



# Neuroprotective Effects of Sildenafil on Traumatic Brain Injury in an Experimental Rat Model

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## Abstract

**Objective** Not only primary injuries, secondary injuries such as posttraumatic biochemical cascades, ischemia, and hypoxia also affect the morbidity and mortality of traumatic brain injury (TBI). Sildenafil released the vasodilatation by relaxing the smooth muscle of the systemic artery and vein. Also, the effects of sildenafil are evidenced in multiple sclerosis, Alzheimer's disease, and memory loss as a part of experimental studies. Sildenafil decreases oxidative stress by increasing the cGMP level. We aimed to examine the protective effects of sildenafil on TBI with histopathological and biochemical parameters.

**Methods** 21 Sprague–Dawley rats were separated into three groups ( $n = 7$ ). “The weight drop injury model,” which was described by Marmou, was used for the head injury. Group 1: nontraumatic sham group, Group 2: nontreated TBI group, Group 3: sildenafil (100 mg/kg) treated TBI group. The whole brain and serum were collected for histopathological and biochemical study. The histopathological sections were examined under a light microscope.

**Results** On comparison of total antioxidant status (TAS), total oxidant status (TOS), nitric oxide (NO), and plasma nitrite/nitrate (PNOx) between groups, NO level was significantly high in group 3 ( $p = 0.013$ ). Even though the TAS level was significantly high in group 3 ( $p = 0.02$ ), there were no significant differences in TOS level in groups ( $p = 0.225$ ). Disappearing Nissle granules occurred in a pyknotic situation in the cell nucleus, and acidophilic staining in neuron cells, which describe the neuron degeneration observed in the trauma group. The neuron degeneration markers were not seen in the sildenafil-treated trauma group.

**Conclusion** Our study has shown that sildenafil decreases the oxygen radicals and affects the recovery of experimental TBI in rats.

## Keywords

- Sildenafil
- brain injury
- trauma
- experimental
- neuroprotection
- secondary damage

## Introduction

Head injury is one of the most important health problems that kill, cripple, and require long-term treatment and care. In modern populations, developing technologies and social

life increase TBI incidence and mortality and morbidity risk factors. It especially affects young adults, with loss of production, and acute and chronic treatment costs lead to severe economic losses.<sup>1,2</sup> When examined carefully, it was determined that not only severe head trauma but mild and

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moderate head trauma also cause permanent disability.<sup>3</sup> In the United States, 235,000 people are hospitalized every year, 1.1 million people are treated as outpatients in the emergency department, and approximately 50000 people die.<sup>1,4</sup>

TBI is classified as primary and secondary injury. Although neurodegeneration in head trauma occurs due to primary mechanical movement (nerve cells, vascular structure, shaking, rupture, etc.), it is important to deal with the secondary damage that occurs after the primary damage and the occurrence of pathochemical and pathophysiological cascades that affect the actual prognosis.<sup>5-8</sup> The level of lactic acid increases, and adenosine triphosphate (ATP) and phosphatase decrease at the early periods of TBI. In other words, primary ischemia begins in the early period of TBI. This progressive tissue ischemia is the main cause of secondary tissue destruction. Ischemia needs to be delayed if recovery from head trauma is desired.<sup>9</sup>

The most important secondary damage shown by experimental studies is the oxidative destruction of lipid, protein, and nucleic acids caused by free oxygen radicals.<sup>10</sup> The brain has less tolerance for oxygen deficiency and oxidative stress than other organs. Therefore, the brain must be protected against oxygen radical-induced trauma and ischemia.<sup>10</sup>

Sildenafil was produced in 1998 and used for the treatment of erectile dysfunction. It makes vasodilatation by relaxing the smooth muscle of systemic arterial and venous vessels.<sup>11,12</sup> As a result of this effect, it has been shown experimentally that it is useful in some clinical conditions such as multiple sclerosis, Alzheimer's, and memory losses, as used in the treatment of diseases such as erectile dysfunction and pulmonary hypertension.<sup>13,14</sup>

In this study, we aimed to investigate whether sildenafil inhibits the effect of tissue hypoperfusion in patients with a head injury; thus, preventing secondary destruction.

