Turk Neurosurg 31(1):8-17, 2021

Original Investigation

DOI: 10.5137/1019-5149.JTN.25523-19.3

Received: 15.01.2019
Accepted: 14.04.2019
Published Online: 20.05.2019

Effects of Nimodipine and *Nigella sativa* on Oxidative Stress and Apoptosis in Serum and Brain Tissue of Rats with Experimental Head Trauma

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ABSTRACT

AIM: To investigate whether Nimodipine (N) and *Nigella sativa* (NS) oil have protective, antioxidant effects in brain injury caused by experimental head trauma.

MATERIAL and METHODS: Fifty albino Wistar rats were randomly divided into 5 groups that underwent experimental head trauma. Oxidative parameters were compared in the serum and brain tissue of the different groups. In addition, apoptosis and caspase-3 immunoreactivity were evaluated by histopathological examination.

RESULTS: Serum total antioxidant status (TAS) levels were significantly increased in N and N+NS groups when compared with controls ($p=0.001$, $p<0.01$). Tissue TAS levels were significantly higher in the NS and N+NS groups compared to controls ($p=0.001$, $p<0.01$). Total oxidant status levels in the brain tissue were significantly higher in the NS group than in the control group ($p=0.021$).

CONCLUSION: N and NS were shown to significantly reduce the occurrence of oxidative stress in secondary brain injury due to head trauma. We also found that apoptosis levels decreased in response to N, NS and N+NS treatments after head trauma.

KEYWORDS: Craniocerebral trauma, *Nigella sativa*, Nimodipine, Oxidative stress, Apoptosis, Rats

INTRODUCTION

Head trauma is among the most important health issues contributing to death and disability, requiring long-term treatment and care. Approximately 85% of deaths from traffic accidents are due to traumatic brain injury. In primary damage due to head trauma, brain tissue integrity is impaired, and damage to the vessels, neurons and axonal structures occurs. Secondary damage consists of a chain of biochemical events in response to the primary damage occurring after the initial event (4,33). Since primary tissue damage caused by mechanical head trauma is irreversible, the primary purpose of treatment should be to prevent or eliminate secondary brain damage caused by biochemical or physiological events occurring after the trauma. Secondary damage results in excitotoxicity, ionic imbalance, ATP depletion, proteolysis, and oxidative stress due to the transient release of a large number of excitatory neurotransmitters (3,42).

Brain tissue contains high quantities of polyunsaturated fatty acids; therefore, it is highly vulnerable to reactive oxygen species (ROS) and lipid peroxidation, leading to

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neurodegeneration (12). There is a direct relationship between the degree of oxidative stress and brain damage (40). ROS products cause the deterioration of lipids, proteins, and nucleic acids in brain tissue (1,37,41). Antioxidants protect the brain against oxidative damage through enzymatic or nonenzymatic means (38). Catalase, superoxide dismutase (SOD), glutathione peroxidase (Gpx), and paraoxonase (PON1) are among the enzymes involved in the defense against oxygen radicals (6,8).

Under normal conditions, there is a balance between antioxidant defense mechanisms and free radicals, and oxidative stress is observed if this balance becomes impaired in favor of free radicals. Oxidant and antioxidant molecules can be individually measured in the plasma, but recently, total oxidant status (TOS) and total antioxidant status (TAS) measurements have improved (13,14). ROS are highly reactive and attack biomolecules, such as proteins, DNA, and polyunsaturated fatty acids in their environment. Arachidonic acid, a common unsaturated fatty acid, ultimately peroxidizes to form malondialdehyde (MDA) (44).

Many previous studies have examined various agents, such as N, Citicoline, propofol, halothane, isoflurane, Naringin, Erythropoietin and Thimokinone, for their neuroprotective effects due to their ability to inhibit antioxidant and free radical formation (2,15,19,21,22,23,35,39,43,45,46). SOD forms the first line of defense against ROS. SOD is an enzymatic antioxidant that catalyzes the superoxide radical (O_2), hydrogen peroxide (H_2O_2), and molecular oxygen (O_2) (16). Gpx is found in the cytoplasm of cells and protects them against oxidative damage by preventing the formation of OH from H_2O_2 (18). PON1 is an ester hydrolase associated with high-density lipoproteins (HDL) that can hydrolyze paraoxon, a strong inhibitor of cholinesterases (9). NS, in the Ranunculaceae family, has been widely used in the treatment of many diseases in Eastern Mediterranean countries for some time. Despite the 12 known species of Nigella species growing in Turkey, only NS can be cultivated (30,31).

NS consists of volatile oils, 0.4% to 0.45%, 18.4% to 24%, and 45% to monoterpenes (10). Many studies have shown that NS is a bronchodilator, immunomodulator, antibacterial, hypotensive, antidiabetic, hepatoprotective, gastroprotective, antihistamine, antioxidative and neuroprotective compound (10,11,20,23-29,47).

Nimodipine is an L-type calcium channel blocker, which is known to increase cerebral blood flow in humans and animals by expanding cerebral arterioles. Apoptosis is a physiological event also known as cell suicide. After receiving signals for apoptosis, many biochemical events (DNA fragmentation, excretion of phosphatidylserine molecules on the outer surface of the cell membrane) and morphological changes (cell shrinkage, chromatin condensation and apoptotic body formation) occur within the cell (17).

