ISSN 1220-8841 (Print)
ISSN 2344-4959 (Online)

ROMANIAN
NEUROSURGERY

Vol. XXXVII | No. 1 March 2023

Black cumin 0.3 gram and 0.4 gram on apoptotic levels in Cerebral Contusions Rattus Norvegicus Wistar

Tommy Alfandy Nazwar,
Farhad Balafif,
Donny Wisnu Wardhana,
Muhammad Annas,
Agus Chairul Anab,
M. Istiadjid E.S.

DOI: 10.33962/roneuro-2023-007

Romanian Neurosurgery (2023) XXXVII (1): pp. 54-58
DOI: 10.33962/roneuro-2023-007
www.journals.lapub.co.uk/index.php/roneurosurgery

Black cumin 0.3 gram and 0.4 gram on apoptotic levels in Cerebral Contusions Rattus Norvegicus Wistar

Tommy Alfandy Nazwar[1,2], Farhad Balafif[1,2], Donny Wisnu Wardhana[1,2], Muhammad Annas[2], Agus Chairul Anab[3], M. Istiadjid E.S.[4]

[1] Division of Neurosurgery. Department of Surgery. Brawijaya University/Saiful Anwar Hospital Malang East Java, INDONESIA
[2] Department of Surgery. Brawijaya University/Saiful Anwar Hospital Malang East Java, INDONESIA
[3] National Hospital Surabaya, INDONESIA
[4] Department of Neurosurgery and Plastic Surgery. Faculty of Medicine, Brawijaya University, Malang, INDONESIA

Keywords
black cumin,
cerebral contusion,
apoptosis,
neuronal injury

ABSTRACT

Background. Apoptosis is one of the indicators to check for following brain damage. Along with this trend, treatment in the form of herbal and phytopharmaca therapy is required more frequently to treat brain injury complications. Black cumin possesses a function that opposes the apoptotic mechanism.

Objectives. This study sought to determine the effect of black seed on an animal model of brain damage using apoptotic measures.

Methods. Four treatment groups were created from the experimental animals as follows: Group BC1: For 7 days following the brain contusion, they were given [0.3 gram] g/kg bw of black cumin extract daily. Group BC2: For 7 days following the brain contusion, they were given [0.4 gram] g/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 TUNEL DNA fragmentation method was used to count the amount of apoptotic cells and analysis was conducted using ANOVA with F-test and Tukey HSD.

Results. The control group had the greatest amount of apoptosis at 30.4. Apoptosis averages for BC1 (0.3 g), and BC2 (0.4 g) groups of rats were 25.0, and 18.8, respectively. Black cumin anova test with apoptosis was present while a higher dose of black cumin will minimize the amount of apoptosis.

Conclusions. Injecting black cumin extracts into rats with head injuries reduced apoptosis, albeit not significantly. In rats with experimental head injuries, black cumin extract induces a connection through the apoptosis mechanism.

INTRODUCTION

The progression of a brain injury is not random, but rather a continuous process between primary and secondary brain injuries[1]. Consequently, the initial diagnosis, treatment, and prognosis of brain injury are not

Corresponding author:
Tommy Alfandy Nazwar

Brawijaya University/Saiful Anwar Hospital Malang East Java, Indonesia

nsubtommy@gmail.com

ISSN online 2344-4959
© Romanian Society of Neurosurgery

First published
March 2023 by
London Academic Publishing
www.lapub.co.uk

simple, despite the fact that methods of diagnosis and management of brain injury are constantly evolving[2]. 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 [3–5]. Experimental animals receiving black cumin seed extract for cerebral ischemia have lower levels of MDA (malondialdehyde)[6]. Black cumin prevents formaldehyde from causing neuronal apoptosis when administered to animals[7].

It is believed that black cumin inhibits calcium channel blockers, hence decreasing calcium flow[8]. 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 Apoptotic neuron cells following head damage in Rattus norvegicus wistar rats.

Methods

In this laboratory investigation, mice were utilized as the experimental animals, and the experiment was designed to be entirely randomized. 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. Four treatment groups were created from the experimental animals as follows: Group BC1: For 7 days following the brain contusion, they were given [0.3 gram] g/kg bw of black cumin extract daily. Group BC2: For 7 days following the brain contusion, they were given [0.4 gram] g/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 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).

