshold was detected with 10 dB intensity changes. At least two traces were created for each measurement to ensure the reliability of the wave. The ABR thresholds were evaluated separately by two investigators. The ABR threshold was defined as the lowest intensity level in which Wave II was observable. Since the outer ear channels of the rats were not opened in the ABR test on the 10th day of life of the rats, the ear specimens were applied to the rats' ears by adaptation to the TDH-49 head and applying the serum hoses to the rats' ears[15]. On the 24th day of the birth of rats ABR tests were applied with insert earphone[16]. ABR tests were obtained bilaterally.

### Morris Water Maze Experiment

When the rats were 30 days old, they were subjected to the Water Maze water tank test to evaluate spatial memory. The test was applied according to the previous method[17]. Day 0 of the test was planned as practice, 1, 2 and 3 were for learning and the fourth day was allocated as the test day. The test was aimed to measure the learning and memory ability of the rats based on the differences in the way, and the delay in finding the platform.

### Light Microscopic Evaluation

Tissue samples were taken in 10% formaldehyde and flicked for 48 hours. Then, the tissue was buried in paraffin blocks after a routine tissue follow-up procedure. 5 µm coronal sections were obtained from the blocks. According to rat brain atlas, sections were taken from 16, 17 planes for the globuspallidus, and 21, 23, 25 for the hippocampus (CA1 region)[18].

### Determination and Evaluation of Apoptosis

TUNEL (The DeadEnd Colorimetric TUNEL system kit, Roche, Mannheim, Germany) was used to detect DNA fragmentation in cell nuclei. Sections were deparaffinized, rehydrated and pretreated in proteinase K for 15 minutes. After washing, tissues were incubated TdT at 37°C for 60 minutes. Converter POD (Detection of Peroxidase) solution was then applied to the slides. Sections were stained with diaminobenzidine (DAB) and counter-stained with Mayer hematoxylin and analyzed[19].

### Immunohistochemical Scoring

For immunohistochemical scoring, non-overlapping areas were selected randomly. The grade of positive staining was assessed by semi-quantitative scoring using an indicator scale ranging from 0 to 3 in terms of intensity and distribution. If there was no immune-reactivity, a point of 0 was allocated; very little positive staining was labeled as 1, moderate positive staining as 2, and strong positive staining was labeled as 3. The average of the scores was calculated[20].

### Image Analysis

After the staining process, sections were examined with a light microscope (Olympus BX-51, Tokyo, Japan) and images were transferred to the computer using a high-resolution camera (Olympus DP-71, Japan). All sections were digitally photographed. Measurements were made using the UTHSCA Image Tool (software version 3.0, University of Texas Health Science Center, USA)[21].

### Statistical Analysis

Data were analyzed using SPSS version 22.0 (IBM, Armonk, NY, USA). Values were presented as mean ± SD. Since the data obtained from the study were inconsistent with normal distribution and which had a small sample size, we used the non-parametrical statistical methods. When the sample size is convenient, the assumption of normality of the distribution was evaluated with the Shapiro-Wilk test. One Way ANOVA was used for parametric group comparison with Post-Hoc Games Hovel test for pairwise comparisons. Differences between all groups were examined

with Kruskal Wallis test for non-parametric data and the pairwise comparisons were examined with the Mann-Whitney U-test, with findings of $p < 0.05$ were considered significant.

## Results

There was no difference in gender distribution between the groups. Mean body weights were 10.2 ± 1.5 g and there was no significant difference between the groups. There were no significant differences in brain/body weight ratios of animals sacrificed during acute and chronic periods. There was no significant difference between groups in daily body weight gain.

In early neurological evaluation, the BIND score was higher in the bilirubin group ($p < 0.05$; Table I). The score was significantly lower in the darbepoetin alpha treatment group ($p < 0.05$; Table I). No significant improvement was observed in the topiramate group. When the darbepoetin alpha and darbepoetin alpha+topiramate groups were compared, no synergistic effect was detected. Auditory brainstem potentials obtained from the right and left ear showed normal wave morphology and reverse wave morphology obtained by rarefaction and condensation polarities at 85 dB nHL. There were no reverse waves and significant differences in auditory brainstem potentials between control and bilirubin groups on the 10th and 24th days (Figure 1). On day 4 of the Water Maze experiment, based on the analysis of variance ($p = 0.035$), the time to find the platform in the bilirubin group was significantly higher when compared with the control group. However, neither darbepoetin alpha nor topiramate, nor the combination of the two drugs improved spatial memory (Figure 2). The examination of the globuspallidus and hippocampus by the caspase-3 and TUNEL methods in acute and long-term groups, revealed an increase in apoptosis in the bilirubin group. When examined individually, darbepoetin alpha and darbepoetin alpha did not yield histological improvement, whereas when the two drugs were used in combination, both the hippocampus and the globus pallidus showed a decrease in apoptosis. However, this was not statistically significant (Figures 3, 4, 5, 6).