In our study, we assessed SOD, GPx, PON1, MDA, TOS and TAS levels in brain tissue and serum after experimental head trauma in rats administered Nimodipine and NS. Apoptosis and caspase-3 immunoreactivity were also compared among groups by histopathological examination. We aimed to investigate the effects of Nand NS on oxidative stress and brain injury in rats after experimental head trauma.

MATERIAL and METHODS

Ethics approval for this study was obtained from the Dicle University Animal Experiments Local Ethics Committee. The study was performed at the Dicle University Medical Faculty's Experimental and Clinical Research Center between 29/04/2010 and 29/04/2011. All experimental, surgical and laboratory studies were performed at the Dicle University Experimental Research Centre, Dicle University Medical Faculty Biochemistry and Pathology Laboratories.

Experimental materials and laboratory environment

Fifty male Wistar albino rats weighing 250-340 g were used in the study. Rats were housed in groups of two or three in wire cages under standard laboratory conditions (23 ± 3°C in a 12 hours light/dark cycle). Animals were fed a standard pellet diet and water. Rats suspected of having an underlying disease were not included in the experiments. Through a simple random sampling method, rats were divided into five groups, with ten rats per group.

Surgical procedures and experimental protocols

Experimental groups were organized as follows:

Control Group (C): Head trauma was not induced, and intrathecal saline was administered.

Head Trauma Group (HT): Experimental head trauma was induced, and no medication was given.

Nimotidipine Group (N): Experimental head trauma was induced, and N was administered subcutaneously at a dose of 50 mg/kg twice at 12-hour intervals.

Nigella sativa Group (NS): Experimental head trauma was induced, and NS was administered orally at 400 mg/kg by intragastric intubation 1 day before trauma.

Nimodipine + *Nigella sativa* Group (N+NS): Experimental head trauma was induced, and N and NS were administered together at the previously mentioned dosages and times.

Head Trauma Model

In this study, the weight drop method, developed by Marmarou et al., was used to induce head trauma (32). Rats were sacrificed 24 hours after the induction of head trauma. Before any experimental surgical procedures, anesthesia was administered with 75 mg/kg intramuscular ketamine hydrochloride (Ketalar 500 mg; Pfizer, Istanbul, Turkey) and 10 mg/kg xylazine hydrochloride (Rompun 2%; Bayer, Istanbul, Turkey). Some of the samples were separated for biochemical examination, and others were fixed in 10% buffered formalin solution for histopathological examination.

Histopathology:

Tissue samples for histopathological evaluation were fixed in 10% formaldehyde solution for 24 hours. Samples were

evaluated by the same pathologist under light microscopy, and apoptosis levels were assessed in brain tissue. In addition, Hematoxylin Eosin (HE) and caspase 3 staining were used to identify apoptosis. Apoptotic cells were evaluated according to nuclear morphology and stained with hematoxylin in HE staining. Observable changes included cell shrinkage or cytoplasmic shrinkage, chromatin condensation, and the reduction or division of the nucleus (17). Active caspase-3 method was detected by immunohistochemical staining in apoptotic cells. For this measurement, to determine whether the tissue expresses caspase-3 or the agent that causes apoptosis in the treated tissue induces caspase-3, T immunoperoxidase immunohistochemical staining was observed in apoptotic cells (36).

Biochemical Analyses

Brain tissue was frozen at -80°C immediately after removal. Then, after being separated into small pieces, tissue was homogenized using 1/5 (w/v) phosphate solution. Samples were centrifuged for 10 min at 4000 rpm, and serum was separated and stored at -80°C until analysis. For TAS and TOS analyses, the method developed by Erel was used. For this purpose, a completely automated RelAssay kit (Rel Assay Diagnostics kit, Mega Tip, Gaziantep, Turkey) was used with the colorimetric method using the VitalScientific, Selectra/Flexor E (The Netherlands) autoanalyzer. TAS and TOS results are presented as μmol H_2O_2 equivalent/L (13,14). In the samples, MDA was measured according to the method of Draper and Hadley. The principle of this method is based on the spectrophotometric measurement of pink color absorbance resulting from the thiobarbituric acid (TBA) reaction with MDA (7). Spectrophotometric measurements were performed on the Perkin Elmer Lambda 20 UV/VIS spectrometer (Lake Michigan Computers, Saint Joseph, Michigan, USA). The results are shown as nmol/g protein. PON1 levels were measured using a fully automatic RelAssay® (RelAssay Diagnostics kit, Mega Tıp, Gaziantep, Turkey) commercial kit in an Abbott Architect® c16000 autoanalyzer. The results are shown as units/mol. In tris buffer, the calcium ion-activated PON1 enzyme catalyzes paraoxane into p-nitrofenole (diethyl-p-nitrophenylphosphate). The molar absorbance of P-nitrofenole is 18.290 M-1 cm-1, and one unit of PON1 activity equals 1 mol of product generated in 1 min at 37°C. The results are shown as units/mol. For assessment of SOD, a kit was utilized and measured by ELISA (Cayman Chemicals, Ann Arbor, MI, USA). The results are shown as U/g protein.

Statistical Analysis

Data were analyzed using Statistical Packages for the Social Sciences (version 22.0, SPSS Inc, Chicago, IL). Descriptive statistics are presented as the mean ± standard deviation and median (min-max) values. Significance between difference groups was evaluated using the Kruskal-Wallis test (nonparametric analysis of variance) because data did not meet the assumptions of the parametric test analysis of variance. Post hoc pairwise comparison tests were used to determine which groups differed significantly. A p-value < 0.05 was considered statistically significant.

