RESEARCH ARTICLE

Propofol and *Nigella sativa* L. Seeds Ethanol Extract Enhance Neuroprotection: A Histopathological Study in Rat Models with Traumatic Brain Injury

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Abstract

BACKGROUND: *Nigella sativa* has been known for its neuroprotective properties, while propofol is an anesthetic induction drug that has been widely used in the treatment of traumatic brain injury (TBI). To determine the effectiveness of both active ingredients, it is necessary to compare their neuroprotective effects. This study was performed since not many studies have compared the effect of propofol and *Nigella sativa* seeds ethanol extract (NSSEE) or their combination on histopathological features in TBI cases.

METHODS: Thirty male rat models were divided into 5 groups. Four groups received TBI induction with the methods of Feeney's weight drop model, while another group (control group) did not receive TBI induction. Groups with TBI induction, later received no treatment, treatment with 500 mg/kg NSSEE orally, 10 mg/kg propofol intravenously, or a combination of NSSEE and propofol. After 8 days, rats were euthanized by cervical dislocation. Subsequently, a craniotomy was performed to obtain brain samples. The brain sample was placed in 10% neutral buffered formalin for histopathological examination, which includes brain hemorrhage, congestion, inflammatory cells, necrosis, apoptosis, and degeneration.

RESULTS: The present study found that NSSEE showed greater efficacy in histopathological features (brain hemorrhage, congestion, inflammatory cells, necrosis, apoptosis, and degeneration) in rat models with TBI compared to propofol or a combination of propofol and NSSEE.

CONCLUSION: NSSEE has superior potential compared to propofol and the combination of both in providing neuroprotection in TBI cases.

KEYWORDS: traumatic brain injury, propofol, *Nigella sativa* seeds ethanol extract, histopathology, neuroprotective

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Introduction

Traumatic brain injury (TBI), an acquired brain injury caused by external mechanical force, is gaining attention due to its potential for long-term effects and temporary or permanent impairment.(1,2) TBI involved brain parenchyma and blood vessel damage, causing cerebral ischemia, glutamate release, and calcium and sodium ion influx, resulting in mitochondrial damage, neuroinflammation, and neuronal apoptosis.(3,4) TBI is like ischemic reperfusion injury, thus inducing inflammation and immune responses.(5,6)

After TBI, neuronal cells are damaged through an inflammatory process as the body's reaction to trauma.

(7) To protect neurons and improve neuroplasticity, active ingredients that provide neuroprotection are needed.(8) Propofol, an anaesthetic agent commonly used in TBI treatment, is favoured for its quick induction and potential to reduce ischemic brain damage. Propofol is an intravenous anaesthetic drug that has interesting characteristics that are used in neuroanaesthesia. Propofol is a strong hypnotic drug, does not increase intracranial pressure and has neuroprotective effects.(6) Propofol has a beneficial sedative effect in TBI management because of its relatively rapid onset and offset of action, facilitating neurological assessment.

Meanwhile, *Nigella sativa* L. (black cumin) has been noted in various studies for its neuroprotective properties by enhancing immunity, maintaining mucosal integrity, improving organ blood flow, supporting healing, and reducing infectious complications. *Nigella sativa* has a strong influence as a neuroprotective by reducing the levels of malondialdehyde (MDA) lipid peroxidation in the hippocampus because it has antioxidant properties that can increase protection and resistance to oxidative stress. The neuroprotective effect on transient brain damage caused by ischemic conditions in the rat hippocampus occurs through its antioxidant mechanism. This is indicated by a decrease in MDA levels and an increase in glutathione stimulating hormone (GSH), catalase (CAT) activity and super oxide dismutase (SOD).(5,9–11) *Nigella sativa* also has the potential to accelerate healing because it has various active ingredients that are potential in the treatment of TBI.

Due to various benefits that propofol and *Nigella sativa* have as potential TBI treatments, and since not many studies have assess its effect in providing neuroprotection and enhancing neuroplasticity in cases of TBI, therefore this study was conducted to investigate the effects of propofol and *Nigella sativa* seeds ethanol extract (NSSEE) on histopathological features of haemorrhage, congestion, inflammatory cells, necrosis, apoptosis, and degeneration in TBI rat models.