## Discussion

Kernicterus is a devastating, chronic disabling neurological disorder that reflects both a predilection of bilirubin toxicity for neurons and a regional topography of bilirubin-induced neuronal injury that is characterized by prominent basal ganglia, cochlear, and oculomotor nuclei involvement[22]. UCB inhibits synaptic connections in the long term, leading to learning and memory disorders[23]. Considering the potential molecular mechanisms of bilirubin toxicity, this study evaluated the neuroprotective effects of darbepoetin alpha, topiramate and a combination of the two, on bilirubin induced neurotoxicity at both histological and functional level and demonstrated a partial benefit.

UCB inhibits protein kinase C activity in cultured neurons. This activity is essential for eliciting calcium influx through the high conductance cation channels in the plasma membrane of the postsynaptic neuron and maintaining synaptic activity. Membrane depolarization and binding of glutamate to N-methyl-D-aspartate (NMDA, class 1) receptors allow this channel to open[24]. In addition, the exposure of astrocytes to UCB decreases the uptake of glutamate, and conse-

**Table I.** BIND scores according to groups*.

| Groups | aBIND 0 hour | aBIND 6 hours | aBIND 24 hours |
|---|---|---|---|
| 1: Control | 0 (0.00) | 0 (0.00) | 0 (0.00) |
| 2: Bilirubin | 0 (0.00) | 2.4 (0.55) | 3.6 (0.55) |
| 3: Darbepoetin alpha | 0 (0.00) | 0.6 (0.55) | 1.4 (0.55) |
| 4: Topiramate | 0 (0.00) | 1.4 (0.55) | 2.2 (0.45) |
| 5: Darbepoetin alpha + Topiramate *p*-value < 0.05 | 0 (0.00) | 0.6 (0.55) | 1 (0.00) |
| 1 *vs*. 2 | 1 | 0.004** | 0.004** |
| 2 vs. 3 | 1 | 0.008** | 0.008** |
| 2 *vs*. 4 | 1 | 0.056 | 0.016** |
| 2 *vs*. 5 | 1 | 0.008** | 0.008** |
| 3 *vs*. 5 | 1 | 1 | 0.31 |

*Values are expressed as mean (± SD), **$p < 0.05$.

