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The Effect of Pycnogenol® on Spatial Learning and Memory in Rats with Experimental Closed Head Injury

Deneysel Kapalı Kafa Travması Oluşturulan Sıçanlarda Piknogenol'ün® Uzaysal Öğrenme ve Bellek Fonksiyonlarına Etkisi

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Abstract

Aim: Trauma is a leading cause of emergency admissions. In this study, we investigated the effect of Pycnogenol® on spatial learning and memory (SLM) function in rats subjected to closed head injury.

Methods: The study was a randomized, experimental study of four groups, each containing six rats. Pycnogenol® was administered to rats in two groups (group three and four) daily for five days starting on day one. A Barnes maze was used to test SLM in the rats in all four groups. Group 1: These rats did not have a closed head injury and were not administered Pycnogenol®. Group 2: On the day three, closed head trauma was inflicted. Group 3: Pycnogenol® was administered to the rats. On day three, closed head trauma was inflicted. Group 4: Only Pycnogenol® was administered. At the end of day five, the brain tissue of the 24 rats was removed.

Results: There were no significant differences between the groups in mean SLM durations on days one through five. No significant differences were detected in the pathological examination between of the four groups.

Conclusion: Future studies that employ biochemical markers and free radical levels in the brain are needed.

Keywords: Antioxidant, pycnogenol, secondary brain damage

Öz

Amaç: Travma acil başvurularının önde gelen bir nedenidir. Bu çalışmada, kapalı kafa travması oluşturulan sıçanlarda Piknogenol'ün® uzaysal öğrenme ve bellek (UÖB) üzerine etkisini araştırdık.

Yöntemler: Çalışma her biri altı sıçan içeren dört gruptan oluşan randomize, deneysel bir çalışmadır. Piknogenol®, iki gruptaki sıçanlara (grup üç ve dört) birinci günden başlayarak beş gün boyunca uygulandı. Dört gruptaki sıçanların tamamında UÖB fonksiyonlarını test etmek için beş gün boyunca Barnes labirenti kullanıldı. Grup 1: Kontrol grubu olarak belirlenen bu gruptaki sıçanlara travma ve Piknogenol® uygulanmadı. Grup 2: Üçüncü gün kapalı kafa travması uygulandı. Grup 3: Her gün Piknogenol® ve üçüncü günde kapalı kafa travması uygulandı. Grup 4: Yalnızca Piknogenol® uygulandı, kafa travması uygulanmadı. Beşinci günün sonunda 24 sıçanın beyin dokusu perfüzyon-fiksasyon yöntemiyle çıkartıldı.

Bulgular: Gruplar arasında ortalama UÖB süreleri yönünden, birinci günden beş günün sonuna kadar istatistiksel olarak anlamlı fark saptanmadı. Ayrıca patolojik incelemede dört grup arasında belirgin fark yoktu.

Sonuç: Beyin dokusunda biyokimyasal belirteçlerin ve serbest radikal düzeylerinin bakıldığı Piknogenolle® ilgili yeni çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Antioksidan, piknogenol, sekonder beyin hasarı

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Introduction

Trauma is a leading cause of emergency department admissions. In a retrospective study, it has been reported that 12.2% of all emergency department admissions (n=110.495) were due to trauma (1-3). Considering its clinical consequences, emergency and neurosurgery specialists pay special attention to head trauma (4-6). The morbidity and mortality risk associated with head trauma can be decreased by implementing effective measures (7). In head trauma, primary injury develops as a result of direct mechanical trauma, and the clinical profile is determined by injury location and severity (8). The prognosis is affected by whether the head injury is focal or diffuse. However, secondary injury is caused by oxidative stress, apoptosis, and decreased cellular energy production by brain cells, particularly in the mitochondria (8). On the basis of this knowledge, antioxidant-mediated prevention of the secondary injury cascade caused by medium-intensity head trauma has recently drawn significant attention (9). Ansari et al. (10) showed that Pycnogenol®, an antioxidant made of pine bark extract, demonstrated some neuroprotective properties in traumatic brain injury. In this study, we investigated the effect of Pycnogenol® on spatial learning and memory (SLM) function in rats subjected to experimental low-to-moderate intensity closed head injury.

