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Potential of Nigella Sativa on Anti or Pro-apoptotic Pathway After Brain Injury Via Brain-derived Neurotrophic factor on Rattus Norvegicus Wistar

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ABSTRACT

Black cumin may affect BDNF production to achieve the anti-apoptotic pathway due to its molecular structure. Black cumin as a neuroprotectant in brain injury (cerebral contusion) or trauma models is unusual. This study examined how black cumin affected BDNF neuron cells in Rattus norvegicus wistar head damage. The experimental animals were divided into the following four treatment groups: Group JH1: after the brain contusion was conducted, they were fed with black cumin extract 200 mg/kg bw every day for 7 days. After a brain contusion was conducted on Group JH2, they were given black cumin extract 300 mg/kgbw each day for seven days. Group JH3: After the brain contusion was conducted, they were fed with black cumin extract 400 mg/kg bw every day for 7 days. Group K: after the brain contusion was given Nacl 0.9% 3 ml per day for 7 days. JH3 group (400mg/kgbw) with an average BDNF (65.46 ng/ml), JH1 group (200mg/kgbb) (14.93 ng/ml) when compared to control (18.96 ng/ml), and JH2 group (300mg) with BDNF levels (22.04 ng/ml) that were nearly the same as the control. 400 mg of black cumin boosted BDNF levels dramatically (P=0.000). The higher the quantity of black cumin administered, the lower the amount of apoptosis seen (P=0.076). And as BDNF levels increased, neuronal apoptosis decreased (P = 0.004). In experimental rats who had suffered head trauma, the injection of black cumin extract led to an increase in BDNF levels. In experimental rats who had suffered head trauma, the administration of black cumin extract led to a reduction in apoptosis.

1. INTRODUCTION

Head injuries account for fifty percent of trauma-related fatalities and eighty percent of cases treated in the emergency department. Head traumas can damage the entire head, including the scalp, skull, and brain [1- 3]. The progression of a brain injury is not random, but rather a continuous process between primary and secondary brain injuries [4]. Consequently, the initial diagnosis, treatment, and prognosis of brain injury are not simple, despite the fact that methods of diagnosis and management of brain injury are constantly evolving [1], [5]. Until recently, BDNF has been widely utilized as a seromarker in numerous studies involving nerve cells. A higher level of BDNF indicates that cell healing will occur more effectively, [6] than in those with lower BDNF levels. BDNF also functions as an anti-apoptotic agent by inhibiting caspase-3 [7]. Infusing BDNF into a patient with a spinal cord injury will enhance the recovery of neurological function [8]. In order for neurons to thrive and generate new synapses, it is necessary to provide them with an adequate supply of brain-derived neurotrophic factor (BDNF) [9], [10]. Numerous research have demonstrated the advantages of black cumin seeds, which include analgesic, antibacterial, anti-inflammatory, anti-microbial, antioxidant, anti-pyretic, anti-tumor, immunomodulatory, and neuroprotective activities [11- 13]. Experimental animals receiving black cumin seed extract for cerebral ischemia have lower levels of MDA (malondialdehyde) [14]. Black cumin prevents formaldehyde from causing neuronal apoptosis when administered to animals [15]. Through the caspase-3 blocking pathway, which is comparable to neurotropin, the mechanism of black cumin as an anti-apoptotic neuron was examined (BDNF) [7], [15], [16]. Black cumin and BDNF have different chemical structures, therefore it is plausible that black cumin influences the production of BDNF to activate the anti-apoptotic pathway [17]. It is believed that black cumin inhibits calcium channel blockers, hence decreasing calcium flow [18]. Black cumin research as a neuroprotectant in non-traumatic settings has been validated. Black cumin has not yet been investigated as a neuroprotectant in models of head injury (cerebral contusion) or trauma. This study aimed to examine the effect of black cumin on BDNF neuron cells following head damage in Rattus norvegicus wistar rats.

2. MATERIAL AND METHODS

In this laboratory investigation, rats were utilized as the experimental animals, and the experiment was designed to be entirely randomized. This research has obtained ethical permission from the institution of Dr. SAIFUL ANWAR General Hospital Malang Indonesia with serial number 351/KEPK/VII/2012. Four treatment groups were created from the experimental animals as follows: Group JH1: For 7 days following the brain contusion, they were fed black cumin extract at a dose of 200 mg/kg bw each day. Group JH2: For 7 days following the brain contusion, they were given 300 mg/kg bw of black cumin extract daily. Group JH3: For 7 days following the brain contusion, they were given 400 mg/kg bw of black cumin extract daily. Following the brain contusion, Group K received 3 ml of Nacl 0.9% daily for 7 days. The Ethical Clearance No. 351/KEPKVII/2012 Commission for Health Research Ethics granted permission for this line of investigation. Dr Saiful Anwar General Hospital Malang Indonesia. The research was conducted in the Faal laboratory, Pharmacology laboratory, and Anatomical Pathology laboratory, Faculty of Medicine, Brawijaya University Malang, from January 2012 to February 2012.

The Rattus norvegicus wistar strain was used in this experiment, and the average age and weight of the animals used were 12-14 weeks and 200-250 grams, respectively. The male experimental animal was in good health and was freely moving around. Supplies needed to sustain experimental animals for 10 days. Black cumin extract is manufactured by. Salinity of Seawater, typical of normal (0.9% Saline) (Otsuka). Ketamine injection was utilized for the euthanasia of experimental animals. Materials for the fabrication of histopathological preparations included rat brain tissue, 10% buffered formalin, hematoxylin-eosin dye, paraffin, xylol, and alcohol at 80%, 95%, 96%, and 100% concentrations. Acid alcohol, counter staining, Canadian balm, egg white, water. Apoptosis examination material: mouse brain tissue, Apoptec (Proteinase-K Enzyme, Apoptag, DAB liqud). Reagents using Ray Bio® BDNF Elisa Kit, Ray Biotech Inc, consisting

of: BDNF microplate, Buffer concentrate, Standards: Recombinant BDNF, Assay diluent A, Assay diluents B, Detection Antibody BDNF, HRP Streptavidin Concentrate, Tetramethyl benzidine (TMB) One Step Substrate Reagent, Stop Solution.