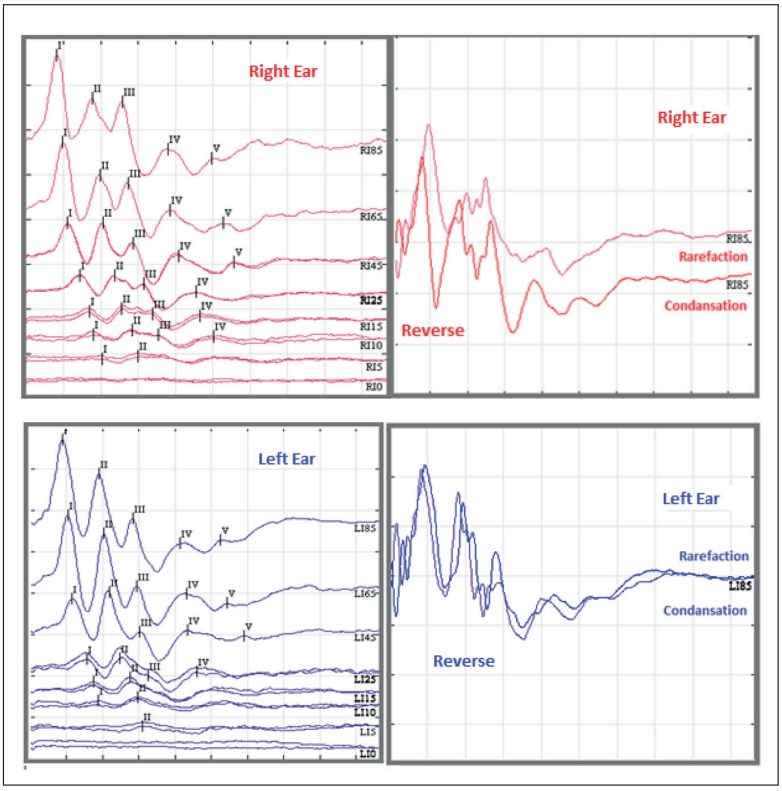
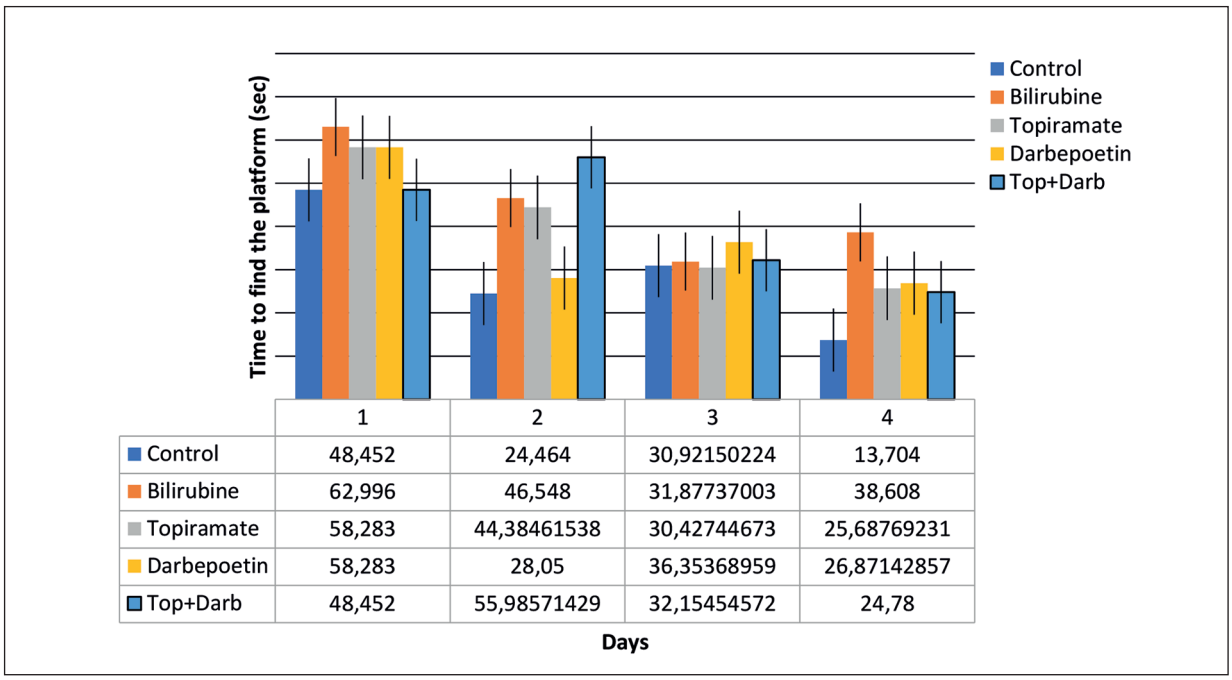



**Figure 1.** F ABR tests obtained from the right and left ear normal wave morphology and reverse wave morphology obtained by Rarefaction and Condensation polarities at 85 dB nHL.

quently, the duration of glutamate in the synaptic cleft increases. These engender overstimulation of NMDA receptors and excitotoxicity[25]. Excitotoxicity induces neuronal cell swelling due to excessive influx of $Na^{+}$, $Ca^{+2}$, $Cl^{-}$ and water, triggering cell death by both apoptosis and necrosis[26]. UCB may directly damage the nerve cell membrane by increasing both oxidative stress and




| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Control | 48,452 | 24,464 | 30,92150224 | 13,704 |
| Bilirubine | 62,996 | 46,548 | 31,87737003 | 38,608 |
| Topiramate | 58,283 | 44,38461538 | 30,42744673 | 25,68769231 |
| Darbepoetin | 58,283 | 28,05 | 36,35368959 | 26,87142857 |
| Top+Darb | 48,452 | 55,98571429 | 32,15454572 | 24,78 |

