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Original Article *Özgün Araştırma*

PROTECTIVE EFFECTS OF SESAMOL AGAINST SECONDARY INJURY IN THE RAT MODEL OF TRAUMATIC BRAIN INJURY

Travmatik Beyin Hasarı Sıçan Modelinde Sesamol'ün İkincil Yaralanmaya Karşı Koruyucu Etkileri

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ABSTRACT

Objective: Sesamol is a powerful antioxidant, anti-inflammatory, antiapoptotic, and neuroprotective chemical. This study aimed to investigate the histopathological effects of sesamol in a rat traumatic brain injury (TBI) model.

Material and Methods: Thirty-two male rats were divided into the following four groups: control, trauma, vehicle, and sesamol. All groups were subjected to TBI, and immediately after trauma saline and sesamol (100 mg/kg) were administered intraperitoneally to the vehicle and sesamol groups, respectively. At 24th hour of TBI, brain samples were collected, and histomorphological investigation of brain parenchyma was performed using electron and light microscopy.

Results: On histopathological investigation, TBI induced brain injury was lesser than trauma and vehicle groups in the sesamol group. Compared to the trauma group, in the sesamol group there was less perivascular region edema. The neuronal processes of the sesamol group also displayed less intracellular edema and vacuoles.

Conclusion: The results of the current study revealed for the first time that sesamol exhibits neuroprotective effects against TBI.

Keywords: *Neuroprotection, sesamol, traumatic brain injury*

ÖZ

Amaç: Sesamol güçlü bir antioksidan, antiinflammatuar, antiapoptotik ve nöroprotektif bir kimyasaldır. Bu çalışmada, sesamolün sıçan travmatik beyin hasarı (TBH) modelinde histopatolojik etkilerinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Otuz iki erkek sıçan dört gruba ayrıldı: kontrol, travma, sham ve sesamol. Travma, sham ve sesamol gruplarına ağırlık düşme yöntemi ile kapalı kafa travması uygulandı, travmadan hemen sonra sırasıyla sham ve sesamol gruplarına periton içine salin ve sesamol (100 mg/kg) uygulandı. Travmadan 24 saat sonra, beyin örnekleri alındı ve elektron ve ışık mikroskobu kullanılarak beyin korteksi histomorfolojik olarak incelendi.

Bulgular: Histopatolojik değerlendirme sonucunda sesamol grubunda kafa travması ile indüklenen beyin korteksindeki hasar travma ve sham gruplarından daha azdı. Travma grubuna göre sesamol grubunda perivasküler alan ödemi daha azdı. Sesamol grubunun nöronal uzantılarında da daha az hücre içi ödem ve vakuoller izlendi.

Sonuç: Bu çalışmanın sonuçları sesamolün TBH'na karşı nöroprotektif etkiler gösterdiğini ortaya koymuştur.

Anahtar Kelimeler: *Nöroproteksiyon, sesamol, travmatik beyin hasarı*

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INTRODUCTION

Traumatic brain injury (TBI) has a high mortality rate and is one of the leading causes of morbidity (1). Primary injury occurs at the time of the insult and causes direct mechanical injury to the neurovascular and glial tissues. Secondary injury initiates within minutes following TBI causing further neuronal loss due to oxidative, apoptotic, and inflammatory signal cascades (2-4). Despite being preventable, secondary injuries cause disability that may take days or years to heal (5). There is no experimentally studied agent approved for clinical use except amantadine sulfate for the prevention of TBI related secondary injury (2,6-8).

Sesamol is the primary component of the seeds and oil of sesame, *Sesamum indicum* L. (9-11). Sesamol inhibits the breakdown of deoxyribose and scavenges hydroxyl and lipid peroxyl radicals (9,12). Antioxidant, anti-inflammatory, and antiapoptotic properties of sesamol have been demonstrated through experimental models (13,14). Sesamol attenuates superoxide anion radical and nitric oxide (NO) production, induces mitochondria-related antiapoptotic effects, and decreases the levels of proinflammatory proteins (13). Sesamol can cross the blood-brain barrier (BBB) owing to its high lipophilicity (15). Recent studies have shown sesamol to function as a neuroprotective agent in cerebral ischemia and reperfusion models owing to its antioxidant, antiapoptotic, and anti-inflammatory activities (16,17). Sesamol reduced the expression of proinflammatory cytokines, inhibited inflammation and apoptosis, improved neurological deficits, and prevented cognitive decline (16,17). Moreover, in an experimental epilepsy model, sesamol exhibited ameliorative effects against epilepsy, cognitive impairment, and oxidative stress (18).