2.1 Cerebral Contusion Model

The total number of rats used in the study was 20, where each treatment consisted of 5 animals. Cerebral contusion model was done on experimental animals by giving a load of 0.2 kg was dropped through a cylindrical tube from a height of 0.8 m (impact energy of 1.6 Joules) over the head of a stereotactic frame-mounted experimental animal. Previously, 1 mg/kg of body weight (i.m.) of ketamine was administered intramuscularly to sedate the test animals (Ibolja, 2005). Contusions were studied by: Histopathology: with Haematoxylin-Eosin (HE) staining, utilizing the calculation of the edema index subdivided into grades 0.1,2,3, by analyzing 20 visual areas. Grade 0-3 reflects the percentage of edema results (0%, 25%, 50%, > 50%).

2.2 Examination of Apoptotic Cell Count

The TUNEL DNA fragmentation method was used to count the amount of apoptotic cells and the results are as follows: Slides were cleaned in PBS pH 7.4 and then treated with proteinase K (20 ug/mL) for 15 minutes at 37 °C. Three times, each for five minutes, wash with PBS pH 7.4. 15 minutes of 3% H2O2 incubation. Three times, each for five minutes, wash with PBS pH 7.4. Incubation with fragmented DNA labeling from Tunel for sixty minutes at a temperature of 370 degrees Celsius. Wash using PBS with a pH of 7.4, doing so three times for a period of five minutes each. Incubate at 37 degrees Celsius for forty minutes with a peroxidase solution. Wash with PBSpH 7.4, doing so three times for a period of five minutes each. Twenty minutes at room temperature were spent with the drops utilizing a substrate for peroxidase known as DAB, which is diamino benzidine. Rinse with tap water, then wash with dH2O after counterstaining for 10 minutes with Mayer hematoxylene and washing with PBS pH 7.4. Dry off, then shut the coverglass. then take a 1000x magnification look at it with a light microscope. The nucleus of apoptotic cells is illustrated in brown. Using ovulated mouse ovaries as a positive control. In order to determine the cell count, the number of brown apoptotic cells among 100 seen cells was determined by moving the field of observation. The average value was then calculated after observations were made on additional slides belonging to the same treatment group. The researcher's findings were subjected to a double-blind verification test with assistance from a verifier from the anatomical pathology Faculty of Medicine laboratory, Brawijaya University.

2.3 Examination of BDNF

BDNF was evaluated using ELISA, as reported by Ray Biotech, Inc. BDNF evaluation protocol utilizing ELISA technique: Antibody-filled wells that also contain a reference sample that has been prepared to a maximum volume of 100ul. 150 minutes of incubation, followed by 150 minutes of room-temperature stirring and a buffer wash. After washing, the wells are dripped with 100ul of biotin-labeled antibody solution and incubated for 60 minutes at room temperature while being stirred. After adding 100ul of streptavidin, the mixture was left to incubate for 45 minutes in a mortar at room temperature before being cleaned. After 30 minutes of incubation, 50ul of stop solution was applied to the wells that had been washed with subtract solution. The Elisa reader utilizes a 450nm wavelength to read the wells. BDNF diperiksa dengan ELISA menurut Ray Biotech, Inc.

2.4 Black Cumin Extract Preparation

Typical commercially available black cumin extract formulations contain 100 mg/cc of a suspension prepared by dissolving 600 mg of black cumin extract from capsules in 6 cc of 0.9% NaCl. Group JH1 received 200 milligrams (mg), group JH2 300 milligrams (mg), and group JH3 400 milligrams (mg) via nasogastric tube.

2.5 Experiment Implementation

Following the administration of black cumin extract, specimen collection (harvesting) was performed on the seventh day for each group (n=5) in the study. Infusions of ketamine at a dose of 1 mg/kg body weight were used to induce anesthesia. After performing a decapitation on the patient, a ventriculostomy procedure with a spinal needle placed 27.3 mm in front of the central sulcus and 3 mm lateral to the fissure was used to withdraw cerebrospinal fluid from the patient. Half of the right and left brains were removed sterilely and placed in a petri dish with 10% formalin. The removed brain tissue was stained with haematoxylin eosin (HE), sliced, and then prepared with paraffin blocks before being inspected under a light microscope. uses 20 fields of view to calculate the edema index, which is divided into grades of 0.1, 2, 3, and 4. The percentage of edema results (0%, 25%, 50%, > 50%) is shown for grades 0–3. Based on the presence of vacuoles containing intracellular and interstitial fluid, edema is evaluated.

2.6 Data Analysis

The computation method utilizes SPSSTM software tools. A statistical analysis was conducted: Examine the difference between Black Cumin extract treatments. In each group, analysis was conducted using ANOVA with F-test and Tukey HSD for multiple comparisons

3. RESULTS

In this study the data were presented according to the 4 treatment groups. The JH1 group was the experimental animal group with head injury fed black cumin at 200mg/kgbw, the JH2 group fed black cumin at 300mg/kgbw, the JH3 group fed 400mg/kgbw, and the control group fed 0.9% 3cc NS.

3.1 Brain tissue on a macroscopic level from rats who had head trauma

A head injury model was used for the experimental group JH1, JH2, JH3, as well as the control, and it had an energy of 1.6 joules. A macroscopic examination of the patient's brain tissue did not reveal any signs of cranial fracture, subdural hemorrhage, subarachnoid or intracerebral bleeding (Figure 1). On a macroscopic scale, there was no discernible difference between the treatments; more specifically, the structural characteristics of the brain parenchyma were identical across all treatment groups.