**Figure 2.** Comparison of Morris Water Maze Test.

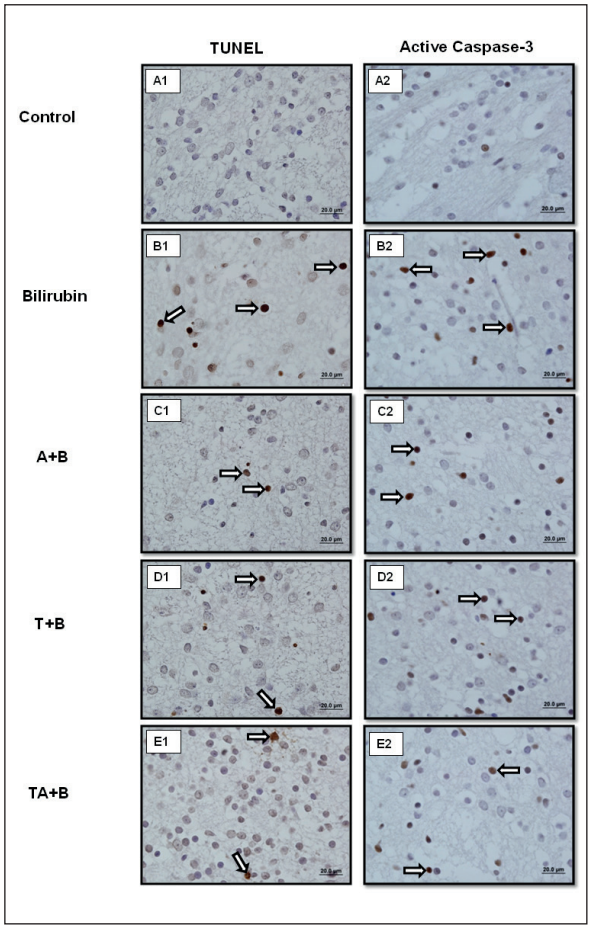



**Figure 3.** Representative light-microscopic images of TUNEL (A1,B1,C1,D1,E1) and anti-active caspase-3 (A2,B2,C2,D2,E2) immunoreactivity of early stage group in globus pallidum of brain tissue; in the control group (A1-2), bilirubin group (B1-2), A+B group (C1-2), T+B group (D1-2) and TA+B group (E1-2). Arrows (→) indicate TUNEL-positive cells and anti-active caspase-3 immune positive cells.

membrane permeability[27]. In developing rat brain neurons, UCB passes through the mitochondrial membranes causing mitochondrial swelling and the release of cytochrome-c into the cytosol[28]. This triggers activation of caspase-3 and translocation of Bax resulting in cell death by apoptosis via the mitochondrial pathway[29].

In this study, the BIND score was higher in the bilirubin group in early neurological evaluation demonstrated that an experimental model had been generated.

Darbepoetin alpha was found to improve the BIND score in the acute phase, and this early benefit was attributed to being possibly due to the anti-inflammatory and antioxidant effects of darbepoetin alpha. Memişoğluet al[30] demonstrated the anti-inflammatory effect of recombinant darbepoetin alpha for the rat brain and they found that serum TNF alpha, IL-10 and glutathione levels were decreased with darbepoetin alpha.

Bilirubin neurotoxicity in Gunn rat pups, as well as human newborns, produces specific, progressive, potentially reversible increases in the inter-wave intervals and decreases in wave amplitudes of the auditory brainstem response[31]. Brainstem cochlear nuclei are the first structures affected by elevated unconjugated bilirubin levels, followed by the auditory nerve[32]. However,