RESULTS

TAS

Serum TAS levels were significantly higher in NS and N + NS groups compared to controls (p=0.001, p<0.01). The mean serum TAS levels were 0.04 ± 0.03 mmol/L in the Control group, 0.26 ± 0.13 mmol/L in the HT group, 0.28 ± 0.63 mmol/L in the N group, 0.45 ± 0.39 mmol/L in the NS group and 0.52 ± 0.67 mmol/L in the N+NS group (Table I, Figure 1A). In brain tissue, TAS levels were higher in NS and N + NS groups than in controls (p=0.001, p<0.01). In the N group, TAS levels were lower than in the control group (p=0.001). The mean tissue serum TAS levels were 0.03 ± 0.26 mmol/L in the C group, 0.20 ± 0.98 mmol/L in the HT group, 0.22 ± 0.05 mmol/L in the N group, 0.36 ± 0.31 mmol/L in the NS group, and 0.41 ± 0.05 mmol/L in the N+NS group (Table II, Figure 2A).

TOS

There were no significant differences in serum TOS levels between any groups (p=0.154). The mean serum TOS levels were 34.27 ± 11.29 mmol/L in the C group, 28.45 ± 1.15 mmol/L in the HT group, 31.44 ± 8.09 mmol/L in the N group, 26.09 ± 7.09 mmol/L in the NS group, and 25.17 ± 12.28 mmol/L in the N+NS group (Table I, Figure 1B). TOS levels in the brain were significantly higher in the NS group than in the control group (p=0.021). The mean tissue serum TOS levels were 122.85 ± 40.47 mmol/L in the C group, 101.98 ± 4.14 mmol/L in the HT group, 92.75 ± 23.89 mmol/L in the N group, 76.97 ± 20.91 mmol/L in the NS group and 74.26 ± 36.24 mmol/L in the N+NS group (Table II, Figure 2B).

GPx

There was no significant difference in the serum GPx levels between groups (p=0.090). The mean serum GPx levels were 104.34 ± 37.76 mmol in the C group, 122.17 ± 18.94 mmol in the HT group, 91.83 ± 35.97 mmol in the N group, 85.57 ± 31.67 mmol in the NS group and 89.10 ± 36.50 mmol in the N+NS group (Table I, Figure 1C). There was no significant difference in the brain tissue GPx levels between groups (p=0.034). The mean tissue serum GPx levels were 163.80 ± 59.28 mmol in the C group, 191.80 ± 29.73 mmol in the HT group, 133.87 ± 52.44 mmol in the N group, 124.74 ± 46.17 mmol in the NS group and 129.88 ± 53.20 mmol in the N+NS group (Table II, Figure 2C).

MDA

There was a statistically significant difference in MDA levels between groups in both brain tissue and serum (p<0.001). MDA levels were reduced in N and N + NS groups compared to controls (p<0.001, p=0.001). MDA levels were significantly lower in N, NS, and N + NS groups compared to the CT group (p=0.002, p=0.004, p=0.014). The mean serum MDA level was 37.20 ± 8.92 mmol in the C group, 33.79 ± 13.65 mmol in the HT group, 15.97 ± 2.1 mmol in the N group, 16.73 ± 2.43 mmol in the NS group and 16.84 ± 3.17 mmol in the N+NS group (Table I, Figure 1D). Tissue MDA levels were significantly higher in N, NS, and N + NS groups than in the control group (p=0.001, p=0.001, p=0.011). In addition, MDA levels were higher in the N group compared to the HT group (p=0.032).