Methods

Study Design

An experimental study using post-test-only control group design was conducted at Laboratory of Clinical Animal Study, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia in May 2023. This study was prepared in accordance with Animal Research: Reporting of *in vivo* Experiments (ARRIVE) Guidelines.(12) Study protocol of this study was approved by Ethical Committee of Animal Research, Faculty of Veterinary Medicine, Universitas Syiah Kuala (Approval No.: 208/KEPH/III/2023).

Animal Inclusion

Thirty healthy male rats (*Rattus norvegicus*) aged 5-10 weeks and weighed 150-250 grams were included in this study. Rats with presence of structural or functional abnormalities, and ongoing infection were excluded. Rats were housed in groups of 6 in well-shaded, tranquil rooms, with a consistent environment. Rats were provided the same quantity and type of food, and a tray beneath the cages collected urine and feces, cleaned daily. Ventilated cages were maintained at a temperature of 25-27°C and humidity of 50-60%. A 12:12 light-dark cycle was maintained, with lights on at 5:30 AM. Rats were fed formulated pellet feed, at 10% of body weight, twice a day. Clean water was provided *ad libitum* through specialized bottles. Acclimatization period lasted for 7 days. Rats were considered to be dropped-out if they were deceased, infected during study period, inactive, or refused to eat.

The determination the minimal sample size of each group was calculated using the Federer formula, as follow: $(r - 1)(t - 1) \geq 15$, with t = number of treatments (5 groups) and r = number of repetitions/minimal subjects in each group. Based on this calculation, it was obtained that the required number of subjects was minimal 5 rats per group. To anticipate drop-outs, sample size was increased by 10%, resulting in 6 rats included in each group. Microsoft Excel v.2021 (Microsoft, Redmond, WA, USA) was used for sample randomization into each group.

TBI Induction

TBI induction protocol in this study utilized a modified Feeney's weight drop model. Rats were injured by a controlled fall from a height of 1 meter using an additional weight of 40 grams, with a weight diameter of 2.5 cm, which was placed around the rat's neck, resulting in cortical contusion. Before dropping, the rats' scalps were shaved and treated with topical anesthesia. Following anesthesia, a 4 mm incision was made in the right front of parietal area to expose skull bones.

Preparation of Propofol

The propofol was purchased from the pharmaceutical company PT Kalbe Farma (Jakarta, Indonesia) and further processed in the laboratory of Faculty of Veterinary Medicine, Universitas Syiah Kuala. The calculation of

propofol dosage each administration was calculated using formula: (body weight x dose)/concentration. With a propofol concentration of 10 mg/mL and rats each weighing 200 g (0.2 kg), the calculated dose was 0.2 mL which was administered intravenously in the rat's tail.

Preparation of NSSEE

Extraction of *Nigella sativa* seeds was performed using maceration with n-hexane, ethanol, and methanol. Fifty grams of seed powder were macerated in 200 mL n-hexane with a magnetic stirrer at 200 rpm for 24 hours, repeated three times. The residue was dried and re-macerated with ethanol, then with methanol. After extraction, solvents were evaporated under vacuum to obtain a crude extract, which was then weighed and stored in a freezer for testing. Similar to the calculation of propofol dosages, the dosage of NSSEE was calculated using formula: (body weight x dose)/ concentration. NSSEE in a concentration of 50 mg/mL was prepared by mixing 500 mg extract in 10 mL of solvent, resulting in a dose of 2 mL orally for each administration.

Animal Model

Thirty rats were divided into 5 groups with 6 rats in each group; rats that was not TBI-induced and not treated (control group); TBI-induced rats that received no treatment (TBI-induced group), TBI-induced rats that treated with 500 mg/kg NSSEE orally (TBI+NSSEE group), TBI-induced rats that treated with 10 mg/kg propofol intravenously (TBI+Propofol group), and TBI-induced rats that treated with the combination of 500 mg/kg NSSEE orally and 10 mg/kg propofol intravenously (TBI+NSSEE+Propofol group). Administration of propofol and/or NSSEE was given 7 times a day for 7 days.