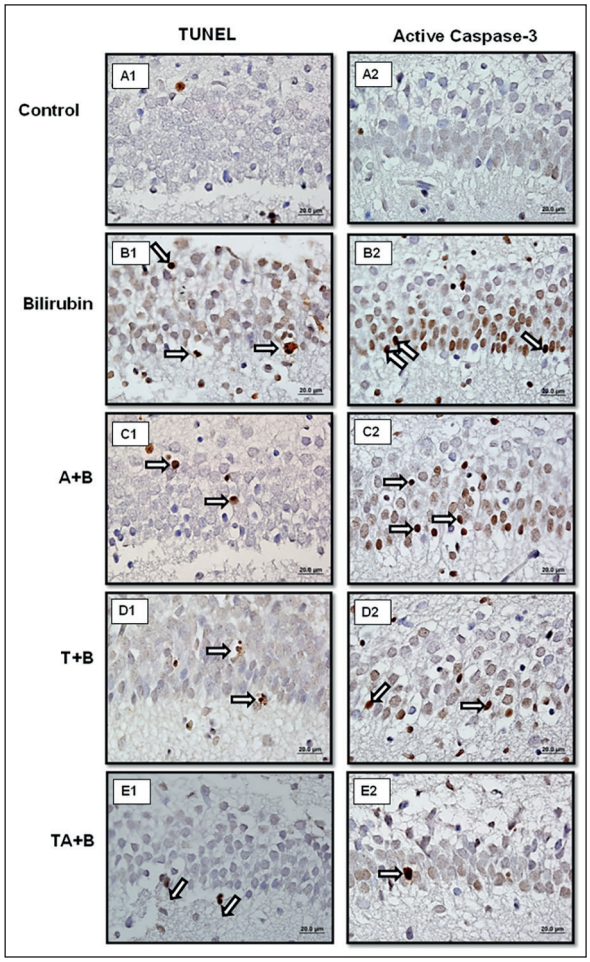



**Figure 4.** Representative light-microscopic images of TUNEL (A1,B1,C1,D1,E1) and anti-active caspase-3 (A2,B2,C2,D2,E2) immunoreactivity of early stage groups in CA1 region of hippocampus; in the control group (A1-2), bilirubin group (B1-2), A+B group (C1-2), T+B group (D1-2) and TA+B group (E1-2). TUNEL and anti-active caspase-3 immunoreactivity were increased in bilirubin group compared to control group whereas the immunoreactivity was decreased in TA+B group compared to bilirubin group. Arrows (→ indicate TUNEL-positive cells and anti-active caspase-3 immune positive cells.

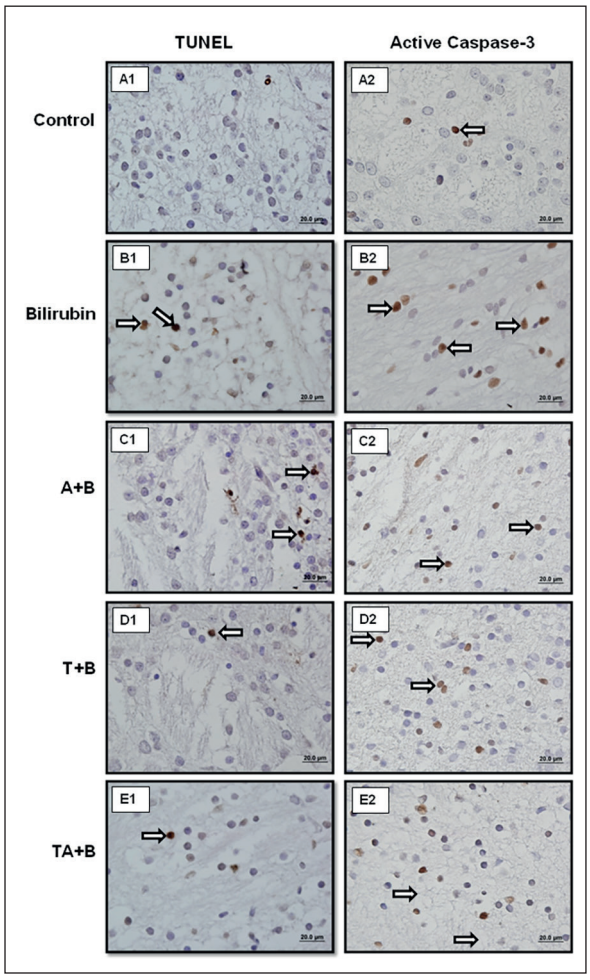



**Figure 5.** Representative light-microscopic images of TUNEL (A1,B1,C1,D1,E1) and anti-active caspase-3 (A2,B2,C2,D2,E2) immunoreactivity of long term groups in globus pallidum of brain tissue; in the control group (A1-2), bilirubin group (B1-2), A+B group (C1-2), T+B group (D1-2) and TA+B group (E1-2). TUNEL and anti-active caspase-3 immunoreactivity were increased in bilirubin group compared to control group whereas the immunoreactivity was decreased in TA+B group compared to bilirubin group. Arrows (→) indicate TUNEL-positive cells and anti-active caspase-3 immune positive cells.

we could not find any difference in the ABR test between the bilirubin group and the control group, possibly due to the inability of bilirubin to penetrate the cochlear nucleus.

In this study, the time spent in the bilirubin group to find the platform in the Morris Water Maze test was significantly higher compared to the control group. This suggests that the neurotoxic effects of bilirubin administered exogenously to the brain spinal fluid negatively affect learning and especially spatial memory. Similar results of a previous study demonstrated that the bilirubin-treated rats had significantly worse Water Maze test results than the controls. These results indicate that the bilirubin-treated rats experienced learning and memory deficits at the age of 28 days; this is comparable to the clinical features in preschool children with kernicterus[12]. In our study, neither darbepoetin alpha nor topiramate nor the combined use of both was found to improve spatial memory. Our dosage may have been too low or repeated doses may have been necessary in order to yield the protective effects