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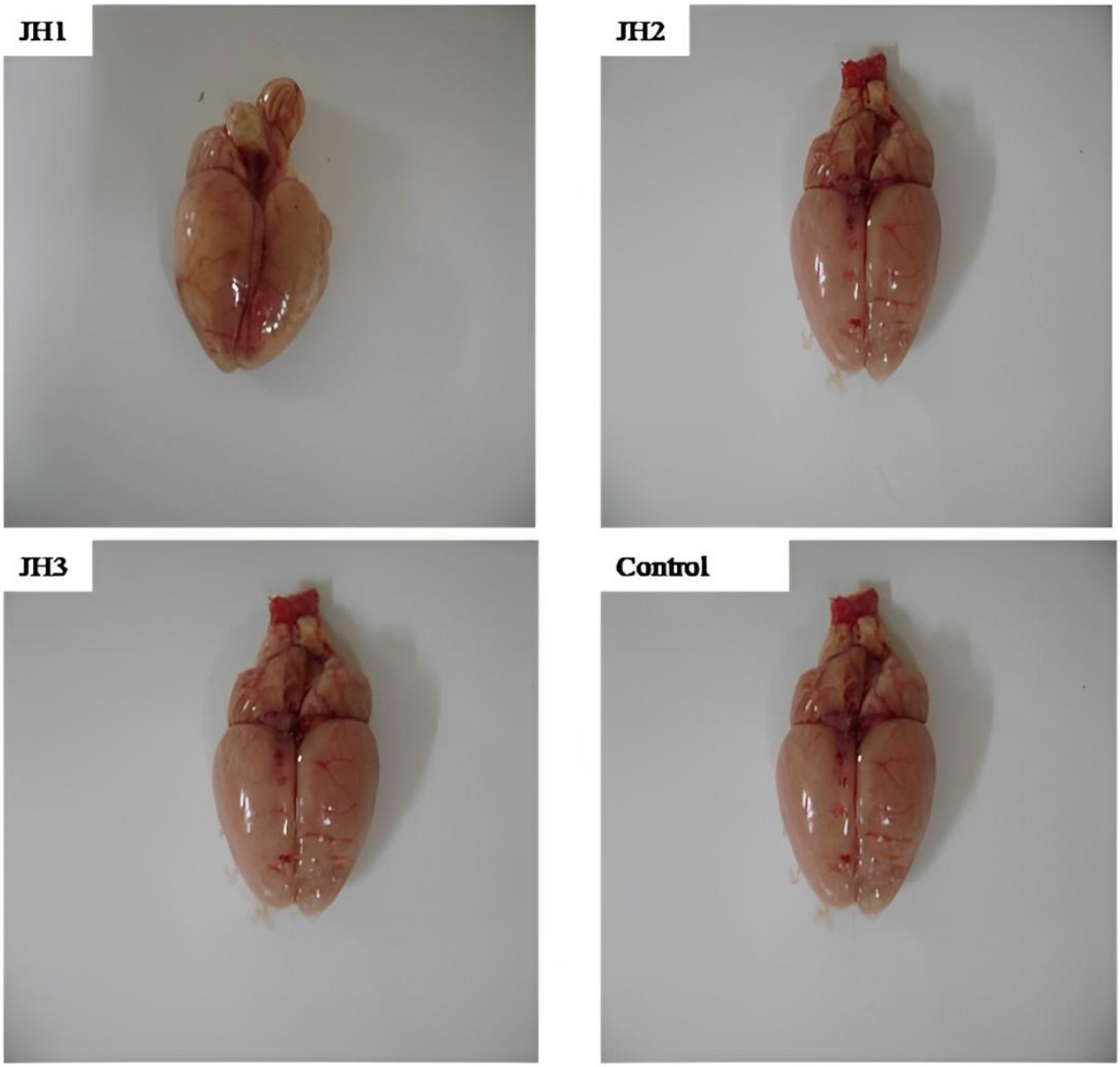

Figure 1. Macro view of the parenchyma of rat brain tissue. After seven days of treatment with either black cumin (JH) or a placebo consisting of NS, the macroscopic picture of the rat brain parenchyma did not reveal any differences between the two groups

3.2 The Relationship of Black Cumin on the Number of Apoptotic Neurons and BDNF levels

After 7 days of treatment with black cumin, cerebrospinal fluid BDNF levels were determined using ELISA. Following ventricular puncture, cerebrospinal fluid was extracted from the rat brain. Meanwhile, the TUNEL DNA fragmentation method was used to count the number of apoptotic cells. Average BDNF concentrations and the amount of apoptosis in the treatment group versus the control group are described as shown in the diagram (Figure 2).