After 8 days of treatment, rats were euthanized by cervical dislocation. Subsequently, a craniotomy was performed to obtain brain samples, which were then weighed. The brain was placed in 10% neutral buffered formalin for the histopathological examination. All microsurgical procedures were conducted by a professional veterinarian. The histopathological features observed were used to determine the neuroprotective function, by assessing brain hemorrhage, congestion, inflammatory cells, necrosis, apoptosis, and degeneration in rats' brain.

Observation of Hemorrhagic Parameter

Hemorrhagic was calculated by looking at the blood vessels between neuron cells, namely the release of erythrocytes in the tissue per microscopic field of view marked by ruptured blood vessels so that there were many blood spots outside the blood vessels, but still in the tissue. Blood came out of the vessels towards the nearest tissue. Hemorrhage was quantified by observing erythrocyte release between neuron cells, indicating ruptured blood vessels and blood flow into nearby tissue.(7,13)

Observation of Congestion Parameter

Congestion was calculated by looking at the blood vessels between neuron cells. The number of neuron cells per microscopic field of view was marked by capillaries in hyperaemic tissue that look dilated and full of blood. Cell congestion mean that there was a dam at the cellular level. To see microvascular congestion, blood vessels were carefully examined whether they are small, medium or large containing red blood cells. Congestion was assessed by observing dilated capillaries filled with blood, indicating cellular damming.(14)

Observation of Inflammatory Cell Parameter

Inflammatory cells were calculated by looking at inflammatory cells in the tissue marked by cell nuclei. The number of neuron cells per microscopic field of view was marked by the presence of many inflammatory cells, such as polymorphonuclear leukocytes (PMN) and mononuclear cells (MNCs) accompanied by dilation of their blood vessels/ capillaries. Inflammatory cells were counted by identifying nuclei-marked cells, with an increase in polymorphonuclear cells, mono morphonuclear cells and dilated blood vessels/ capillaries.(15)

Observation of Necrosis Parameter

Necrosis was calculated by looking at the number of neuron cells per large field of view microscopically, marked by the rupture of the cell membrane so that the nucleus was lysed, only the structure of the cytoplasm could be seen which was more acidophilic (red) but without a nucleus, because it had been lysed. Necrosis was determined by lysed cell membranes and loss of nuclei, leaving only acidophilic cytoplasm.(16)

Observation of Apoptosis Parameter

Apoptosis was calculated by looking at the number of neuron cells per large field of view microscopically, marked by the intact cell membrane, so that the contents of the cytoplasm were still intact. The intact nucleus would form apoptotic bodies. The appearance of the cells was cleaner. Apoptosis was identified by intact cell membranes and intact cytoplasm, with intact nuclei forming apoptotic bodies.(17)

Observation of Degeneration Parameter

Degeneration was calculated by looking at the cell nucleus of pyknosis, cryoeclysis, karyolysis, the number of neuron cells per field of view microscopically indicated by swollen cells, also indicated by pale cytoplasm, there was vacuolization in the cytoplasm which indicated a decrease in the number of nerve cells microscopically. Degeneration was assessed by observing swollen cells, pale cytoplasm, vacuolization, and reduced neuron cell count.(18)

Results

In the present study, the intended sample size was thirty rats, but two rats died shortly after TBI-induction due to severe head injuries before receiving any NSSEE or propofol treatment; therefore, these rats were replaced. Average number of neuronal cell damage for each histopathological features (congestion, hemorrhage, inflammatory cells, necrosis, apoptosis, and degeneration) in the rat brain was lower in control group compared to TBI+NSSEE group. Additionally, TBI+NSSEE group was lower than TBI+NSSEE+Propofol group, while TBI+NSSEE+Propofol group was lower than TBI+Propofol group. Furthermore, TBI+Propofol group was lower than TBI-induced group (Table 1). These findings indicated significant differences in the potential for neuroprotection among control and experimental groups (groups induced with TBI).