Methods

Animals

The study was a randomized, experimental study of four groups, each containing six Sprague-Dawley rats. The experiments were conducted at and the study animals were provided by the Başkent University Laboratory Animal Production and Research Center, Ankara, Turkey. The study protocol was approved by the University Animal Studies Ethics Committee (Project No: DA16/13), and the study was funded by Başkent University Research Fund.

Experimental Treatment

The experimental treatment started with Pycnogenol® (30-mg capsules) (Solgar, Leonia, New Jersey, USA). The contents were removed from the capsules and homogenized using an electromagnetic mixer. The Pycnogenol® treatment was administered at the dose described by Huang et al. (11) (40 mg per kg daily) using orogastric gavage. Pycnogenol was administered to rats in two groups daily for five days starting on day one.

Outcome Assessment

A Barnes maze was used to test the SLM of the rats in all four groups (12). A 122-centimeter diameter wooden plate was used to constitute the maze. The plate had 18 vents with nine centimeter diameter. A drawer was located under one of the vents. The rats were left in the center of the maze randomly for five consecutive days to find the drawer in 120 seconds.

Study Groups

Group 1 (Control): These rats did not have a closed head injury and were not administered Pycnogenol®. The rats' SLM functions were tested in a Barnes maze for five consecutive days.

Group 2 (Head Trauma): SLM functions of the rats in this group were tested on days one and two. On the day three, using a 50-g weight, closed head trauma was inflicted under general anesthesia using the procedure described by Marmarou et al. (13). On the days four and five, the Barnes maze was used to investigate whether SLM functions were affected by the trauma during the acute and subacute periods.

Group 3 (Head trauma + Pycnogenol®): Pycnogenol® was administered to the rats in this group using the protocol described above. SLM functions of the rats in this group were tested in the Barnes maze on days one and two. On day three, closed head trauma was inflicted under general anesthesia using the method described for Group 2. SLM test was administered using the Barnes maze on days four and five.

Group 4 (Pycnogenol®): Pycnogenol was administered to the rats in this group using the protocol described above. SLM functions were tested in the Barnes maze daily for five days. No head trauma was inflicted on this group.

Pathological Examination

At the end of day five, the brain tissue of the 24 rats was removed and fixed in 10% formaldehyde using the perfusion and immersion-fixation process for pathological examination. The brain tissue sections, with an average thickness of three mm, were fixed and stained with hematoxylin and eosin. The signs of apoptosis were assessed under a light microscope.

Statistical Analysis

We analyzed SLM test results using SPSS (version 17.0). The normal distribution of the continuous variables was tested using the Shapiro-Wilk test. The four groups were compared with respect to SLM functions using one-way analysis of variance (ANOVA) tests. The differences between the tests results obtained on different days from the same rat were compared with ANOVA for repeated measurements. A p-value of less than 0.05 was considered statistically significant.

Results

The mean weight of the rats was 363±28.3 g. The mean SLM durations (duration of time to complete the Barnes maze) of the groups are presented in Table 1. There were no significant differences between the groups regarding mean SLM durations on days 1 through 5 (p=0.540, 0.222, 0.962, 0.227, and 0.184, respectively). There were no significant differences between mean SLM durations of rats in each group, measured for 5 consecutive days (p=0.488). No significant differences were detected in the pathological examination between the four groups. Apoptosis was not observed in any rat brain. Figures 1-4 show the brain tissues of the second rat in each group.

Table 1. Mean spatial learning and memory durations of the rats

Group	Day 1	Day 2	Day 3	Day 4	Day 5
Control	33.6	77.6	39	22	37
Head trauma	53	44.5	42	49	44
Head trauma + Pycnogenol®	–	31.5	51	56	51
Pycnogenol®	59.6	60	35.8	29	17

Discussion

Findings from previous studies suggested that further studies are needed regarding the *in vivo* use of Pycnogenol® in humans. Among the first studies on this subject was Van Jaarsveld's investigation of Pycnogenol® in myocardial mitochondrial ischemia. Pycnogenol® alone did not decrease tissue injury. However, when Pycnogenol® was administered with catechin, a flavonoid, Pycnogenol® produced a strong defensive reaction. van Jaarsveld et al. (14) concluded that the antioxidant action of Pycnogenol® did not have a significant effect *in vivo*.