## Materials and Methods

Twenty-one Sprague–Dawley rats (250–300 gr) were housed in an airconditioned room with 12 hours light and dark cycles, where the temperature ( $23 \pm 2^\circ\text{C}$ ) and relative humidity (65–70%) were kept constant. All experimental protocols were approved by the Medicine Animal Care and Use Committee of the local university.

Rats were separated randomly into three experimental groups ( $n = 7$ ). Group 1: nontraumatic control group, Group 2: nontreatment after TBI, and Group 3: treated with sildenafil (100 mg/kg) after TBI.<sup>16,20</sup> Sildenafil was dissolved in ethanol/serum physiologic (1:1) and administered by the intraperitoneal way.

All rats were in normal motor functions. Anesthesia was induced by intramuscular injections of ketamine (60 mg/kg) and xylazine (9 mg/kg). Animals were allowed to breathe spontaneously. The core temperature was monitored with a rectal probe. "The weight drop injury model," which was described by Marmou, was used for the formation of head injury.<sup>15</sup>

The rats were sacrificed after 24 hours by cardiac blood extraction. The blood was centrifugated, and the plasmas

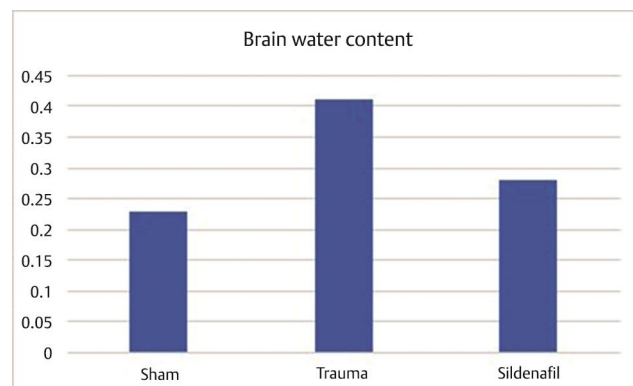
were frozen at  $-80^\circ\text{C}$  in the freezer. Total antioxidant status (TAS), total oxidant status (TOS), nitric oxide (NO), and plasma nitrite/nitrate (PNOx) were examined from plasma by the same biochemist who was blind to the groups. Brain tissue was removed and fixed with formaldehyde 10%. Twenty-four hours later, the tissues were dehydrated in routine alcohol series (70, 80, 90, 96, 100%) and embedded in paraffin. Five-mm sections from paraffin blocks were constituted and stained with hematoxylin and eosin (H&E). The sections were examined by the same pathologist who did not know the groups. Brain edema was evaluated by the drying-weighting method.<sup>33</sup> The whole brain was weighed and then dried for 48 hours at  $100^\circ\text{C}$ ; afterward, it was reweighed. The percentage of water was calculated according to the following formula:  $\% \text{H}_2\text{O} = ([\text{wet weight} - \text{dry weight}] / \text{wet weight}) \times 100$ .

Mann–Whitney U test was used to analyze the difference between groups. SPSS program was used for statistical tests.