Macroscopic Brain Lesions

Macroscopic examination revealed differences among five groups (Figure 1). Lesions were observed in the right brain of rats subjected to TBI, characterized by bruising, cerebral edema, extensive hemorrhage, and dilated blood vessels (hemorrhagic). While, in the control group, no brain lesions were observed.

Histopathological Features of The Brain Tissue

The features of histopathological changes in brain tissue in the control group, neuron cells appeared to be normal. Changes in TBI-induced group showed a lot of damage to neuron cells that were necrotic and some experience apoptosis, in addition to blood clotting (congestion), hemorrhagic and visible degeneration in the brain. In the TBI+NSSEE group, the changes appeared to be better with the administration of NSSEE when compared to the treatment of propofol only. This was seen as fewer neuron cells experiencing necrosis and apoptosis, and a combination of propofol with NSSEE when compared to the level of neuron cell damage in the TBI+Propofol group. In the TBI+NSSEE+Propofol group, there were several neuron cells that appeared to be normal, only a few cells that experienced necrosis and apoptosis (Figure 2).

Effect of Propofol and NSSEE on Brain Haemorrhage

NSSEE protected neuronal cells significantly compared to propofol or its combination (Table 1). No significant difference was observed between control group and TBI+NSSEE group, nor between TBI+Propofol group and TBI+NSSEE+Propofol group. However, TBI-induced, TBI+Propofol, and TBI+NSSEE+Propofol groups were significantly differed from the TBI+NSSEE group (Figure 3A).

Effect of Propofol and NSSEE on Brain Congestion

NSSEE protected neuronal cells significantly compared to propofol or its combination (Table 1). TBI+Propofol and TBI+NSSEE+Propofol groups did not significantly differ from each other but significantly differed from the TBI-induced group. Meanwhile, TBI+NSSEE group significantly differed from both TBI-induced and control group (Figure 3B).

Table 1. Histopathological features after experimental exposure.

Histopathology Variables	Control Group	TBI-induced Group	TBI+NSSEE Group	TBI+Propofol Group	TBI+NSSEE+ Propofol Group	*p*-value
Hemorrhage (mean±SD)	2.42 ±1.11	10.08±2.18	2.92±0.97	8.00±1.45	7.17±1.47	<0.001*
Congestion (mean±SD)	0.92 ±0.49	2.50±0.32	0.42±0.38	0.75±0.27	0.67±0.26	0.001*
Inflammatory cells (mean±SD)	4.58±11.42	11.42±1.72	6.58±1.11	10.58±1.99	8.92±1.02	<0.001*
Necrosis (Mean±SD)	5.75±0.99	36.67±2.89	12.25±1.81	27.50±2.00	19.25±2.02	<0.001*
Apoptosis (mean±SD)	2.92±0.80	18.33±0.98	5.50±1.14	14.08±2.01	11.50±0.89	<0.001*
Degeneration (mean±SD)	21.17±2.56	48.42±3.28	24.17±2.82	36.75±1.81	34.58±1.53	<0.001*

Data presented as mean±SD. *Considered to be significant if $p<0.05$, tested with One-way ANOVA on normally distributed data and Kruskal-Wallis test on non-normally distributed data.

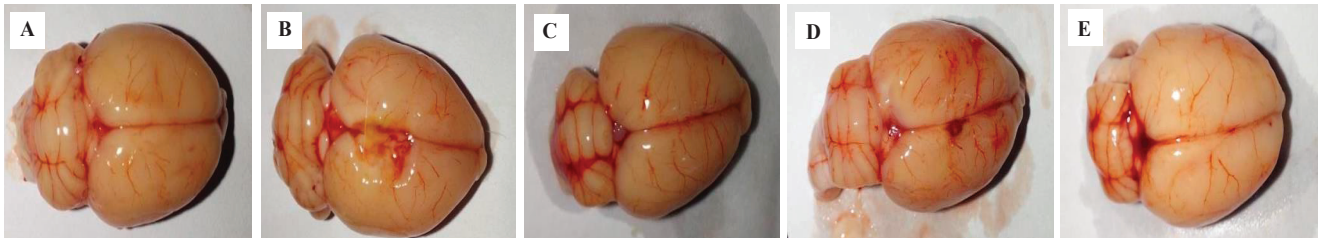

Figure 1. Macroscopic brain lesions observed in control group and TBI-induced groups. A: control group; B: TBI-induced group; C: TBI+NSSEE group; D: TBI+Propofol group; E: TBI+NSSEE+Propofol group.