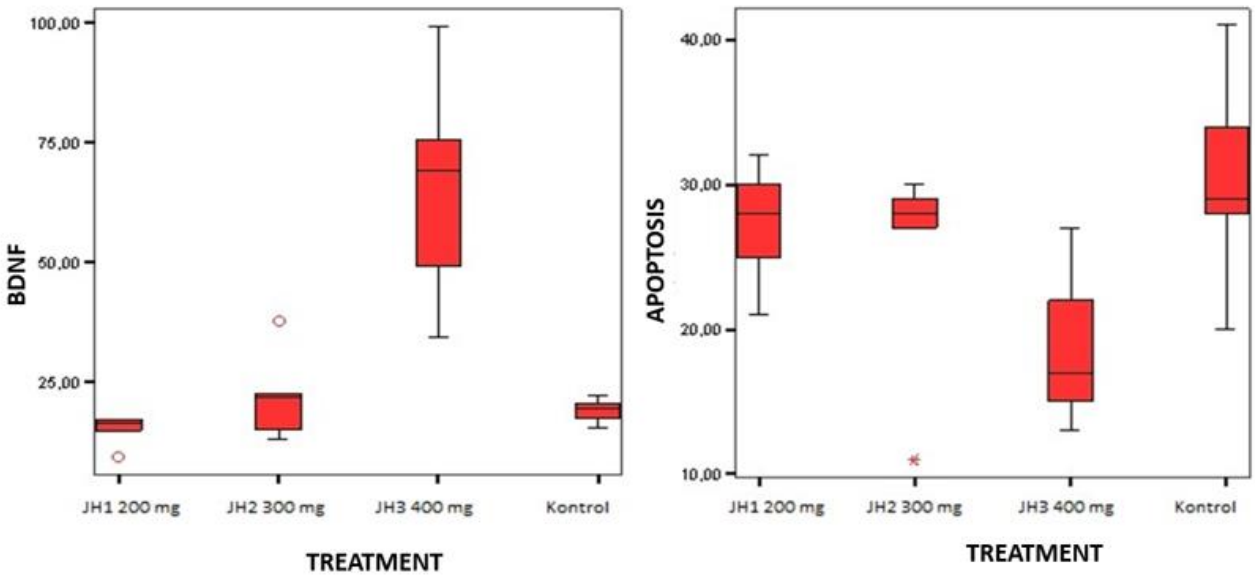

Figure 2. Data on cerebrospinal fluid BDNF levels and the number of apoptotic neurons in rat brain after treatment with black cumin and controls. Rats received black cumin feeding of 200mg/kgbw (JH1), 300mg/kgbw (JH2), 400mg/kgbw and 3 cc of NS (control) after head injury for 7 days

In contrast to the other categories, the JH3 group that was administered black cumin at a dose of 400 mg/kg body weight per day had the highest average level of BDNF (65.46 ng/ml) (Figure 2). When compared to the control group, which had an average level of BDNF of 18.96 ng/ml, the JH2 group (300mg) had BDNF levels of (22.04 ng). /ml, which is almost the same as the control group's level. Giving a dose of black cumin 200mg/kgbw turned out to have a lower average level of BDNF (14.93 ng/ml). In other side, the graph above shows that the quantity of apoptosis decreases with increasing doses of black cumin. Comparing all of the groups of rats administered black cumin, the control group had the greatest amount of apoptosis at 30.4 (Figure 2). Apoptosis averages for the JH1 (200 mg), JH2 (300 mg), and JH3 (400 mg) groups of rats were 27.2, 25.0, and 18.8, respectively. The table below displays the outcomes of the black cumin anova test with apoptosis, even though descriptively it was discovered that the higher the dose of black cumin will minimize the amount of apoptosis (Table 1).

Table 1. The results of the mean, ANOVA statistical test, and Tukey Test on the Number of Apoptotic Neurons and BDNF levels

		Anova Statistical Test				Tukey HSD Test			
Variable	Group	Mean (ng/ml)	Standard Deviation	F-value	p	N	Subtealpha=0,05 1	Subtealpha=0,05 2	Sig
BDNF	JH1	14,93	3,34	15,08	0,000	5	14,927		1,000
	JH2	22,04	9,68			5	22.040		
	JH3	65,46	25,01			5		65,456	
	Control	18,96	2,75			5	18,95		
Apoptosis	JH1	27,20	4,32	2,761	0,076	5	18,800		0,058
	JH2	25,00	7,91			5	25,000		
	JH3	18,80	5,67			5	27,200		
	Control	30,40	7,77			5	30,400		

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Based on the ANOVA test results in the table above, the BDNF variable has a significance value of 0.000, because p<0.05, it can be concluded that there are significant differences between treatments. The results of the Tukey BDNF test showed that the JH3 (400mg) treatment group had a different effect compared to the other three treatment groups. The group receiving 300 mg of JH3 had the highest average BDNF value (65.46), compared to the other groups. The average level of brain-derived neurotrophic factor (BDNF) was decreased in the JH1 group (200 mg) compared to the control group; however, the dose of black cumin was not significantly different from the experimental group without black cumin. The administration of 400 mg of black cumin boosted BDNF levels dramatically (Table 1).

On the other side, in the apoptosis variable it was found that the control group had the highest degree of apoptosis (mean 30.40) followed by JH1 (mean 27.20), JH2 (mean 25.00) and JH3 (mean 18.80). The results of the ANOVA test with p=0.076 are not significant. Although descriptively, the control group and feeding normal saline caused the most apoptosis (average 30.4), followed by the group JH1 with black cumin feeding 200 mg/kg body weight per day for 7 days (average 27.2), followed by the group JH2 with black cumin feeding 300 mg/kg body weight per day for 7 days (average 25), and the group JH3 with (average 18.8). The difference between the means of apoptosis in each treatment group was not statistically significant, but p = 0.076 indicates that the risk of failureof 7 treatments in 100 clinical trials is extremely high (Table 1).