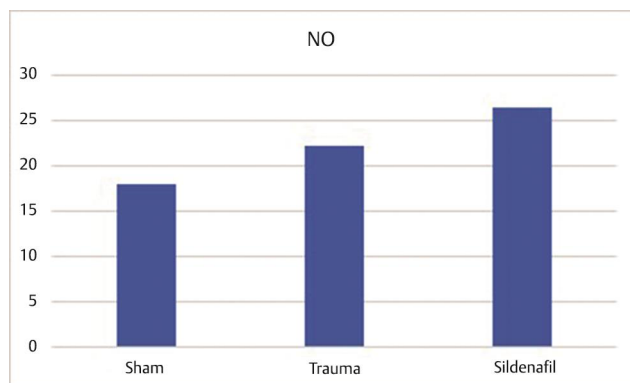
## Results

The mortality rate was 1/7 (14%) in group 2 and group 3. Deaths occurred in the first minutes after the induction of trauma in group 2, and 24 hours later in group 3. The edema was evaluated by the drying-weighting method, and the water content of the brain was significantly increased in the trauma group when compared with the sildenafil-treated trauma group ( $p = 0.01$ ; ► **Fig. 1**). The results of NO level in plasma were significantly increased in the sildenafil group when compared with the trauma group ( $26.8 \pm 2$ ,  $p = 0.001$ ; ► **Fig. 2**). In groups 1 and 3, the results showed that sildenafil was significantly increased the NO level ( $p = 0.002$ ). The results of the TAS level were significantly increased in the sildenafil group when compared with the trauma group ( $1.57 \pm 0.2$ ,  $p = 0.02$ ; ► **Fig. 3**). There was no significant difference in TOS levels between groups 2 and 3 ( $17.79 \pm 3$ ,  $p = 0.225$ ; ► **Fig. 4**). Similarly, there was a statistically significant increase in PNOx values between groups in comparison with TAS. ( $115.73 \pm 12$ ,  $p = 0.338$ ; ► **Fig. 5**)

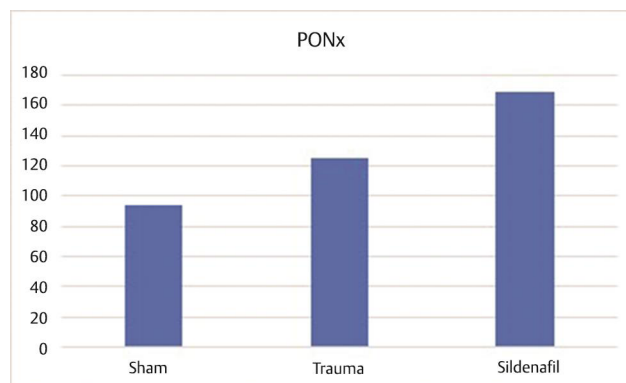
Morphological examination revealed normal histological structure in brain tissues taken from the sham group



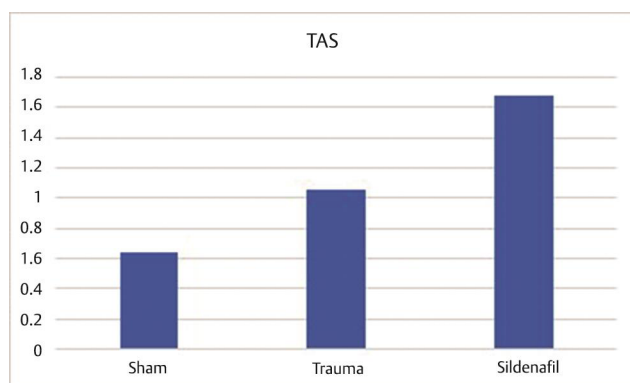
**Fig. 1** The water content of the brain was significantly increased in the trauma group when compared with sildenafil-treated trauma group ( $p < 0.01$ ).



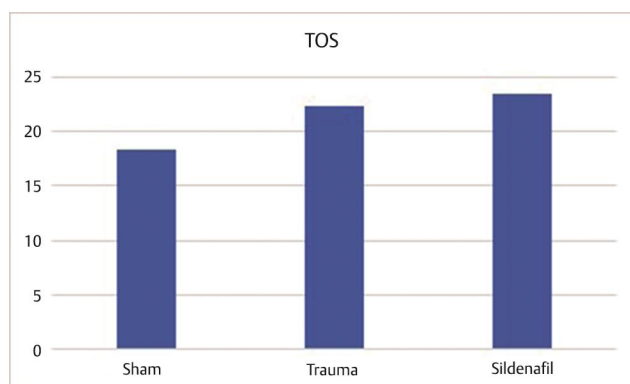
**Fig. 2** No level in plasma was significantly increased in the sildenafil group when compared with the trauma group ( $26.8 \pm 2$ ,  $p < 0.001$ ). The comparison with group 1 and 3 shows that sildenafil was significantly increased the NO level ( $p = 0.002$ ).