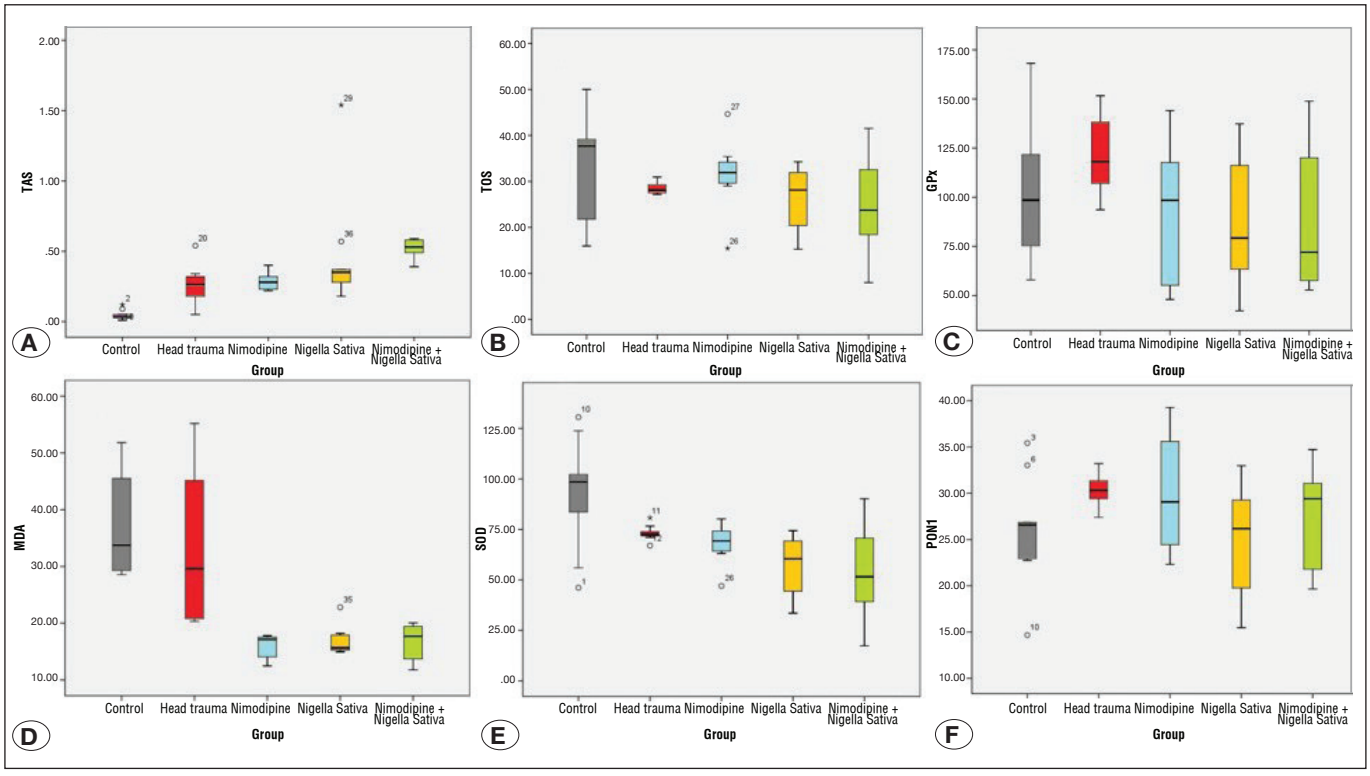

Figure 1: Box plot graphs of TAS, TOS, GPx, MDA, SOD and PON1 levels in serum are shown.

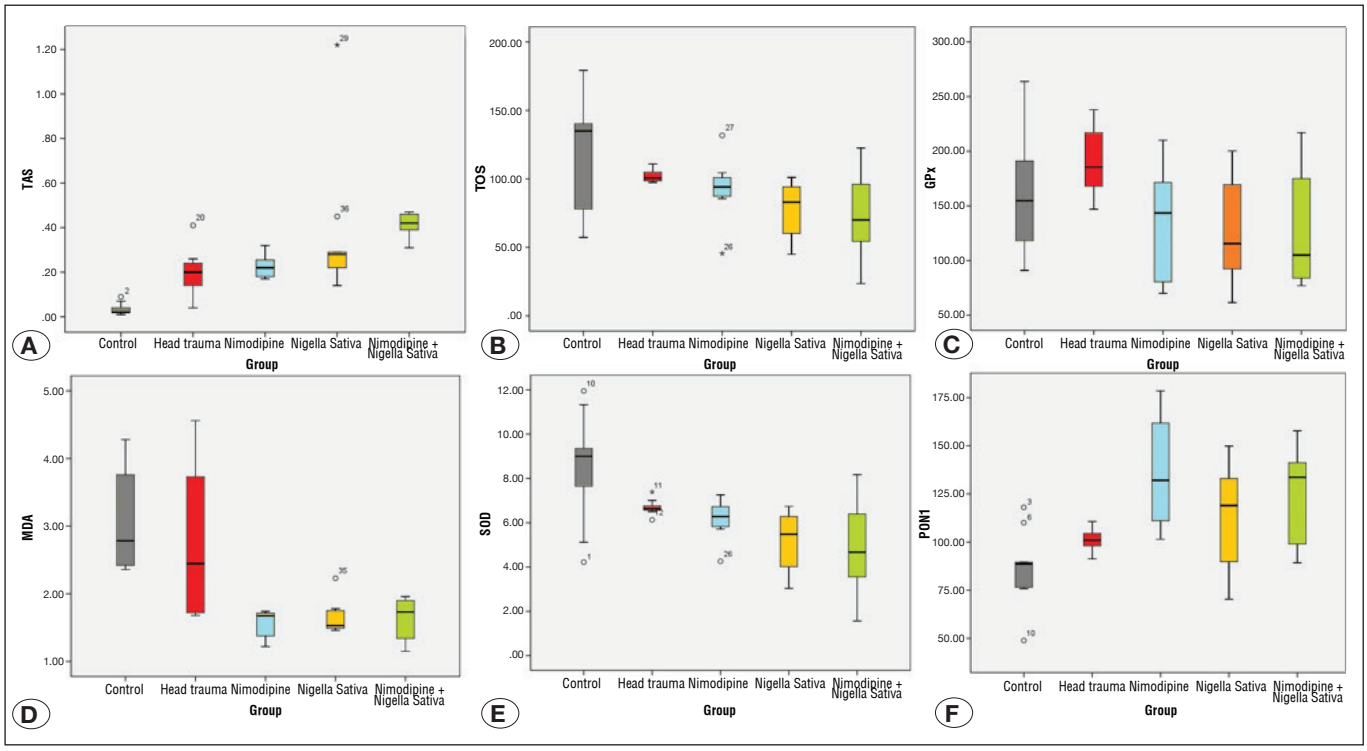

Figure 2: Box plot graphs of TAS, TOS, GPx, MDA, SOD and PON1 levels in tissue.

The mean tissue serum MDA level was 3.07 ± 0.73 mmol in the C group, 2.79 ± 1.12 mmol in the HT group, 1.56 ± 0.20 mmol in the N group, 1.63 ± 0.23 mmol in the NS group and 1.64 ± 0.30 mmol in the N+NS group (Table II, Figure 2D).