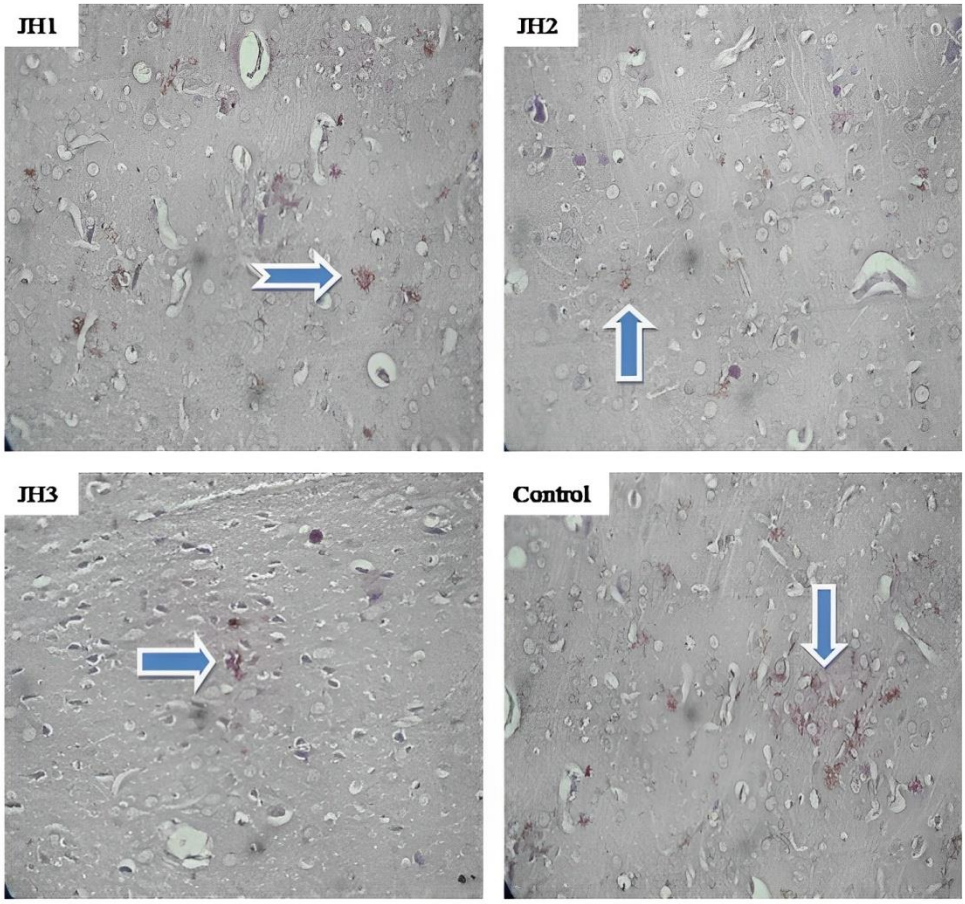

Figure 3. An overview of neuronal cell apoptosis from brain-injured rat. Black cumin was administered orally to the treatment group for seven days at doses of 200 mg/kgbw, 300 mg/kgbw, 400 mg/kgbw, and 3 cc of ordinary saline (control). With a 1000x magnification, cells coloured brown using the DNA fragmentation technique were seen to be undergoing apoptosis.

Histopathological sections stained with methyl green to show DNA break age by the terminal nucleotide dUTP nick end labeling method (Figure 3). The specimens were studied using a ligh tmicroscope with a 1000x magnification. The percentage of dying cells (brown) in a sample of 100 was used to estimate the number of apoptotic neurons. Descriptively, more cells were dyed brown on the control slide than they were with any of the other three treatments (Figure 3).

3.3 Correlation of BDNF with neuronal apoptosis in head injury after black cumin administration

The Spearman correlation test was used to ascertain the correlation between BDNF and neuronal apoptosis following head damage after injection of black cumin because the BDNF variable was not normally distributed (Table 2).

Table 2. Spearman correlation analysis BDNF and Apoptosis

Variable	N	Average	r-value	Sig	Notes
BDNF	20	30,35	-0,614	0,004	Significantly correlated
Apoptosis	20	20,35			

Notes: the results of the correlation analysis sig 0.004 ($p < 0.05$)

The correlation between the BDNF variable and apoptosis has an r-count value of -0.614 and a significance value of 0.004 according to the results of the correlation study presented in the table above. Because (p0.05), Ho is rejected, and it may be argued that BDNF and apoptosis have a substantial association. If the r-count is negative, there is an inverse link between BDNF and apoptosis; the higher the level of BDNF, the less apoptosis will occur, and the lower the level of BDNF, the more apoptosis will occur (Table 2).

Based on graph above, the correlation between BDNF and neuronal apoptosis following brain damage after administration of black cumin revealed a straight line with a negative correlation between BDNF and apoptosis. This indicates that as BDNF levels in crease, neuronal apoptosis declines. According to the results of the Spearman correlati on test (table 2), where the r-value -0.614, BDNF and apoptosis have an inversely proportionate association (Figure 4)

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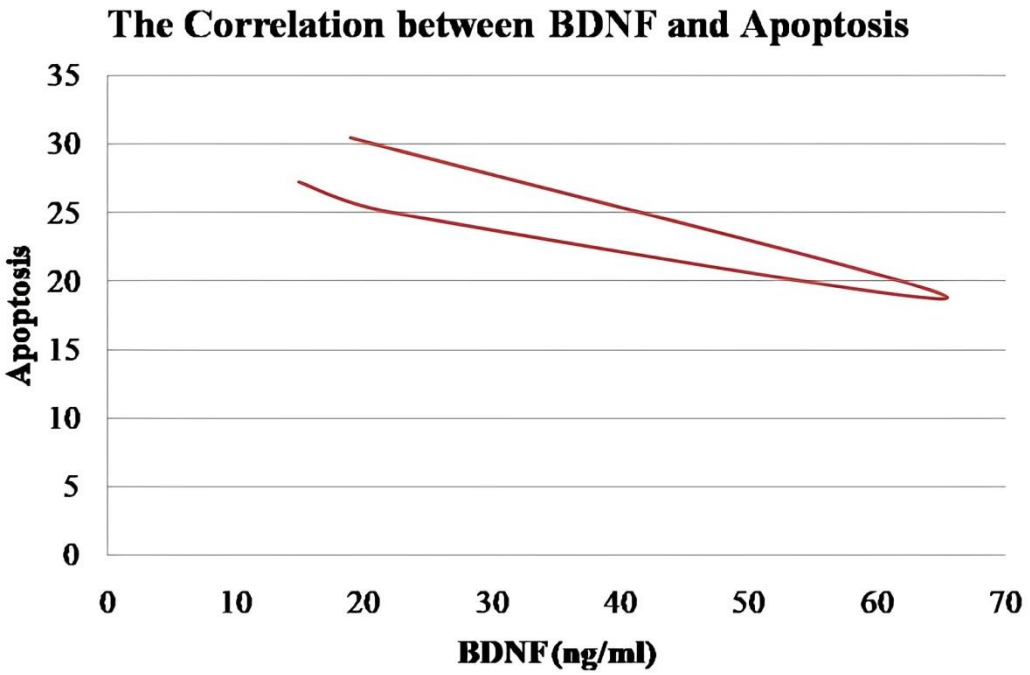

Figure 4. Graph of correlation of BDNF and brain apoptosis of rats with head injury after administration of black cumin. Apoptosis was found to be reduced in proportion to the concentration of BDNF.