**Fig. 5** The results of plasma nitrite/nitrate (PNOx) were significantly increased in the sildenafil group when compared with the trauma group ( $115.73 \pm 12$ ,  $p = 0.038$ ).

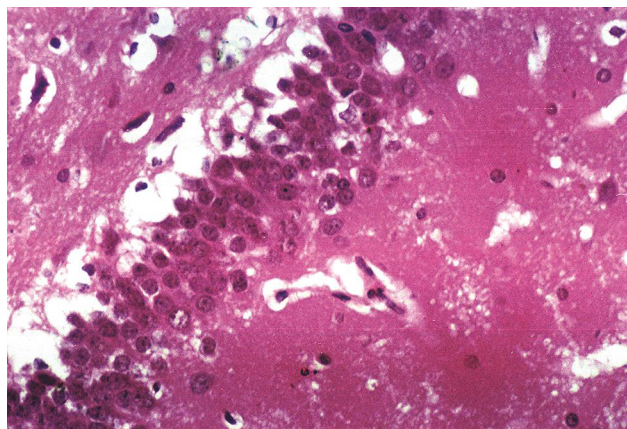


**Fig. 3** The results of the total antioxidant status (TAS) level were significantly increased in the sildenafil group when compared with the trauma group ( $1.57 \pm 0.2$ ,  $p = 0.02$ ).



**Fig. 4** There was no significant difference between groups 2 and 3 about total oxidant status (TOS) level ( $17.79 \pm 3$ ,  $p = 0.225$ ).

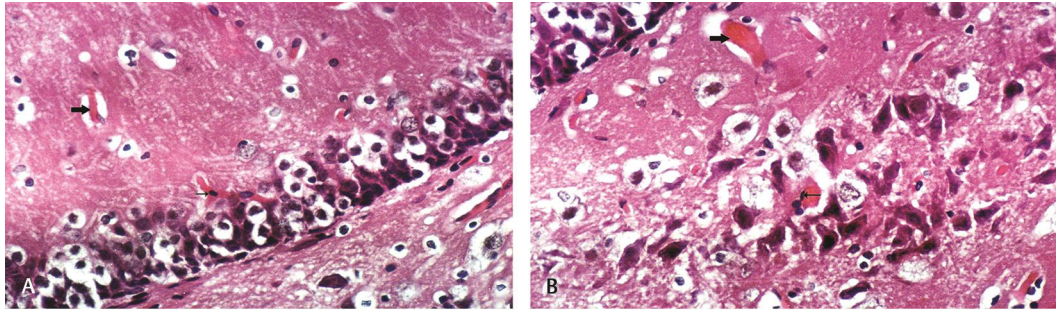
(►**Fig. 6**). Brain cortex examinations of the head trauma group revealed the loss of Nissle granules, which characterize neuron damage, the pyknotic status of nuclei, and acidophilic shift of cytoplasm (►**Fig. 7 a, b**). The neuron degeneration markers were not seen in the sildenafil-treated trauma group (►**Fig. 8**).



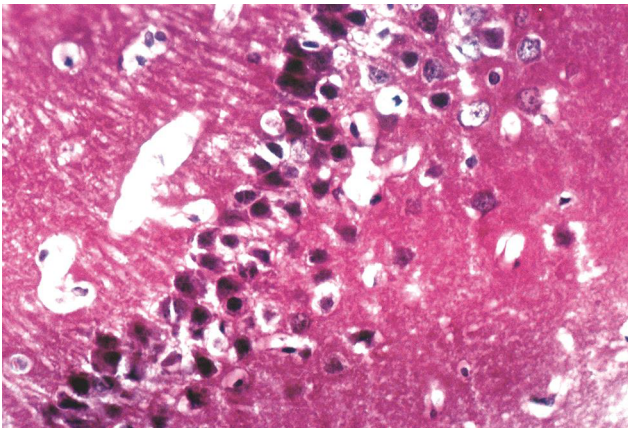
**Fig. 6** Sham group: Normal histological contents have been seen in sham group rats (hematoxylin and eosin [H&E], magnification  $\times 160$ ).