SOD

There was a statistically significant difference between groups in SOD levels in both brain tissue and serum (p<0.05). The mean serum SOD level was 92.65 ± 26.45 mmol in the C group, 73.16 ± 3.62 mmol in the HT group, 67.86 ± 10.16 mmol in the N group, 57.02 ± 15.10 mmol in the NS group and 54.53 ± 26.52 mmol in the N+NS group. SOD levels were significantly lower in N and N + NS groups compared to the control group (p<0.001, p=0.003) (Table I, Figure 1E). The mean tissue SOD values in N and N + NS groups were higher than those in the control group (p=0.004, p=0.014). The mean tissue SOD level

Table I: Mean Comparisons for Serum Biochemical Parameters Between Groups (Kruskal-Wallis Analysis of Variance)

Biochemical Parameters	Groups	n	Mean ± SD	Median	Min	Max	p	Post-hoc p
TAS	Control (1)	10	0.04 ± 0.35	0.03	0.01	0.12		**1-4: p=0.001**
	HT (2)	10	0.26 ± 0.13	0.26	0.05	0.54		**1-5: p<0.001**
	N (3)	10	0.28 ± 0.63	0.28	0.22	0.40	**<0.001**	**2-5: p=0.024**
	NS (4)	10	0.45 ± 0.39	0.35	0.18	1.54		
	N+NS (5)	10	0.52 ± 0.67	0.53	0.39	0.59		
TOS	Control	10	34.27 ± 11.29	37.70	15.96	50.01		
	HT	10	28.45 ± 1.15	28.06	27.19	30.93		
	N	10	31.44 ± 8.09	31.91	15.44	44.67	0.154	
	NS	10	26.09 ± 7.09	28.13	15.26	34.27		
	N+NS	10	25.17 ± 12.28	23.73	8.00	41.54		
GPx	Control	10	104.34 ± 37.76	98.54	57.97	168.10		
	HT	10	122.17 ± 18.94	118.16	9.64	151.61		
	N	10	91.83 ± 35.97	98.44	48.02	144.06	0.090	
	NS	10	85.57 ± 31.67	79.23	42.26	137.34		
	N+NS	10	89.10 ± 36.50	72.03	52.82	148.86		
MDA	Control	10	37.20 ± 8.92	33.70	28.56	51.79		**1-3: p<0.001**
	HT	10	33.79 ± 13.65	29.58	20.33	55.18		**1-4: p=0.001**
	N	10	15.97 ± 2.11	17.13	12.48	17.80	**<0.001**	**2-3: p=0.002**
	NS	10	16.73 ± 2.43	15.65	14.94	22.81		**2-4: p=0.004**
	N+NS	10	16.84 ± 3.17	17.70	11.76	20.05		**2-5: p=0.014**
								1-5: p=0.003
SOD	Control	10	92.65 ± 26.45	98.49	46.13	130.64		**1-4: p=0.004**
	HT	10	73.16 ± 3.62	72.42	67.01	80.79		**1-5: p=0.015**
	N	10	67.86 ± 10.16	69.33	47.03	80.15	**<0.001**	
	NS	10	57.02 ± 15.10	60.50	33.56	74.41		
	N+NS	10	54.53 ± 26.52	51.53	17.33	90.20		
PON 1	Control	10	25.99 ± 5.68	26.60	14.67	35.42		**1-2: p<0.001**
	HT	10	30.43 ± 1.67	30.30	27.39	33.20		**1-3: p=0.001**
	N	10	29.97 ± 6.35	29.05	22.32	39.27	**<0.001**	**1-4: p=0.034**
	NS	10	25.34 ± 5.71	26.17	15.48	32.97		**1-5: p=0.004**
	N+NS	10	27.44 ± 5.53	29.44	19.64	34.72		

Min: *Minimum,* ***Max:*** *Maximum,* ***SD:*** *Standard deviation.* ***C:*** *Control group,* ***HT:*** *Head trauma group,* ***N:*** *Nimodipine group,* ***NS:*** *Nigella Sativa group,* ***N+NS:*** *Nimodipine + Nigella Sativa group.*

was 8.47 ± 2.42 mmol in the C group, 6.69 ± 0.33 mmol in the HT group, 6.47 ± 0.92 mmol in the N group, 5.16 ± 1.36 mmol in the NS group and 4.94 ± 2.40 mmol in the N+NS group (Table II, Figure 2E).

PON1

Serum PON1 values were significantly different between groups (p<0.05). Serum PON1 levels were significantly reduced in N, NS, and N + NS groups compared to the control group (p=0,001, p=0.034, p=0.004). The mean serum PON1 level was 25.99 ± 5.68 mmol in the C group, 30.43 ± 1.67 mmol in the HT group, 29.97 ± 6.35 mmol in the N group, 25.34 ± 5.71 mmol in the NS group and 27.44 ± 5.53 mmol in the N+NS group (Table I, Figure 1F). Tissue PON levels were significantly different between groups in brain tissue (p<0.05). PON1 levels were significantly higher in N and N + NS groups than in controls (p=0.001, p=0.011). The mean tissue PON1 level was 86.65 ± 18.95 mmol in the C group, 101.43 ± 5.58

Table II: Mean Comparisons for Tissue Biochemical Parameters Between Groups (Kruskal-Wallis Analysis of Variance)