4. DISCUSSION

In this experiment, a male Rat Rattus Norvegicus weighing between 250 and 300 grams was employed. The choice of these experimental animals was made in accordance with [13] research. The low mortality rate of rats, cost-effectiveness, and simplicity of the brain contusion model all contribute to the usage of this experimental rodent [19].

In this particular experiment, only male experimental animals were utilized. When there is a difference between the sexes, the results will be different. Although the precise mechanism is still poorly known, it is believed that males and females activate X-linked inhibitor of apoptosis protein (XIAP) in distinctive ways, even if the pathomechanism is poorly understood. XIAP production was more in female rats than in male rats, resulting in reduced apoptosis in female rats compared to male rats. It is believed that increased estrogen levels in female rats contribute to higher XIAP levels protein [20].

The head injury model was executed by impacting the head of the experimental animal with an energy of 1.6 Joules, specifically by lowering a 200-gram weight through a 0.80-meter-tall cylindrical pipe. This agrees with Ibolja 21 model of brain contusion, which assumes an impact of 1.62–1.89 Joules. There is bleeding inside the skull (either subdural, subarachnoid, or intracerebral) but no visible fractures of the skull. Increased permeability of the cerebral vasculature, decreased cerebral blood flow, and raised intracranial pressure are all possible outcomes of the contusion model. Mild bleeding will start after 48 hours of impact. These animal models are reproducible and can be used to simulate mild or moderate head trauma in humans, depending on the weight of the load, the height of the fall, and the weight of the experimental animal. This study employs 1.6 Joules of energy, hence the brain contusion model in this study is consistent with the usual cerebral contusion model [21].

Using a probe, black cumin (JH) was administered orally using the following dosages: JH1 200 mg/kgBW, JH2 300 mg/kgBW, JH3 400 mg/kgBW, and a control with 3 cc Normal saline. The 400 mg/kg bw dosage is

based on research conducted by Kanter 15 In order to evaluate whether or not very low dosages of black cumin have the effect of elevating BDNF and inhibiting apoptosis, a dose of 200 milligrams per kilogram of body weight was used. However, a dose of 300 milligrams per kilogram of body weight is a dose that falls between a low dose and a high dose [13]. Since black cumin's use is predicated on the idea that scavengers need to be present before free radicals appear or are generated, it is administered as soon as possible after injury. After 4 hours post-traumatically, cerebrovascular leakage and iNOS/NO expression both began to rise [19], [22].

4.1 Black cumin extract increases BDNF levels after head injury

An endogenous molecule called brain derived neurotrophic factor has a role in sustaining synaptic plasticity, neuron function, and the structural integrity of the adult brain. Numerous events, such as head trauma, raise BDNF levels. Additionally, ischemia and hypoxia raise BDNF levels. The range of human BDNF concentrations in cerebrospinal fluid under typical circumstances is 6.16 pg/ml.9 BDNF is involved in neuronal growth, defense, and differentiation, but it is also involved in neuronal cell death.9 It is unknown how black cumin increases BDNF levels, however it is probable that the antioxidant content (thymoquinone) in black cumin functions as a free radical scavenger, hence preventing neuronal damage [16].

In this investigation, it was determined (table 1) that a group of JH3 rats administered 400 mg/kg body weight per day of black cumin extract for seven days had significantly higher BDNF levels than the other groups ($p = 0.000$). If we examine the Tukey HSD test results (table 1), the JH1 200 mg/kgbw, control, and JH2 (300mg) were not statistically significant, but the average value of control BDNF levels was higher than JH1 (200mg), indicating that the administration of black cumin at that dose had no effect on increasing BDNF levels. The administration of 300 mg/kgbw black cumin did not differ significantly from the control group (table 1). However, there was a considerable increase in BDNF levels at a dose of 400 mg/kgbw; the source of the sudden increase in the JH3 treatment group was apparently related to the cumulative dose dependence of black cumin. The ability of black cumin to scavenge free radicals is still outstripped by the creation of free radical ions at doses less than 300 mg, meaning that many free radical ions are not caught by the antioxidant molecule of black cumin (thymoquinone), and there are still radical ions/molecules. Unpaired free radicals continue to oxidize neuronal membranes, causing several more neurons to lyse as a result of free radical oxidation. The amount of neuron cells that lysis causes to diminish also causes a decrease in the ability of cells to generate cytokines, including cytokines that are produced by the central nervous system such as BDNF [23].

There is definitely a stimulating mechanism for boosting the concentration of BDNF in the central nervous system without the administration of any antioxidant preparations (including black cumin). When a person suffers a head injury, the intracranial pressure rises, which can alter the physiology of the brain. Blood flow in the brain is interrupted, which can lead to ischemic processes and brain metabolic diseases. Brain edema will result from secondary brain injury caused by this mechanism up to 48–72 hours after the incident. The load inside the skull will rise as a result. Neuronal death consequently occurs, aggravating the degenerative process of neuronal injury. It is believed that tissue hypoxia and ischemia cause neuronal injury, which stimulates the creation of BDNF [24].