## Discussion

Since the definition of the trauma model, defined by Marmarou et al, several studies have been conducted on the pathophysiology of head trauma injuries.<sup>15</sup> Differentiation of primer injury factors related to trauma from secondary injury factors such as intracranial pressure increase, herniation, brain edema, and brain ischemia in severe head trauma are extremely important for planning treatment.<sup>9,16,17</sup> Although many diagnoses and treatment methods are in use in the treatment of patients with an acute head injury, the morbidity and mortality rate are still high. Because of the mechanical effect of the trauma, brain tissue and neurons are damaged. This is called primer injury. A number of complex physiopathological events such as hypoxia, ischemia, increased intracranial pressure, and brain edema, which develop in response to primary trauma in the following minutes, hours, and even days following trauma and increase neuronal damage. This is called a secondary injury. A cascade of events triggered by secondary injury is the work of activation of endogenous cell death pathways. One of the most important factors in the emergence of secondary injury is the



**Fig. 7 a, b:** Disappearing Nissle granules, the formation of the pyknotic situation in the cell nucleus (□), and acidophilic staining (□) in neuron cells, which describe the neuron degeneration observed in the trauma group (hematoxylin and eosin [H&E], magnification  $\times 160$ ).



**Fig. 8** The neuron degeneration markers were not seen in the sildenafil-treated trauma group.

lack of energy due to ischemia. Ischemia causes a lack of adequate glucose and oxygen uptake to the tissues and indirectly leads to a lack of energy and a decrease in ATP storage. The free radical formation and inflammatory mediator release increase neuron damage.<sup>5,6,18</sup>

NO is an activator of soluble guanylate cyclase (GC) in the target cell and provides cGMP formation from guanosine triphosphate. Phosphodiesterase type 5 (PDE5) is an important enzyme involved in the destruction of cGMP.<sup>19,20</sup> Sildenafil is a highly selective inhibitor of PDE 5 and leads to increased cGMP in the cell. Experimentally, it showed that NO increases the amount of cGMP in the brain in the stroke studies on rats; thus, accelerating cell regeneration and functional recovery.<sup>19</sup> Sildenafil reduces oxidative stress by increasing intracellular cGMP.<sup>13</sup>

Studies on Alzheimer's disease animal models have shown us that sildenafil enhances learning and memory.<sup>21</sup> Despite these studies, the antifatigue and neuroprotective effects of sildenafil are unknown. Brain blood flow studies show that sildenafil decrease transient ischemic attacks in the recovery phase, and they can significantly affect the results of head trauma.<sup>5</sup>

In their study in 2005 and 2006, Whang and Zhang observed that sildenafil can detect the blood-brain barrier in rats, increase the memory and learning capacity, increase neurogenesis, and cause functional recovery of

neurological deficits by affecting glutamate NO cGMP pathway.<sup>22,23</sup> Uthayathas has shown that Parkinson symptoms such as tremor, rigidity, akinesia, and erectile dysfunction, were decreased after sildenafil treatment on Parkinson disease animal model.<sup>13</sup> In another study, Uthayathas also mentioned that sildenafil showed a direct effect on neurogenesis, memory-enhancing synaptic palsy treatment, and stroke treatment, besides the neuroprotective effect by increasing the blood flow.<sup>14</sup>

In conclusion, the results of our study showed that sildenafil given to rats treated with head trauma decreased oxygen radicals and contributed to tissue healing. However, our results require further clinical research for clinical practice.

#### Funding

None.

#### Ethical Approval

All applicable international, national, and institutional guidelines for the care and use of animals were followed.

#### Conflict of Interest

None declared.

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