Biochemical Parameters	Groups	n	Mean ± SD	Median	Min	Max	p	Post-hoc p
TAS	Control (1)	10	0.03 ± 0.26	0.02	0.01	0.09		**1-4: p=0.001**
	HT (2)	10	0.20 ± 0.98	0.20	0.05	0.54		**1-5: p<0.001**
	ND (3)	10	0.22 ± 0.52	0.22	0.17	0.32	<0.001	**2-5: p=0.018**
	NS (4)	10	0.36 ± 0.31	0.28	0.14	1.22		
	NS+ND (5)	10	0.41 ± 0.05	0.42	0.31	0.47		
TOS	Control	10	122.85 ± 40.47	135.12	57.21	179.25		**1-4: p=0.021**
	HT	10	101.98 ± 4.14	100.58	97.44	110.85		
	ND	10	92.75 ± 23.89	94.14	0.17	0.32		
	NS	10	76.97 ± 20.91	83.00	0.14	1.22		
	NS+ND	10	74.26 ± 36.24	70.00	0.31	0.47		
GPx	Control	10	163.80 ± 59.28	154.70	91.00	263.90		
	HD	10	191.80 ± 29.73	185.50	147.00	238.00		
	ND	10	133.87 ± 52.44	143.50	70.00	210.00		
	NS	10	124.70 ± 46.17	115.50	61.60	200.20		
	NS+ND	10	129.88 ± 53.20	105.00	77.00	217.00		
MDA	Control	10	3.07 ± 0.73	2.78	2.36	4.28		**1-3: p=0.001**
	HT	10	2.79 ± 1.12	2.44	1.68	4.56		**1-4: p=0.001**
	ND	10	1.56 ± 0.20	1.67	1.22	1.74		**1-5: p=0.011**
	NS	10	1.63 ± 0.23	1.53	1.46	2.23		
	NS+ND	10	1.64 ± 0.30	1.73	1.15	1.96		
SOD	Control	10	8.47 ± 2.42	9.01	4.22	11.95		**1-4: p=0.004**
	HT	10	6.69 ± 0.33	6.62	6.13	7.39		**1-5: p=0.014**
	ND	10	6.14 ± 0.92	6.28	4.26	7.26		
	NS	10	5.16 ± 1.36	5.48	3.04	6.74		
	NS+ND	10	4.94 ± 2.40	4.67	1.57	8.17		
PON 1	Control	10	86.65 ± 18.95	88.67	48.90	118.07		**1-3: p=0.001**
	HT	10	101.43 ± 5.87	101.00	91.30	110.67		**1-5: p=0.011**
	ND	10	136.23 ± 28.88	132.08	101.46	178.50		
	NS	10	115.22 ± 25.96	118.97	70.35	149.88		
	NS+ND	10	124.73 ± 25.17	133.65	89.28	157.80		

Min: *Minimum,* ***Max:*** *Maximum,* ***SD:*** *Standard deviation.* ***C:*** *Control group,* ***HT:*** *Head trauma group,* ***N:*** *Nimodipine group,* ***NS:*** *Nigella Sativa group,* ***N+NS:*** *Nimodipine + Nigella Sativa group.*

mmol in the HT group, 136.23 ± 28.88 mmol in the N group, 115.22 ± 25.96 mmol in the NS group, and 124.73 ± 25.17 mmol in the N+NS group (Table II, Figure 2F).

Histopathological Investigation

Histopathologically, apoptotic cells were significantly increased in all groups when compared to controls. Compared to the CT group, apoptotic cells were significantly decreased in NS and N+NS groups ($p<0.05$). There was no statistical significance between the N and N+NS groups. The number of apoptotic cells decreased significantly in the N+NS group compared to the NS group ($p<0.05$) (Table III). Compared to controls, there was a statistically significant increase in caspase-3 staining in all other groups ($p<0.05$). The N, NS, and N+NS groups exhibited a statistically significant decrease in caspase-3 compared to the CT group ($p<0.05$). There was also significantly reduced caspase-3 in the NS and N+NS groups compared to the N group ($p<0.05$). There was no statistically significant difference between the NS and N+NS groups (Table I). Caspase 3 reactivity and apoptotic cell counts for all groups are shown as a box plot in Figure 3. Photomicrographs of HE and immunoperoxidase-stained paraffin sections of rat brain tissue are shown in Figure 4A-K.

DISCUSSION

In our study, we found that the use of N, NS and N+NS after experimental head trauma reduces oxidative stress products in both serum and brain tissue and decreases the level of apoptosis in brain tissue. When oxidative stress occurs, mitochondrial function may exhibit impaired migration to synaptic regions, resulting in neurons being physically unable perform their normal function (5,34). These physiopathological events explain the development of secondary mechanisms of traumatic brain injury and guide researchers in developing methods to prevent damage by measuring antioxidants, lipid peroxidation products, and protein damage. There are experimental studies showing that oxidative stress and lipid peroxidation occur in brain tissue after head trauma (43,46). The combination of citicoline and propofol prevents the increase in lipid peroxidation and oxidative damage following such head trauma (43). Yurdakoc et al. reported that MDA is a product of lipid peroxidation in response to experimental head trauma, and isoflurane, a general anesthetic, protects against this by decreasing MDA levels (46). Ustun et al. showed that tissue MDA levels in rabbits exposed to head trauma were reportedly higher than those in the controls (45). In our study, we found that MDA levels in brain tissue were significantly higher in N, NS, and N+NS groups than in the control group. In addition, MDA levels in the N group were higher than those in the HT group. Furthermore, in serum, MDA levels were lower in N and N+NS groups compared to those in the control group. MDA levels in N, NS, and N+NS groups were all significantly lower than those in the CT group.