Effect of Propofol and NSSEE on Brain Inflammatory Cells

NSSEE significantly protected neuronal cells compared to propofol or its combination (Table 1). TBI+Propofol group did not significantly differ from the TBI+NSSEE+Propofol group or the TBI-induced group, but significantly differed from the control group and TBI+NSSEE group (Figure 3C).

Effect of Propofol and NSSEE on Brain Necrosis

NSSEE significantly protected neuronal cells from necrosis compared to propofol or its combination, with all groups showing significant differences among each other. As also can be seen, that both control group and the TBI+NSSEE group had the lower necrosis (Table 1, Figure 3D).

Effect of Propofol and NSSEE on Brain Apoptosis

NSSEE significantly protected neuronal cells from apoptosis compared to propofol or its combination, with all groups showed significant differences among each other. Control group hd the lowest apoptosis followed by the TBI+NSSE group (Table 1, Figure 3E).

Effect of Propofol and NSSEE on Brain Degeneration

NSSEE protected neuronal cells significantly compared to propofol or its combination (Table 1). TBI+Propofol group and TBI+NSSEE+Propofol group showed no significant difference, but differed significantly from the control group, TBI-induced group, and TBI+NSEE group. Meanwhile, the control, TBI-induce, and TBI+NSEE groups were significantly differed from each other among the groups (Figure 3F).

Discussion

In the present study, histopathological effects of TBI were observed in the TBI-induced group, showing significantly higher levels of hemorrhage, congestion, inflammatory cells, necrosis, apoptosis, and degeneration compared to all other groups ($p<0.05$). TBI induces parenchymal and vascular damage, leading to increased calcium and sodium influx, glutamate release, and subsequent neuroinflammation. (11,19) TBI also causes cerebral ischemia, mitochondrial damage, and release of reactive oxygen species (ROS), resulting in neuronal apoptosis.(20,21) These biochemical processes can lead to cell damage, including apoptosis and necrosis, and clinical symptoms of neurological deficits. (21,22)

The mentioned effects of TBI (neuroinflammation, ROS, ischemia) are part of the secondary brain injury. Secondary injury is an injury that occurs due to various pathological processes that arise as advanced primary brain damage in the form of bleeding, brain oedema, ongoing nerve damage, ischemia, and changes in neuronal chemistry. Abnormalities in brain tissue in humans can result in changes in the physiological function of the brain and can result in pathological conditions and histopathology of the brain. It is very important to reduce or prevent secondary brain injury because clinically these changes in brain function can cause clinical symptoms in the form of periods of loss or decreased consciousness, signs of memory loss shortly before or after the trauma, neurological deficits in the form of muscle weakness, balance disorders, aphasia, visual disturbances/impediments, muscle paralysis, loss of sensory function, and changes in mental status at the time of injury such as confusion, symptoms of disorientation, slow thinking, and so on. These disorders can be permanent or temporary.(19–22)

NSSEE showed a greater reduction in hemorrhagic features, congestion, inflammatory cells, necrosis, apoptosis, and degeneration (histopathological features) compared to the TBI-induced group, with a significant difference. This result might be due to its anti-inflammatory and antioxidant properties.(10,11) Propofol administration also decreased these features compared to the TBI-induced group, but less than TBI+NSSEE group, with a significant difference.