Despite exploring and demonstrating the antioxidant, antiapoptotic, and neuroprotective activities of sesamol in animal models in previous studies, its effects have not been studied in TBI. The present study investigated the possible neuroprotective activity of sesamol with the histopathological examination in a TBI rat model for the first time.

MATERIALS AND METHODS

Experimental Groups

All tests were carried out in accordance with the protection of animals used for experimentation as outlined in European Parliament and Council Directive 2010/63/EU of September 22, 2010. We used 32 adult male Wistar albino rats, which weigh between 350-450 g. The temperature and relative humidity were kept constant at 22±2 °C and 65%–70%, respectively, in an air-conditioned room with 12-hour light and dark cycles. Rats were given free access to water and were fed standard laboratory food.

The rats were randomly divided into four groups:

1. Control group (n=8): Rats simply had a skin incision made. Brain tissue samples taken 24 hours after surgery were not damaged. The brain was cut into 1-mm^3 pieces and preserved in glutaraldehyde for light and electron microscopy.

2. Trauma group (n=8): TBI was performed on rats as explained below. During the operation, samples of the brain were taken for histological examination 24 hours later.

3. Vehicle group (n=8): TBI was performed on rats as explained below. After TBI, a single intraperitoneal dose of vehicle (0.9% NaCl, 0.1 ml/100 gr) was given. 24 hours after the damage, brain samples were taken and used for histological examination.

4. Sesamol group (n=8): Sesamol (100 mg/kg; Sigma-Aldrich, St. Louis, Missouri, USA) was administered intraperitoneally to rats one time after TBI. The sesamol dosage was determined on the basis of earlier studies, (19,20).

Anesthesia and Induction of TBI

The animals were given an intraperitoneal injection of a combination of 50 mg/kg of ketamine (Ketalar, Parke-Davis, Turkey) and 10 mg/kg of xylazine (Rompun, Bayer, Turkey) to induce anesthesia. Head trauma was treated using the mild brain injury model that Marmarou et al. (21) and Ucar et al. (22) adapted. To provide deceleration after the hit, the rats were supported on a 10-cm foam bed while lying flat on a table. In the scalp, a midline incision was made, and the coronal and

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lambdoid sutures were noted. Between the two sutures in the midline, a metallic disk of 10 mm in diameter and 3 mm in thickness was attached to the skull using bone wax. In the location where the disk was positioned on the midline, trauma was applied. A copper tube was used to let a 300 g lead object to fall freely from a height of 1 m onto the metal disk covering the rat's skull. The iron disk was taken out, the surgical site was cleansed, and the skin was stitched after the damage was induced. 24 hours after being injured, all of the animals were decapitated, and the brains were carefully removed.

Sample Preparation for Electron and Light Microscopy

The brain tissue samples were fixed with 2.5% glutaraldehyde, postfixed with 1% osmium tetroxide, dehydrated in a graded alcohol series, cleaned with propylene oxide, and embedded in Epon for transmission electron microscopy analysis (EMS, Cat No: 13940).

Using an ultramicrotome (Leica EM UC7, Leica Microsystems GmbH, Vienna, Austria), semi-thin sections (2000 nm) were cut and stained with toluidine blue. These sections were viewed using an Olympus BX50 light microscope and captured on camera (Olympus LC30).

Using an ultramicrotome, thin sections (70 nm) were cut, and they were contrasted with uranyl acetate and lead citrate. Using a transmission electron microscope, these sections were analyzed and captured on camera (JEOL JEM-1011, Jeol Ltd., Tokyo, Japan).

All experimental techniques utilized in this work were reviewed and approved by the Saki Yenilli Animal Care and Use Committee (0001.01.03).