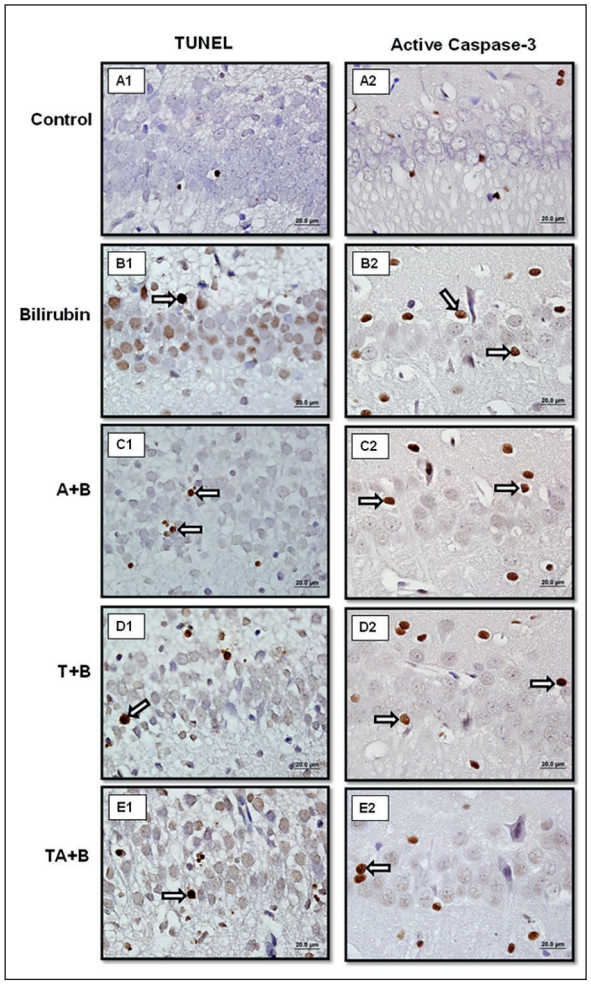



**Figure 6.** Representative light-microscopic images of TUNEL (A1,B1,C1,D1,E1) and anti-active caspase-3 (A2,B2,C2,D2,E2) immunoreactivity of long term groups in CA1 region of hippocampus; in the control group (A1-2), bilirubin group (B1-2), A+B group (C1-2), T+B group (D1-2) and TA+B group (E1-2). TUNEL and anti-active caspase-3 immunoreactivity were increased in bilirubin group compared to control group whereas the immunoreactivity was decreased in TA+B group compared to bilirubin group. Arrows (→) indicate TUNEL-positive cells and anti-active caspase-3 immune positive cells.

of EPO and/or topiramate. Possibly due to the repeated doses, a study[30] determined EPO treatment demonstrated a neuro-protective effect against phenylhydrazine induced hemolytic hyperbilirubinemia in neonatal rats when treatments were administered on three consecutive days.

In acute and long-term groups, the evaluation of the globuspallidus and hippocampus using the caspase-3 and TUNEL methods revealed an increase in apoptosis in the bilirubin group. When darbepoetin alpha and topiramate were used together in both acute and long-term applications, compared to the bilirubin group, there was a slight decrease in apoptosis. The anti-apoptotic effect of topiramate has previously been shown in hypoxia-ischemia (HI) studies. The neuro-protective effect of TPM is linked to influencing the amount of excessive glutamate release, because TPM is able to suppress pre-synaptic voltage sensitive sodium channel of excitatory synapses[9]. The protective effect of EPO against neurotoxicity is associated with the inhibition of NF-κB activation and the suppression of apoptosis that involves the down-regulation of apoptotic molecules by preventing caspase-3 activation[30]. It is possible that darbepoetin alpha and topiramate may have a synergistic effect against bilirubin induced apoptosis[33-36].

There are a few limitations of this study. This was a preliminary study and molecular methods explaining the bilirubin toxicity and potential neuro-protective effects of darbepoetin alpha and topiramate are not sufficient. The model used in this study is a newly established bilirubin encephalopathy model, and it does not reflect a human since the given bilirubin bypasses the blood brain barrier directly which is an exception to normal physiology. A positive aspect of the model is that bilirubin is administered directly and equally to the central nervous system of each animal, regardless of bilirubin metabolism, and thus standardization between groups is ensured. Furthermore, considering the indicated doses were neuro-protective in previous studies, standard darbepoetin alpha and topiramate doses were used and results might have varied if the drugs were administered at different doses and repeatedly.