Apoptosis examination material: mouse brain tissue, Apoptec (Proteinase-K Enzyme, Apoptag, DAB liqud), Assay diluent A, Assay diluents B, Tetramethyl benzidine (TMB) One Step Substrate Reagent, Stop Solution.

Cerebral contusion model: Cerebral contusion was done on experimental animals [9], which has been modified. 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 [10].

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. 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 BC1 0.3 grams (g), and group BC2 0.4 grams (g) via nasogastric tube. 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 animal model, 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 animal model. Half of the right and left brains were removed sterilely and placed in a petri dish with 10% formalin.

The computation method utilizes *SPSS™* 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.

Results

Four treatment groups present data. The BC1 group fed black cumin at 0.3 g/kgbw, the BC2 group fed 0.4 g/kgbw, and the control group fed 0.9% 3cc NS.

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

A head injury model was used for the experimental

group BC1, BC2, as well as the control, and it had an energy of 1.6 joules. A macroscopic examination of the animal model's brain tissue did not reveal any signs of cranial fracture, subdural hemorrhage, subarachnoid or intracerebral bleeding. 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 (Figure 1).

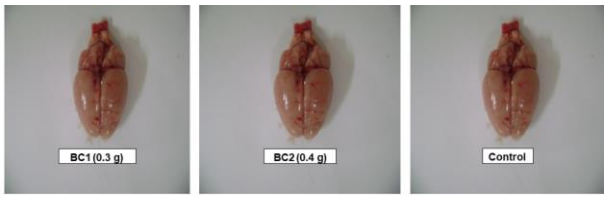

Figure 1. shows a macro view of the parenchyma of rat brain tissue. After seven days of treatment with either black cumin (BC), the macroscopic picture of the rat brain parenchyma did not reveal any differences between the two groups.

Table 1. Average and One Way ANOVA test for apoptosis

Treatment	Average	Standart deviation	F value	p
BC1 (0.3 g)	25,00	7,91	2,761	0,076
BC2 (0.4 g)	18,80	5,67		
Control	30,40	7,77		
Total	25,35	6,42		

Table description; From the table above, it was found that the control group had the highest degree of apoptosis (mean 30.40) BC1 (mean 25.00) and BC2 (mean 18.80). The results of the ANOVA test with p=0.076 are not significant.

The correlation between black cumin and the death of neurons (apoptosis)

The analysis of TUNNEL apoptosis revealed that there were differences between the treatment groups, with the amount of apoptosis decreasing with increasing the dose of black cumin.

The table 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. Apoptosis averages for BC1 (0.3 g), and BC2 (0.4 g) groups of rats were 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 2 shows that no significant differences between treatments were found at the 0.05 level or lower, although descriptively, the control group and feeding normal saline caused the most apoptosis (average 30.4), the group BC1 with black cumin feeding 0.3 g/kg body weight per day for 7 days (average 25), and the group BC2 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 failure of 7 treatments in 100 clinical trials is extremely high.

Table 2. Tukey HSD Apoptosis test results

Treatment	N	subset alpha=0.05
		1
BC1	5	18,8000
BC2	5	25,0000
control	5	30,4000
Sig		,058

Note: from the Tukey HSD test, it appears that the three treatments have no significant difference even though they have different mean values.

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 Kanter[7] 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[11]. In this particular experiment, only male experimental animals were utilized. When there is a difference between the sexes, the results will be different. 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[12]

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 model of brain contusion of previous study[10], 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[10].

Using a probe, black cumin (BC) was administered orally using the following dosages: BC1 0.3 g/kgBW, BC2 0.4 g/kgBW, and a control with 3 cc Normal saline. The 0.4 g/kg bw dosage is based on research conducted by Kanter[7].

However, a dose of 0.3 grams per kilogram of body weight is a dose that falls between a low dose and a high dose[7]. 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 [11,13].