RESULTS

Electron microscopic findings

The control group showed normal myelin sheath, axon, and neuronal processes as well as neuron and glial cell morphology (Figure 1).

Examining the trauma group revealed substantial edema in the perivascular regions. In the neuron processes, vacuoles and intracellular edema were visible. In the neural processes next to the oligodendrocytes, there was considerable intracellular edema and vacuoles. Axons and the myelin sheath showed signs of degeneration (Figure 2).

The perivascular area of the vehicle group showed edema as well, and the neuronal processes showed intracellular edema and vacuoles (Figure 3).

The sesamol group showed myelin injury. Compared to the trauma group, there was less perivascular region edema. The neuronal processes of the sesamol group also displayed less intracellular edema and vacuoles (Figure 4).

Light microscopic evaluation

When study groups' semi-thin sections were examined, the sesamol group showed less perivascular edema than the trauma and vehicle groups (Figure 5).

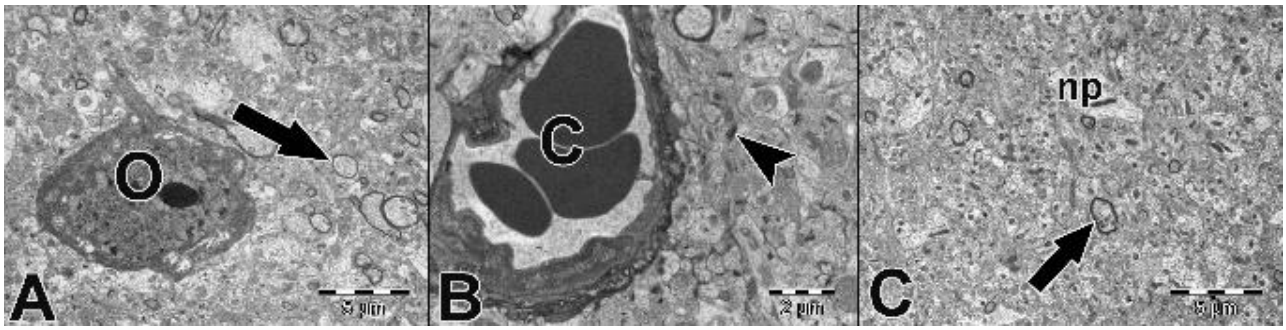

Figure 1: Control group electron microscopic image. The appearance of normal morphology of Neuron (N), Oligodendrocyte (O), Capillary (C), Neuronal processes (np). A and B X5000, C X7500.

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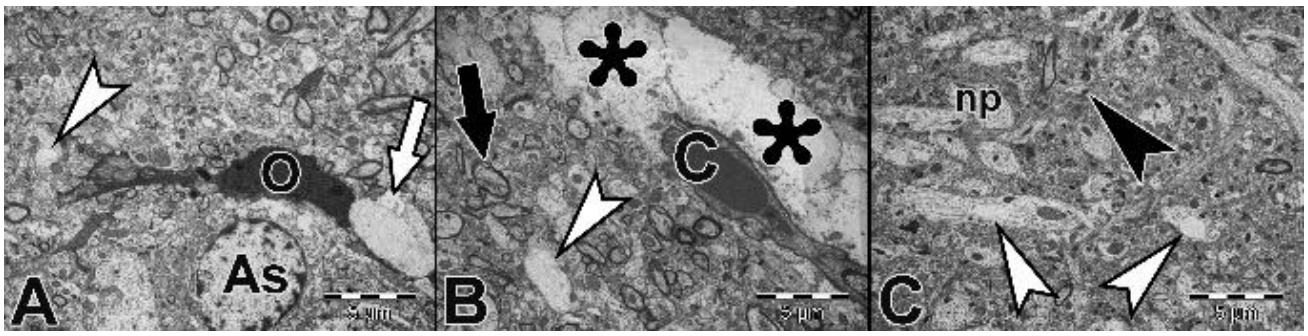

Figure 2: Trauma group electron microscopic image. Capillary (C), intracellular vacuole in the neuronal processes (np) (white arrowhead), intracellular edema and vacuoles in the adjacent to the oligodendrocyte (O) (white arrow), perivascular edema (asterisk), synapse (black arrowhead). A X7500, B X4000, C X5000.