The process through which black cumin extract increases the quantities of endogenous proteins that has been researched focuses on its anti-oxidant properties. Black cumin, which acts as a chelating agent against free radicals, boosts the activity of the acetylcholinesterase enzyme in the central nervous system [25]. In experimental chicken erythrocytes, black cumin administration dramatically decreased MDA levels (p0.002) and increased GSH levels (p0.005) [25]. Stress induces BDNF production, hence BDNF is also known as a

stress protein; this stress protein is also found in the liver, intestines, and brain.26 Four controlled promoter regions enhance the mRNA content at multiple places of the 4 noncoding'sexons (I-IV) and the common 3'exon (V) encoding the mature protein of BDNF, hence regulating the transcriptional regulation of BDNF. Ca2+ influx is thought to be one of the physiological stimuli that can stimulate distinct 5' promoters and induce the production of BDNF exon III [9].

Additionally, black cumin suppresses inflammation by inhibiting the 5-lipoxygenase enzyme, hence inhibiting different inflammatory leukotrienes. LPS-induced iNOS (inducible nitric oxide synthase) expression is suppressed, resulting in decreased NO generation by macrophages, which improves the inflammatory response and reduces cell damage due to fewer free radicals [27].

4.2 The role of black cumin in neuronal apoptosis after head injury

Black cumin's anti-apoptotic effects were observed in this investigation; however, they were not statistically significant (p=0.076). An extremely high BDNF level seems to contradict this finding (p=0.000). In JH3 treatment, high BDNF levels are expected to drastically reduce apoptosis because of BDNF's anti-apoptotic neuronal action. Due to the fact that the quantity of apoptotic bodies is read manually under a microscope at 1000x magnification in 100 fields of view, it is possible for human error to account for apoptosis levels that do not match BDNF values. The varying affinity of BDNF for various receptors influences the final outcome. Pro-BDNF has a strong affinity for P75NTR, allowing it to cause apoptosis, but mature-BDNF is more likely to bind to TrkB and promote cell survival. Pro-BDNF is secreted in two forms, mBDNF (14 kDa) and pro-BDNF, which is converted to mature-BDNF by extracellular proteases. The majority of secreted pro-BDNF will bind to pan neurotrophin P75, while the majority of mBDNF will bind to TrkB [28]. Does black cumin administration raise mBDNF? seems to be the question that has to be answered about black cumin and BDNF. It's conceivable that the outcome of neuronal apoptosis is also affected by the action of extra-neuronal BDNF. This process clarifies why BDNF and apoptotic findings could seem at odds with one another. The mechanism through which black cumin extract reduces neuronal cell death is currently unknown. The treatment of black cumin extract to rats with brain injuries was observed to reduce the levels of MDA (malondialdehyde) p0.001, an end product of lipid membrane peroxidation, possibly through its anti-oxidant activity [15]. A dose of 400 mg/kgbb black cumin extract was proven to dramatically reduce apoptosis (p0.0001) in a non-trauma model (formaldehyde induced neuronal damage).

The TUNEL technique revealed brown apoptotic entities, which included condensed cytoplasm, degeneration of cell nuclei, and dark, picnotic nuclei. The mechanism of prevention of neuronal cell apoptosis inhibitory pathways has not been thoroughly disclosed in a study on the effect of black cumin extract as an anti-apoptotic neuron cell model of non-trauma. Degenerative changes in neurons are typically accompanied by elevated oxidative stress. High oxidative metabolic ability, a high concentration of polyunsaturated fatty acids, and a low antioxidant capacity make the brain, and particularly the cortex and hippocampus, particularly vulnerable to oxidative stress [22]. Thymoquinone (2-Isopropyl-5-methylbenzo-1,4-quinone), which makes about 30% of black cumin's composition, has been shown to promote apoptosis in colon carcinoma cells by upregulating the activation of the MAPK pathway and ERK and JNK signaling (mitogen). active protein kinases) [29]. Thymoquinone can also initiate apoptosis by p53-dependent and p53-independent pathways in addition to these methods. Thymoquinone is a double-edged blade that acts as both a pro- and an anti-oxidant due to its two potentials. Thymoquinone may be reduced to semiquinone (1 electron) or thymohydroquinone, depending on the structure of the thymoquinone molecule (2 electrons). Thymohydroquinone has anti-oxidant properties, whereas semiquinone has pro-oxidant properties [29]. This explains why apoptosis with BDNF may not have been statistically significant in this study.

4.3 Relationship of BDNF and neuronal apoptosis after administration of black cumin in head injury

Increases in BDNF levels reduce neuronal apoptosis because this neurotrophin protects neurons from death. A Spearman correlation analysis test was performed, and the results are shown in Table 2. The value of sig -0.614 denotes a statistically significant inverse link between the two variables. With respect to neuronal apoptosis, the association graph similarly reveals an inverse link between BDNF and higher BDNF values. Despite the seeming paradox, increased quantities of BDNF protein will decrease the extent of apoptosis. BDNF is an antiapoptotic neurotrophic neuron, therefore the higher the neurotrophic level, the lower the neuronal apoptosis. According to correlation analysis test (table 2), the value of sig -0.614 indicates a substantial inverse link between the two variables with a sig value of 0.004. The correlation graph also displays the relationship between BDNF and neuronal apoptosis; the higher the BDNF value, the lower the number of neuronal apoptosis. The link between BDNF and apoptosis appears to be counterintuitive, as high amounts of BDNF protein inhibit apoptosis.

5. Conclusion

This study found that giving black cumin extract to experimental rats with head traumas raised their BDNF levels. In experimental rats with head injuries, injection of black cumin extracts reduced apoptosis, albeit not significantly. In experimental rats with head injuries, treatment of black cumin extract causes a connection between BDNF and apoptosis. Further research is required to determine the mechanism or method through which the black cumin extract increases BDNF expression. It is necessary to do pharmacological study on the various methods of extracting black cumin in order to increase levels of understanding regarding thymohydroquinone. To learn more about thymohydroquinone, pharmacological research on black cumin extraction methods is needed. This is because giving black cumin extract orally to humans requires clinical testing.