## Conclusions

Finally, in the presented study we demonstrated that darbepoetin alpha and/or topiramate ameliorates bilirubin induced neurotoxicity in rat model of kernicterus. Further studies with different treatment regimens are needed to clarify the exact role of these two drugs on biluribin induced neurotoxicity are required.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

### Acknowledgements

This study was conducted in the Neonatology Department of the DokuzEylül University School of Medicine, Izmir, Turkey. This work was supported by the Turkish Neonatal Society.

## References

1) Bhutani VK, Johnson-Hamerman L. The clinical syndrome of bilirubin-induced neurologic dysfunction. Semin Fetal Neonatal Med 2015; 20: 6-13.
2) Han GY, Li CY, Shi HB, Wang JP, Su KM, Yin XL, Yin SK. Riluzole is a promising pharmacological inhibitor of bilirubin-induced excitotoxicity in the ventral cochlear nucleus. CNS Neurosci Ther 2015; 21: 262-270.
3) Schiavon E, Smalley JL., Newton S, Greig NH, Forsythe ID. Neuroinflammation and ER-stress are key mechanisms of acute bilirubin toxicity and hearing loss in a mouse model. PLoS One 2018; 13: e0201022.
4) Dani C, Poggi C, Pratesi S. Bilirubin and oxidative stress in term and preterm infants. Free Radic Res 2019; 53: 2-7.
5) Cayabyab R, Ramanathan R. High unbound bilirubin for age: a neurotoxin with major effects on the developing brain. Pediatr Res 2019; 85: 183-190.
6) Watchko JF. Bilirubin-Induced Neurotoxicity in the Preterm Neonate. Clin Perinatol 2016; 43: 297-311.
7) Kumral A, Tüzün F, Oner MG, Genç S, Duman N, Ozkan H. Erythropoietin in neonatal brain protection: the past, the present and the future. Brain Dev 2011; 33: 632-643.
8) Park S, Fenaux P, Greenberg P, Mehta B, Callaghan F, Kim C, Tomita D, Xu H. Efficacy and safety of darbepoetin alpha in patients with myelodysplastic syndromes: a systematic review and meta-analysis. Br J Haematol 2016; 174: 730-747.
9) Motaghinejad M, Motevalian M, Fatima S, Beiranvand T, Mozaffari S. Topiramate via NMDA, AMPA/kainate, GABA(A) and Alpha2 receptors and by modulation of CREB/BDNF and Akt/GSK3 signaling pathway exerts neuroprotective effects against methylphenidate-induced neurotoxicity in rats. J Neural Transm (Vienna) 2017; 124: 1369-1387.

10) Grasso G, Graziano F, Sfacteria A, Carletti F, Meli F, Maugeri R, Passalacqua M, Certo F, Fazio M, Buemi M, Iacopino DG. Neuroprotective effect of erythropoietin and darbepoetin alfa after experimental intracerebral hemorrhage. Neurosurgery 2009; 65: 763-770.

11) Clark AM, Mondick JT, Cloyd JC, Zuppa AF, Raol YH, Clancy RR. Plasma topiramate concentrations resulting from doses associated with neuroprotection against white matter injury and stroke in two strains of rat pups. Pediatr Res 2013; 73: 317-324.

12) Song S, Hu Y, Gu X, Si F, Hua Z. A novel newborn rat kernicterus model created by injecting a bilirubin solution into the cisterna magna. PLoS One 2014; 9: e96171-e96171.

13) Daood MJ, Hoyson M, Watchko JF. Lipid peroxidation is not the primary mechanism of bilirubin-induced neurologic dysfunction in jaundiced Gunn rat pups. Pediatr Res2012; 72: 455-459.

14) Olgun Y, Kırkım G, Kolatan E, Kıray M, Bağrıyanık A, Şerbetçioğlu B, Yılmaz O, Gökmen N, Ellidokuz H, Kumral A. Otoprotective effect of recombinant erythropoietin in a model of newborn hypoxic-ischemic encephalopathy. Int J Pediatr Otorhinolaryngol2013;7: 739-746.

15) Kesici GG, Öcal FCA, Gürgen SG, Erdem ŞR, Öğüş E, Erbek HS, Özlüoğlu LN. The protective effect of metformin against the noise-induced hearing loss. Eur Arch Otorhinolaryngol2018; 275: 2957-2966.

16) Kuriyama M, Konishi Y, Sudo M. Auditory brainstem response in hyperbilirubinemic rat: Part I. Biol Neonate 1990; 58: 32-40.

17) Cai J, Kang Z, Liu K, Liu W, Li R, Zhang JH, Luo X, Sun X. Neuroprotective effects of hydrogen saline in neonatal hypoxia–ischemia rat model. Brain Res2009; 1256: 129-137.