Neuroprotective drugs are used to combat oxidative damage caused by head trauma. One of these drugs is N, which is used both experimentally and clinically. Animal and human studies have shown that N increases cerebral blood flow and dilates cerebral arterioles (2). The effect of N on oxidative stress caused by traumatic brain injury is unclear. In our study, no significant differences were found in TAS, TOS, GPx or PON levels in the brains of rats with induced head injury after N administration compared to controls. MDA levels in both

Table III: Comparison of Apoptotic Cell Numbers and Caspase 3 Reaction Between Groups (Kruskal-Wallis Analysis of Variance)

Pathological Parameter Groups	n	Mean ± SD	Median	Min	Max	p	Post-hoc p
Apoptotic cell Count							
Control (1)	10	0.40 ± 0.51	0.42	0.14	0.91	**<0.001**	**1-2: p=0.010**
Head trauma (2)	10	2.8 ± 0.42	2.7	2.37	3.22		**1-3: p=0.015**
Nimodipine (3)	10	2.37 ± 0.51	2.85	1.81	2.88		**1-4: p<0.001**
Nigellasativa (4)	10	1.55 ± 0.52	1.62	1.00	2.07		**1-5: p=0.001**
Nimodipine + Nigella sativa (5)	10	2.22 ± 0.44	2.19	1.78	2.66		**4-5: p<0.001**
							2-5: p<0.001
Caspase 3							
Control	10	16.20 ± 2.89	17.89	13.27	19.16	**<0.001**	**1-2: p=0.001**
Head trauma	10	77.10 ± 5.13	78.10	71.74	83.10		**1-4: p<0.001**
Nimodipine	10	59.37 ± 6.92	62.54	52.10	66.30		**1-5: p<0.001**
Nigellasativa	10	39.00 ± 7.50	43.00	31.45	46.4		**2-3: p=0.002**
Nimodipine + Nigella sativa	10	40.44 ± 8.38	39.85	31.06	49.80		**2-4: p=0.001**
							2-5: p<0.001

Min: *Minimum,* ***Max:*** *Maximum,* ***SD:*** *Standard deviation.*

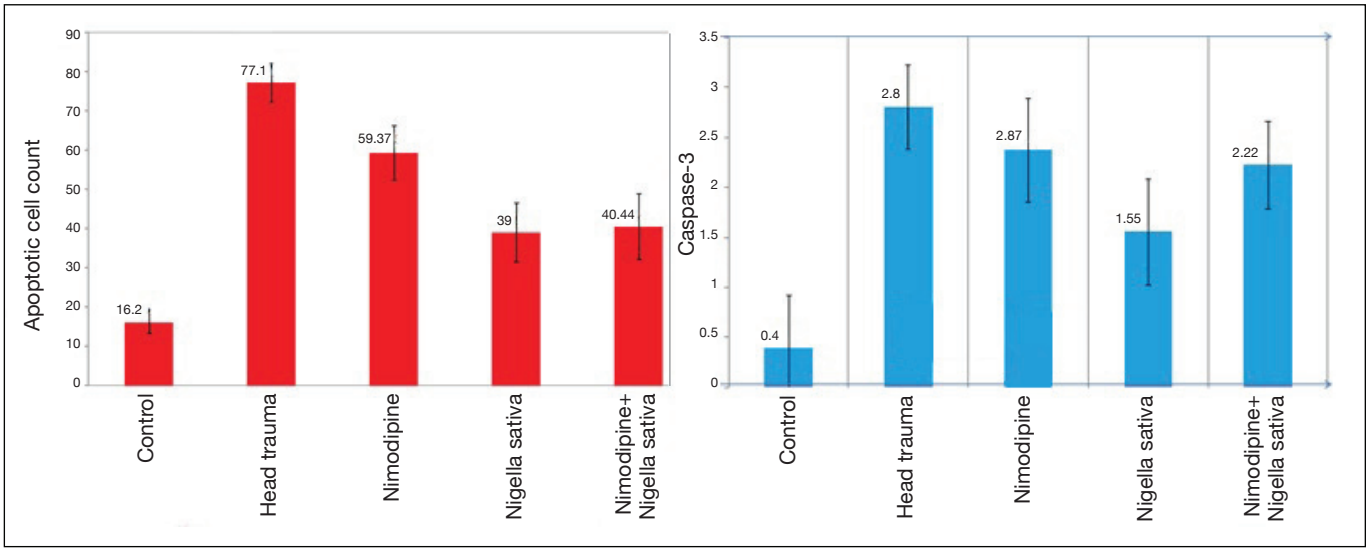

Figure 3: According to groups, caspase-3 reaction and apoptotic cell numbers.