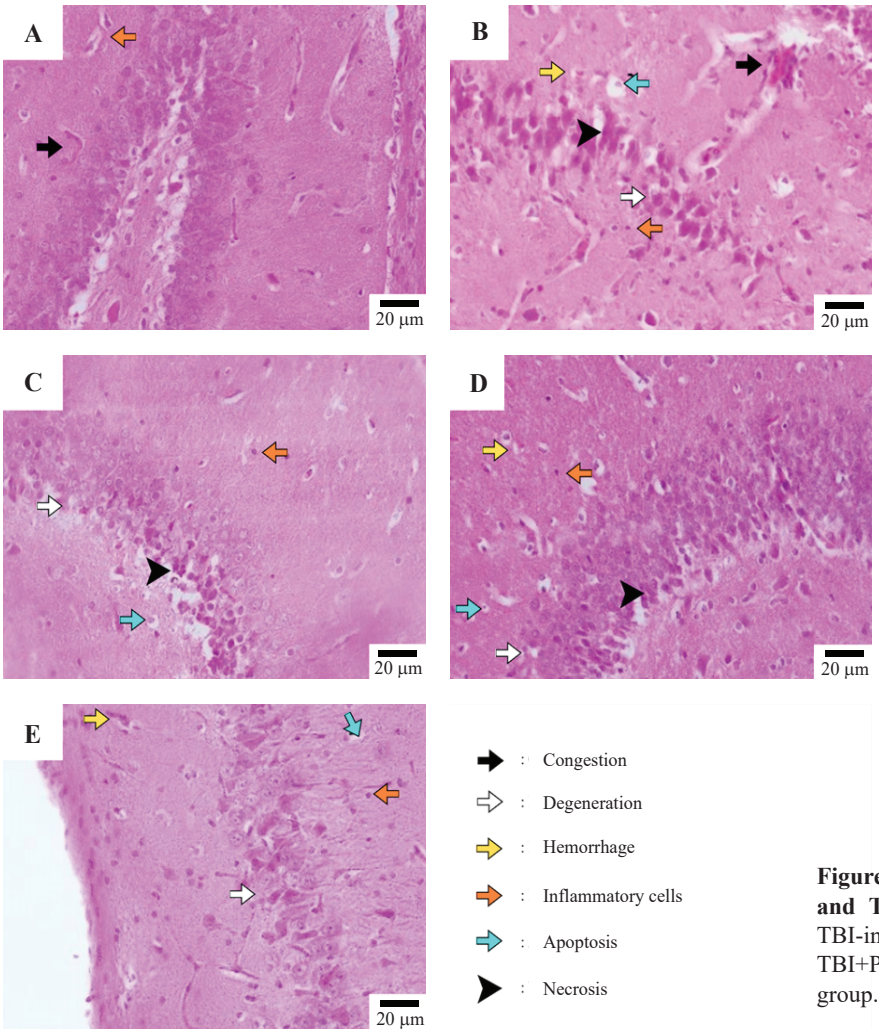

Figure 2. Histopathology images of control group and TBI-induced groups. A: control group; B: TBI-induced group; C: TBI+NSSEE group; D: TBI+Propofol group; E: TBI+NSSEE+Propofol group. Black size bar = 20 µm.

This is because propofol acts as an N-Methyl D-Aspartate (NMDA) antagonist, inhibiting glutamate release and preventing excitotoxicity.(23)

Combination of propofol and NSSEE led to a greater decrease in histopathological features compared to the TBI-induced group. However, it showed a smaller reduction in histopathological features compared to the TBI+NSSEE group, indicating that *Nigella sativa* had a stronger effect on improving neuronal cell damage ($p<0.05$). Combined administration of NSSEE and propofol reduces neuronal cell damage after TBI, diminishing neuroinflammatory and neuro-apoptosis processes and enhancing neuroprotection. (11,19,20)

Hemorrhage, inflammation, necrosis, apoptosis, and degeneration were sequentially higher in TBI-induced group, TBI+Propofol group, TBI+NSSEE+Propofol group, TBI+NSSEE group, and control group. Conversely, congestion was observed to be sequentially higher in the TBI-induced group, TBI+Propofol group, TBI+NSSEE+Propofol group, control group, and TBI+NSSEE group. This suggests that administering NSSEE is more effective in protecting against nerve cell damage in TBI compared to propofol or its combination. (11,19,20) TBI influences histopathological features in brain tissue, with glial reactions playing a role in inflammation and recovery post-TBI.(21)