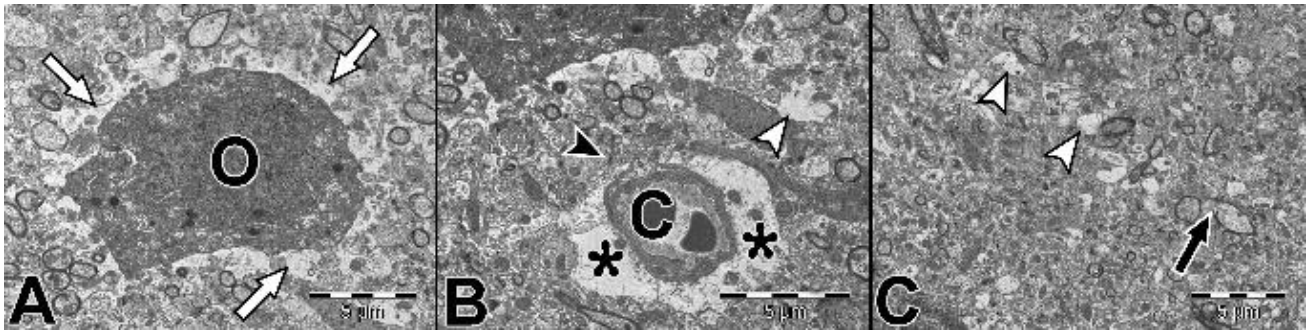

Figure 3: Vehicle group electron microscopic image. Capillary (C), intracellular vacuole in the neuronal processes (white arrowhead), intracellular edema and vacuoles in the adjacent to the oligodendrocyte (O) (white arrow), perivascular edema (asterisk), and synapse (black arrowhead). A X7500, B X7500, C X5000.

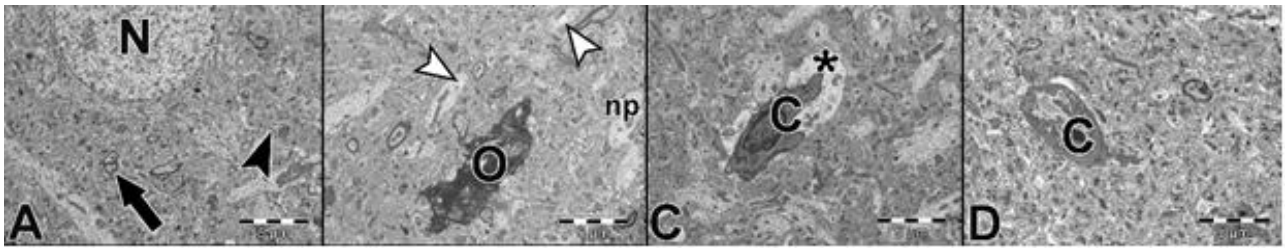

Figure 4: Sesamol group group electron microscopic image. Neuron (N), Oligodendrocyte (O), Capillary (C), intracellular vacuole in the neuronal processes (np) (white arrowhead), myelin sheath (black arrow) and synapse (black arrowhead), perivascular edema (asterisk). A X7500, B X10000, C X6000, D X10000.