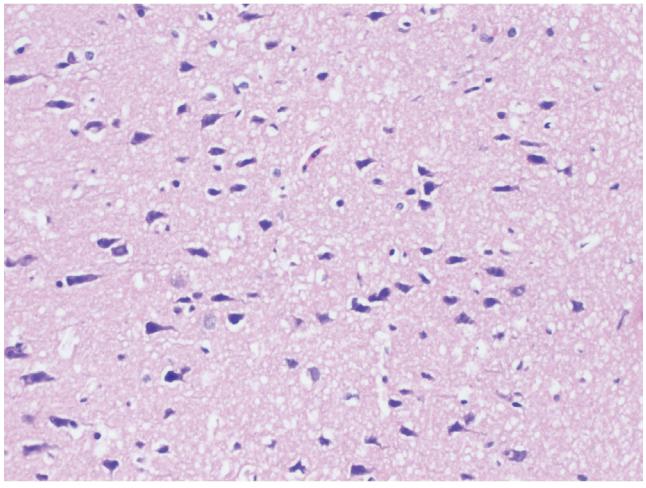

Figure 1. View of the pathological examination of the second rat in Group 1

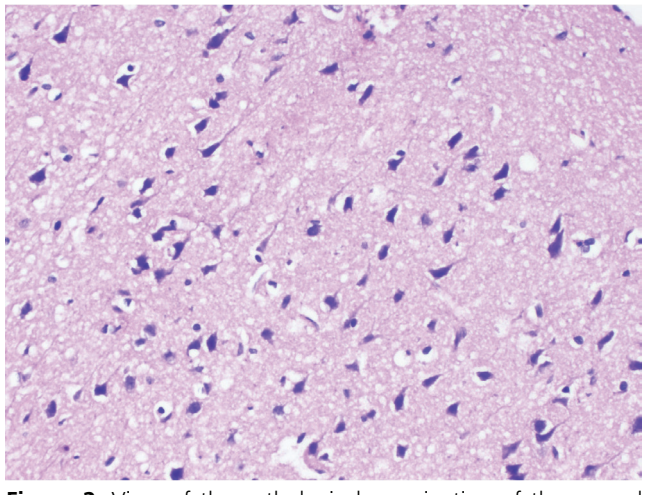

Figure 2. View of the pathological examination of the second rat in Group 2

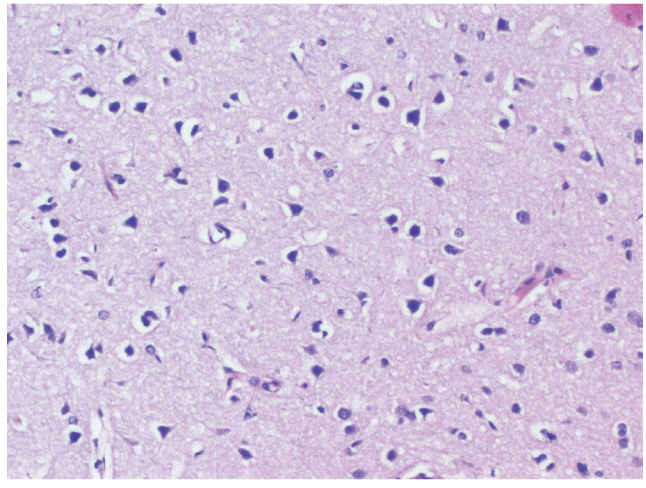

Figure 3. View of the pathological examination of the second rat in Group 3

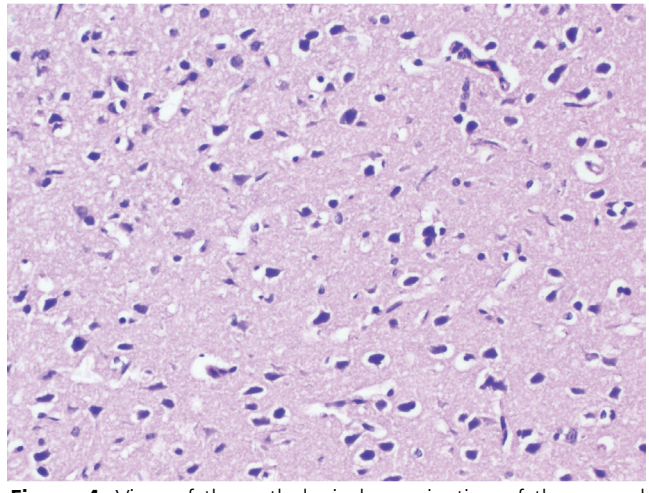

Figure 4. View of the pathological examination of the second rat in Group 4

Pycnogenol® has a beneficial effect on the synaptic proteins of rats with brain injuries. In 2013, Ansari et al. (10) found that Pycnogenol®, administered after a brain contusion, led to a reduction in the intensity of brain injury in young adult rats. Norris et al. (15) found that Pycnogenol preserved CA3-CA1 synaptic functions in rats with traumatic brain injuries and suggested that Pycnogenol® may have a therapeutic role in traumatic brain injuries in humans.

Scheff et al. (16) studied enzymatic and nonenzymatic oxidative stress markers, presynaptic and postsynaptic proteins, and interleukin-6 and tumor necrosis factor-α levels in the cortices and hippocampi of Sprague–Dawley rats. Although we did not find any difference between the groups with regard to SLM functions and pathological examination findings, Scheff et al. (16) observed substantial oxidative stress in the traumatized cortex and ipsilateral hippocampus related to Pycnogenol® administration. Protein carbonyls, lipid peroxidation, and protein nitration were significantly improved in rats treated with Pycnogenol®. Compared with the control group, there was a non significant reduction in loss of presynaptic and postsynaptic proteins in the group treated with Pycnogenol®. Furthermore, although proinflammatory cytokines were increased in both trauma groups, that increase was smaller in the group receiving Pycnogenol® compared with the group that received isotonic saline.