The present study showed that administering NSSEE improves histopathological features and acts as neuroprotection in TBI. Nigella sativa has almost the same therapeutic effect as traditional medicine widely used in Indonesia such as propolis. Just as propolis functions as an antiapoptotic, anti-inflammatory, and antioxidant (24), *Nigella sativa* also has the same effect. In the last few decades, there has been a lot of research on traditional medicine that has been conducted with medical technology to improve the quality and safety of traditional medicine products, which currently have the same quality as modern medicine.(25)

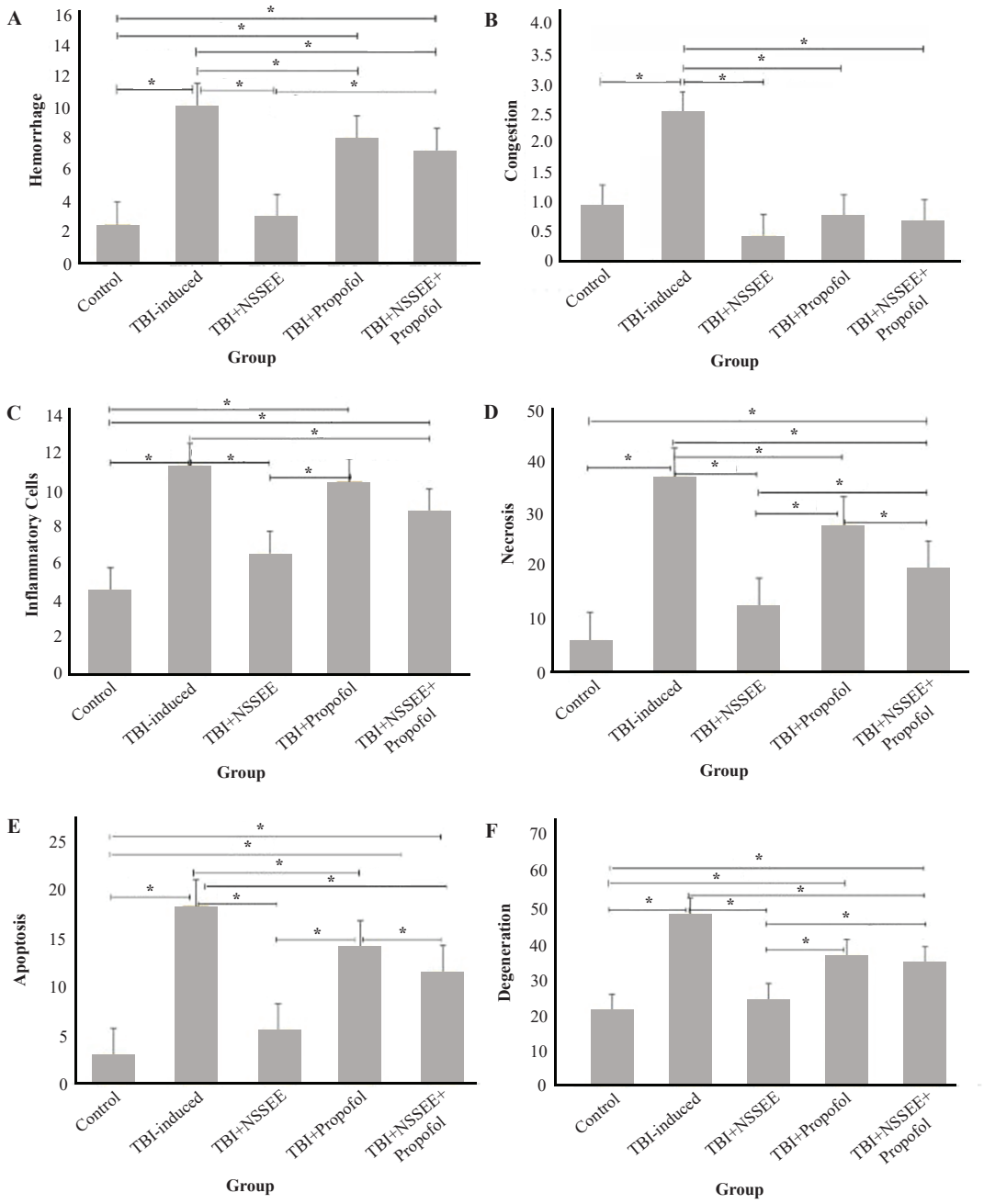

Figure 3. Quantification results of the brain histopathological features after treatment. A: Brain haemorrage; B: Brain congestion; C: Brain inflammatory cells; D; Brain necrosis; E: Brain Apoptosis; F: Brain degeneration. Data presented as mean±SD. *Considered to be significant if $p<0.05$, tested with one-Way ANOVA on normally distributed data or Kruskal–Wallis on non-normally distributed data.