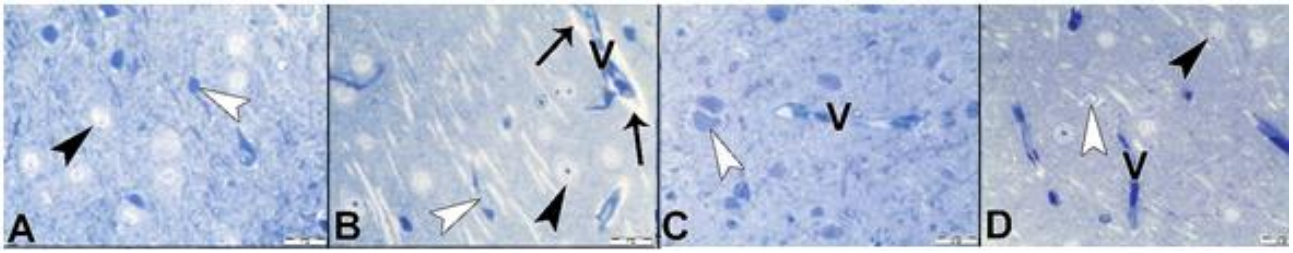

Figure 5: Light microscopic image, semithin sections. (A) Control group, (B) Trauma group, (C) Vehicle group, (D) Sesamol group. Neuron (black arrowhead), glial cells (white arrowhead), perivascular edema (black arrow), vessels (v). Toluidine blue, X400.

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DISCUSSION

Traumatic brain injury is one of the main public health problems and causes of disability all over the world (23). The secondary injury caused by oxidative stress, inflammation, apoptosis, ischemic processes, excitotoxicity, and mitochondrial pathways. The net result of all these mechanisms is neuronal death (2). The last two decades have witnessed an increase in knowledge on the pathogenesis of secondary injury, increased intensive care unit facility, thereby decreasing mortality and improving the patient's quality of life (24). There is no evidence of clinical effectiveness regarding the pharmacological treatment of TBI except amantadine sulfate (2,6-8). Therefore, the treatment of TBI continues to be a topic of interest and several experimental studies are underway to discover a possible therapeutic agent. This study revealed that sesamol exhibits neuroprotective effects against TBI.

Sesamol is a potent phenolic antioxidant—a component of sesame seed and oil—that has antiapoptotic, anti-inflammatory, and membrane stabilizing activities (25-27). It is a potent antioxidant and an inhibitor of cytokine production (9,28,29). Sesamol demonstrates its antioxidant properties by functioning as a chain-breaking agent, a radical scavenger, a metal chelator, and an electron donor during the lipid peroxidation process (25). The neuroprotective effects of sesamol have been investigated in animal models (14,30). Chopra et al. reported that it suppressed neuroinflammation in diabetic neuropathy animal model by modulating increased inflammatory cytokines and apoptosis-related proteins (14). Administration of sesamol treatment in streptozotocin-induced diabetic rats enhanced the antioxidant capacity of the brain and resulted in decreased perturbation in BBB structure and function due to hyperglycemia-induced changes (31).

The evaluation of the brain tissues' histopathology was done at the light microscopic and ultrastructural levels. In contrast to the considerable perivascular region edema seen in the trauma and vehicle groups, the brain morphology was normal in the control group. One of the most severe symptoms of cellular damage is intracellular edema. Importantly, swelling, vacuolar alterations, and the lysis of certain organelles are all part of the overall response to damage. These alterations in traumatic cell damage were identified by our electron microscopic observations. Sesamol reduced the intracellular edema and vacuoles that were shown to be more prevalent in the trauma and vehicle groups.

Nonetheless, our study has certain limitations. Quantitative light and electron microscopy, in-depth inflammatory biomarkers, and functional outcome ratings could all be used to better understand the mechanisms underlying sesamol's effects. Further research using more animals and various dosage regimens administered at various times could be carried out in TBI animal models. Sesamol was given immediately after TBI in this study, however the effect of later applications is also important for clinical settings. Moreover, research could be done using different TBI animal models, such as repetitive TBI models.

In conclusion, the neuroprotective features of sesamol after TBI were shown in this study, which was the first of its kind. Sesamol's potential neuroprotective pathways were described. Sesamol has been proven to be successful in avoiding TBI-related secondary brain damage. Sesamol might be utilized to treat TBI after more experimental and clinical trials. To confirm our findings and establish a safe and effective range of therapeutic dosages for humans, further research and clinical trials are needed.

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Conflict of Interest: The author has no conflict of interest to declare.

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Researchers' Contribution Rate Statement: Concept/Design: PKB; Analysis/Interpretation: PKB,DO; Data Collection: PKB,DO; Writer: PKB; Critical Review: PKB,DO; Approver: PKB.

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Ethics Committee Approval: All experimental techniques utilized in this work were reviewed and approved by the Saki Yenilli Animal Care and Use Committee (0001.01.03).

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