18) Paxinos G, Watson C. The rat brain in stereotaxic coordinates: hard cover edition. Elsevier, 2006.

19) Kumral A, Tuzun F, Ozbal S, Ugur EB, Yilmaz O, Duman N, Ozkan H. Lipopolysaccharide-preconditioning protects against endotoxin-induced white matter injury in the neonatal rat brain. Brain Res 2012; 1489: 81-89.

20) Gencpinar P, Tuzun F, Ozbal S, Tugyan K, Duman N, Ozkan H, Kumral A. Effects of neotrofin on neonatal hypoxic ischemic brain injury. Neurosci Lett 2011; 505: 205-210.

21) Yiş U, Kurul SH, Kumral A, Cilaker S, Tuğyan K, Genç Ş, Yılmaz O. Hyperoxic exposure leads to cell death in the developing brain. Brain Dev 2008; 30: 556-562.

22) Watchko JF. Kernicterus and the molecular mechanisms of bilirubin-induced CNS injury in newborns. Neuromolecular Med 2006; 8: 513-529.

23) Amin SB, Smith T, Timler G. Developmental influence of unconjugated hyperbilirubinemia and neurobehavioral disorders. Pediatr Res 2019; 85: 191-197.

24) Vandresen-Filho S, Severino PC, Constantino LC, Martins WC, Molz S, Dal-Cim T, Bertoldo DB, Silva FRMB, Tasca CI. N-methyl-D-aspartate preconditioning prevents quinolinic acid-induced deregulation of glutamate and calcium homeostasis in mice hippocampus. Neurotox Res 2015; 27: 118-128.

25) Wu Y, Chen C, Yang Q, Jiao M, Qiu S. Endocytosis of GluN2B-containing NMDA receptors mediates NMDA-induced excitotoxicity. Mol Pain 2017; 13: 1744806917701921-1744806917701921.

26) Wang R, Reddy PH. Role of glutamate and NMDA receptors in Alzheimer's disease. J Alzheimers Dis 2017; 57: 1041-1048.

27) Qaisiya M, Coda-Zabetta CD, Bellarosa C, Tiribelli C. Bilirubin mediated oxidative stress involves antioxidant response activation via Nrf2 pathway. Cell Signal 2014; 26: 512-520.

28) Vaz AR, Delgado-Esteban M, Brito MA, Bolaños JP, Brites D, Almeida A. Bilirubin selectively inhibits cytochrome c oxidase activity and induces apoptosis in immature cortical neurons: assessment of the protective effects of glycoursodeoxycholic acid. J Neurochem2010; 112: 56-65.

29) Rodrigues CMP, Solá S, Brites D. Bilirubin induces apoptosis via the mitochondrial pathway in developing rat brain neurons. Hepatology 2002; 35: 1186-1195.

30) Memisoglu A, Kolgazi M, YamanA, Bahadir E, Sirvanci S, Yeğen BÇ, Ozek E. Neuroprotective effect of erythropoietin on phenylhydrazine-induced hemolytic hyperbilirubinemia in neonatal rats. Neurochem Res2017; 42: 1026-1037.

31) Jiang ZD, Wilkinson AR. Impaired function of the auditory brainstem in term neonates with hyperbilirubinemia. Brain Dev 2014; 36: 212-218.

32) Olds C, Oghalai JS. Audiologic impairment associated with bilirubin-induced neurologic damage. Semin Fetal Neonatal Med 2015; 20: 42-46.

33) Celik O, Celik N, Zan E, Dalkilic S, Saglam A, Yurci A, Senturk S, Sahin E, Sahin ME. Genome-wide expression analysis of endometrium before and after endometrioma surgery. Eur J Obstet Gynecol Reprod Biol 2020; 253: 141-147.

34) Ersahin SS, Yurci A. Cord blood and maternal serum preptin and irisin concentrations are regulated independently in GDM. Eur Rev Med Pharmacol Sci 2021; 25: 1954-1958.

35) Yurci A, Dokuzeylul Gungor N, Gurbuz T. Spectroscopy analysis of endometrial metabolites is a powerful predictor of success of embryo transfer in women with implantation failure: a preliminary study. Gynecol Endocrinol 2021; 11: 1-11.

36) Yurci A, Kamar A, Turktekin N, Ozyurt R. Comparison of immediate versus delayed frozen embryo transfer on reproductive outcome. Clin Exp Obstet Gynecol 2021; 48: 263-269.