6. References

[1] Angoa-Pérez M, Anneken JH, Kuhn DM. The Role of Brain-Derived Neurotrophic Factor in the Pathophysiology of Psychiatric and Neurological Disorders. J Psychiatry Psychiatr Disord. 2017 Jul 24;1(5):252-269. doi: 10.26502/jppd.2572-519X0024. PMID: 35098038; PMCID: PMC8793768.

[2] Beal M F, P. T., Kostrzewa R, Archer T 2000. Neuroprotective And Neurorestorative Strategies In Neuronal Injury. Neurotoxicity Research, 2, 71-84.

[3] Binder, D. K. & Scharfman, H. E. 2004. Brain-Derived Neurotrophic Factor. Growth Factors, 22(3), 123-131.

[4] Chen Sd, Wu Cl, Hwang Wc, Yang Di. More Insight Into Bdnf Against Neurodegeneration: Anti-Apoptosis, Anti-Oxidation, And Suppression Of Autophagy. Int J Mol Sci. 2017 Mar 3;18(3):545. Doi: 10.3390/Ijms18030545. Pmid: 28273832; Pmcid: Pmc5372561.

[5] Duan W, L. J., Guo Z, Mattson Mp. 2001. Dietary Restriction Stimulates Bdnf Production In Brain And Thereby Protects Neurons Against Excitotoxic Injury. Jounal Of Molecular Neuroscience, 16, 1-12.

[6] Dash Hh, Chavali S. Management Of Traumatic Brain Injury Patients. Korean J Anesthesiol. 2018 Feb;71(1):12-21. Doi: 10.4097/Kjae.2018.71.1.12. Epub 2018 Feb 1. Pmid: 29441170; Pmcid: Pmc5809702.

[7] El-Najjar N, C. M., Moukadem H, Vuorela H, Ocker M, Gandesiri M, Schneider R, Gali H 2010. Reactive Oxygen Species Mediate Thymoquinon-Induced Apoptosis And Active Erk And Jnk Signaling.

Apoptosis, 15, 183-195.

[8] Graham, D. & Gennarelli, T. 2000. Pathology Of Brain Damage After Head Injury. In: Pr, C. & Golfinos, J. (Eds.) Head Injury. 4 Ed. New York: Mc Graw-Hill Inc.

[9] Helen M.B., O. F. A., George L., Alejandra S.P., Juliana S., Robert W.K.. 2009. Sex Differences In Xiap Cleavage After Traumatic Brain Injury. Neurosci Lett, 461 (1), 58-60.

[10] Holland, M. 2003. Craniocerebral Trauma. In: Way, L. & Doherty, G. (Eds.) Current Surgical Diagnosis And Treatment. 11 Ed. Boston: Mac Graw-Hill Companies, Inc.

[11] Huang, E. J. & Reichardt, L. F. 2001. Neurotrophins: Roles In Neuronal Development And Funtion. Annu. Rev. Neurosci, 24, 677-736.

[12] Japardi 2004. Cedera Kepala: Memahami Aspek-Aspek Penting Pengelolaan Cedera Kepala. Jakarta: Buana Ilmu Populer.

[13] Kanter, M. 2010. Protective Effects Of Nigella Sativa On Formaldehyde-Induced Neuronal Injury In Frontal Cortex. Tip Arstirmalari Dergisi, 8(1), 1-8.

[14] Linsay, K. & Bone, I. 2004. Neurology And Neurosurgery Ilustrated. 4 Ed. Edinburg: Churchill Livingstone.

[15] Lotem J, S. L. 1999. Cytokines As Suppressors Of Apoptosis. Apoptosis, 4, 187-196.

[16] Mousavi, S. H., Tayarani-Najaran, Z., Asgari, M. & Sadeghnia, H. R. 2010. Protective Effect Of Nigella Sativa Extract And Thymoquinone On Serum/Glucose Deprivation-Induced Pc12 Cells Death. Cell Mol Neurobiology, 30, 591-598.

[17] Najaran, Z. T., Sadeghnia, H. R., Asghari, M. & Mousavi, S. H. 2009. Neuroprotective Effect Of Nigella Sativa Hydro Alcoholic Extract On Serum /Glucose Deprivation Induced Pc12 Cells Death. Physiology And Pharmacology, 13(3), 263-270.

[18] Noor, N. A. 2010. Evaluation Of Antioxidant Effect Of Nigella Sativa Oil On Monosodium Glutamat-Induced Oxidative Stress In Rat Brain. Journal Of American Science, 6(12), 13-19.

[19] Padhye, S., Banerjee, S., Ahmad, A., Mohammad, R. & Sarkar, F. H. 2008. From Here To Eternity-The Secret Of Pharaohs: Therapeutic Potential Of Black Cumin Seeds And Beyond. Carcer Therapy, 6, 495-510.

[20] Preeti D., P. G., Uma S., Jagannathan N.R., Madhur R. 2008. Neuroprotective Efficacy And Therapeutic Window Of Curcuma Oil: In Rat Model. Complementary And Alternative Medicine, 8, 55.

[21] Roshan, S., Khan, A., Tazneem, D. & Ali, S. 2010. To Study The Effect Of Nigella Sativa On Various Biochemical Parameters On Stress Induced In Albino Rats. International Journal Of Pharmacy And Pharmaceutical Sciences, 2(4), 185-189.

[22] Tuluce, Y., Ozkol, H., Sogut, B. & Celik, I. 2009. Effects Of Nigella Sativa L. On Lipid Peroxidation And Reduced Gluthation Levels In Erythrocytes Of Broiler Chickens. Cell Membranes And Free Radical Research, 1(3), 95-98.

[23] Yaheya, M. 2009. Therapeutic Role Of Prophetic Medicine Habbat El Baraka (Nigella Sativa L.)-A Review. World Applied Sciences Journal, 7(9), 1203-1208.