The nutritional content of *Nigella sativa*, such as butyl hydroquinone, omega -3, omega-6, glucosamine, stearic acid, palmitic acid, linoleic acid, flavonoids, polyphenols, saponins can reduce the histopathological picture of cell damage so that it has an effect as neuroprotection. *Nigella sativa* also contains bioactive substances such as thymoquinone which is useful as an antioxidant and anti-inflammatory which also functions as a neuroprotective in cases of traumatic nerve injury. Giving thymoquinone immediately after traumatic nerve injury given 6×24 hours after injury can reduce antibodies in the degeneration process.(26)

Because of the content of *Nigella sativa* which functions as an antioxidant, anti-inflammatory, immunomodulator and works on the methyl guanine methyl transferase (MGMT) pathway can activate the mitogen activated protein kinase (MAPK) and nuclear factor-kappaB (NF-κB) pathways so that it influences neuroplasticity.(9) Administration

of 250 mg/kg thymoquinone found in *Nigella sativa*, appeared to be able to reduce immunoglobulin G (IgG) and interleukin (IL)-10 through inhibition of NF-κB expression levels and matrix metalloproteinase (MPP)-9 levels given immediately after traumatic nerve injury.(27) *Nigella sativa* contains antioxidants, anti-inflammatory, antimicrobial, and immunomodulatory properties, including MGMT to reduce neuron cell DNA damage, promote cell regeneration, and decrease necrosis and apoptosis.(9) *Nigella sativa* acts as an antioxidant and anti-inflammatory agent by inhibiting harmful inflammatory mediators such as ROS, nitrous oxide (NO) release, MDA reduction, lipid peroxidation (via lipoxygenase and cyclooxygenase inhibition), and deoxyribose degradation.(11,22) It also enhances antioxidant enzyme activity, including glutathione transferase, SOD, glutathione peroxidase, GSH, and CAT.(11,28)

Propofol administration reduces histopathological damage to TBI neuron cells in rats by decreasing hemorrhage, congestion, necrosis, inflammatory cells, apoptosis, and degeneration. Its protective effect may vary depending on the timing of administration after traumatic brain injury. Propofol functions as neuroprotection with the working mechanism of reducing brain oxygen consumption, cerebral metabolism rate oxygen ($CMRO_2$) and intra cranial pressure (ICP) so that it can reduce the histopathological picture of cell damage such as hemorrhage, congestion, necrosis, inflammation, apoptosis, degeneration.(29–31)

There might be some differences in treatment stress between groups that not receiving medication (control group *vs.* TBI-induced group) and variations in drug administration methods (oral for NSSEE administration, and intravenous for propofol administration) in this study. The observations were also only performed through hematoxylin and eosin staining. Further study which includes more specific examination using immunohistochemistry to see each bleeding, edema, or inflammatory cells might be beneficial. In addition, more researches are needed to explore other plant-based extracts for enhancing neuroplasticity and providing neuroprotection.

Conclusion

NSSEE showed greater efficacy in histopathological features (brain hemorrhage, congestion, inflammatory cells, necrosis, apoptosis, and degeneration) in rat models with TBI compared to propofol or a combination of propofol and NSSEE, indicating its superior potential in providing neuroprotection in TBI cases.

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Authors Contribution

KK, SS, KH, and UB were involved in concepting and planning the research, KK performed the data acquisition/collection, KK, KH and UB calculated the experimental data and performed the analysis, KK and SS drafted the manuscript and designed the figures, KH and UB aided in interpreting the results. All authors took parts in giving critical revision of the manuscript.

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