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. 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[14]. In experimental chicken erythrocytes, black cumin administration dramatically decreased MDA levels (p0.002) and increased GSH levels (p0.005)[15]. 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[16]. Black cumin's anti-apoptotic effects were observed in this investigation; however, they were not statistically significant (p 0.076). 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[7]. A dose of 0.4 g/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[13]. 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)[17]. 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[17]. This study found that giving black cumin extract to experimental rats with head traumas raised neuronal apoptotic 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 through apoptosis mechanism. 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.

Acknowledgment

The authors would like to thank of the contribution of all the members of the neurosurgery Team in Saiful Anwar Hospital RSSA, Malang, East Java, Indonesia.

References

1. Dash, H. H. & Chavali, S. Management of traumatic brain injury patients. Korean J. Anesthesiol. 71, 12–21 (2018).
2. Graham, D., Cooper P, & Golfinos G ed. Pathology of brain damage after head injury. (McGraw-Hill Professional, 2000).
3. Ismail, M. Therapeutic Role of Prophetic Medicine Habbat El Baraka (Nigella sativa L.) - A Review. (2009).
4. Roshan, Abdullah Khan, Tazneem, & Sadath Ali. To study the effect of nigella sativa on various biochemical parameters on stress induced in albino rats. Int. J. Pharm. Pharm. Sci. 2, (2010).
5. Padhye, S., Banerjee, S., Ahmad, A., Mohammad, R. & Sarkar, F. H. From here to eternity - the secret of Pharaohs: Therapeutic potential of black cumin seeds and beyond. Cancer Ther. 6, 495–510 (2008).
6. Hosseinzadeh, R., Shokrieh, M. M. & Lessard, L. Damage behavior of fiber reinforced composite plates subjected to drop weight impacts. Compos. Sci. Technol. 66, 61–68 (2006).
7. Kanter, M. Protective effects of Nigella sativa on the neuronal injury in frontal cortex and brain stem after chronic toluene exposure. Neurochem. Res. 33, 2241–2249 (2008).
8. Najaran, Z. T.-, Hamid Reza Sadeghnia 1,2, , Mozhgan Asghari 3, & Seyed Hadi Mousavi. Neuroprotective effect of Nigella sativa hydro alcoholic extract on serum/glucose deprivation induced PC12 cells death. Physiol. Pharmacol. 13, 263–270 (2009).
9. Bates, J. F. & Goldman-Rakic, P. S. Prefrontal connections of medial motor areas in the rhesus monkey. J. Comp. Neurol. 336, 211–228 (1993).
10. Cernak, I. Animal models of head trauma. NeuroRx J. Am. Soc. Exp. Neurother. 2, 410–422 (2005).
11. Dohare, P., Garg, P., Sharma, U., Jagannathan, N. R. & Ray, M. Neuroprotective efficacy and therapeutic window of curcuma oil: in rat embolic stroke model. BMC Complement. Altern. Med. 8, 55 (2008).
12. Bramlett, H. M. et al. Sex differences in XIAP cleavage after traumatic brain injury in the rat. Neurosci. Lett. 461, 49–53 (2009).
13. Noor Neveen, A. & Iman, M. Mourad. Evaluation of Antioxidant Effect of Nigella sativa oil on Monosodium Glutamate-Induced Oxidative Stress in Rat Brain. J. Am. Sci. 6, (2010).
14. YASSIN, M. M. Prophylactic Efficacy of Crushed Garlic Lobes, Black Seed or Olive Oils on Cholinesterase Activity in Central Nervous System Parts and Serum of Lead Intoxicated Rabbits. Turk. J. Biol. (2005).
15. Tuluce, Y., Halil ÖZKOL, & Bünyamin SÖĞÜT. Effects of Nigella sativa L. on Lipid Peroxidation and Reduced Glutathione Levels in Erythrocytes of Broiler Chickens. CELL Membr. FREE Radic. Res. 1, (2009).
16. Ilaiyaraja, N & Khanum, F. Nigella sativa L: a review of therapeutic applications. J. Herb. Med. Toxicol. (2010).
17. El-Najjar, N. et al. Reactive oxygen species mediate thymoquinone-induced apoptosis and activate ERK and JNK signaling. Apoptosis Int. J. Program. Cell Death 15, 183–195 (2010).