Hasegawa and Mochizuki assessed orchiectomized rats in a radial arm maze for three months to measure the number of reference memory errors, working memory errors, the baseline number of correct choices, and to determine motor functions (17). Three months after the surgical procedure and behavioral test, Pycnogenol® was administered via gavage, as was done in our study, for three weeks. Castration impaired the rat's working memory and reference memory function, but it did not affect motor function. Overall, the differences between working memory, reference memory function, and the number of correct choices diminished in animals treated with Pycnogenol®. Pycnogenol® markedly increased the level of neural growth factor in the hippocampus and cortex but did not affect motor function or testosterone concentration.

Conclusion

We determined that Pycnogenol® did not affect SLM functions in rats with low-intensity closed head trauma. Pathological examination did not reveal any significant differences between the groups with respect to apoptosis. Future studies that employ biochemical markers and free radical levels in the brain are needed. Studies with head traumas of varying intensities would also contribute to our understanding of the subject.

Ethics

Ethics Committee Approval: The study protocol was approved by the University Animal Studies Ethics Committee (Project No: DA16/13).

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.E.K., E.E.D., A.Ö., İ.G., E.C., F.S.K., Ö.G. Concept: A.E.K., R.E., C.Y., C.K. Design: A.E.K., R.E., A.Ö., İ.G., E.C., F.S.K., Ö.G. Data Collection or Processing: A.E.K., R.E., A.Ö., İ.G., E.C., F.S.K., Ö.G. Analysis or Interpretation: A.E.K., R.E., C.Y., C.K. Literature Search: A.E.K., C.K., A.Ö., İ.G., E.C., F.S.K., Ö.G. Writing: A.E.K., C.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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