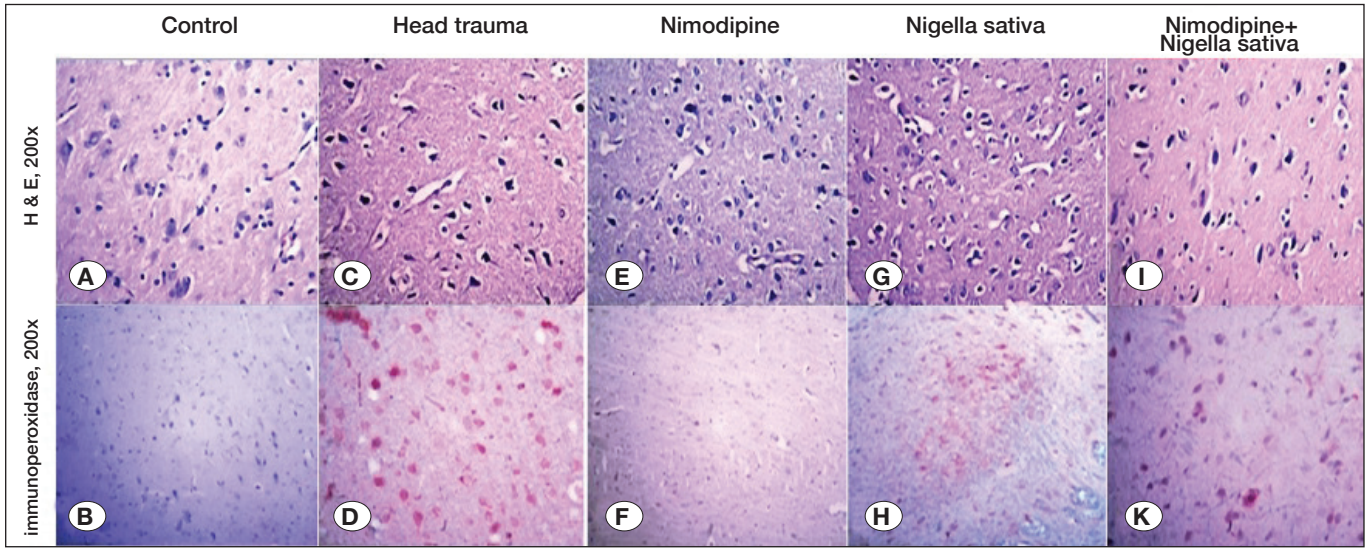

Figure 4: A) Normal brain tissue in a rat in the control group [Hematoxylin Eosin (HE), 200x]. **B)** In the control group, apoptotic cell is not observed in the caspase-3 staining of brain tissue (immunoperoxidase, 200x). **C)** Many traumatic cells are observed in head trauma group (HE, 200x). **D)** The head trauma group shows a large number of apoptotic cells stained with caspase-3 (immunoperoxidase, 200x). **E)** There are few degenerated cells in the Nigella Sativa group (HE, 200x). **F)** In the Nigella sativa group, a small number of apoptotic cells (immunoperoxidase, 200x) were observed in caspase-3 staining. **G)** Moderate degenerated cells in Nimodipine group (HE, 200x). **H)** In the nimodipine group, moderate apoptotic cell staining with caspase-3 is observed (immunoperoxidase, 200x). **I)** Nimodipine + Nigella sativa group has moderate degenerate cells (HE, 200x). **K)** In the nimodipine + Nigella sativa group, moderate apoptotic cell staining with caspase-3 is observed (immunoperoxidase, 200x).

brain tissue and serum were significantly lower than those in controls, and PON1 levels were higher than those in controls.

NS has been shown to exert protective effects against experimental ischemia reperfusion injury and has strong oxygen radical scavenging ability due to its antioxidant properties (35). NS inhibits diabetic experimental neuropathy, decreases oxidative stress, protects against brainstem and frontal cortex damage due to chronic toluene use, and protects dopaminergic neurons in an experimental Parkinson's disease model (19,39). NS has been shown to reduce lipid peroxidation in the hippocampus after cerebral ischemia reperfusion injury (21). The antiepileptic and antioxidant effects of NS were also demonstrated in an experimental epilepsy model (22).

Kanter et al. demonstrated the neuroprotective effects of NS on experimental spinal cord injury (27). The antioxidant and neuroprotective effects of NS on brain trauma have not

been investigated until now. In our study, we found that the levels of TAS in both the serum and tissue of NS and N+NS groups increased compared to those in the control group. Although no difference was found in serum TOS levels in NS-treated rats compared to the other groups, TOS levels were significantly decreased in brain tissue in NS treated rats compared to those in controls. MDA levels were reduced in the NS group compared to those in the control group. In addition, MDA levels in NS and N+NS groups were lower than those in the control group. SOD levels were reduced in NS and N+NS groups compared to those in controls in both serum and tissue. Furthermore, serum PON1 levels were increased in NS and N+NS groups compared to those in the control group.

Histopathologically, the number of apoptotic cells was statistically higher in all groups than in controls. Compared to the HT group, the number of apoptotic cells was significantly decreased in NS and N+NS groups. The number of apoptotic cells decreased significantly in the N+NS group compared to the group that received NS alone. Compared with controls, there was a statistically significant increase in caspase-3 staining in all groups. We found a statistically significant decrease in caspase 3 reactivity in N, NS and N+NS groups compared to the CT group. In summary, we found that apoptosis and caspase 3 levels increased after histopathological head trauma, but this increase was attenuated by N, NS, and N+NS administration.

CONCLUSION

N and, particularly, NS show efficacy in reducing oxidative stress and lipid peroxidation products in rats exposed to experimental head trauma. Furthermore, these agents prevented apoptosis in the brain after the induction of trauma. There are many studies on the protective effects of N in the context of head injury, but there are not sufficient data on NS. Therefore, further studies with larger samples sizes are needed. We hope that these substances, whose role in neuroprotection cannot be denied, will shed light on future studies.

ACKNOWLEDGEMENTS

This study was funded by Dicle University Scientific Research Projects (Decision no: 2010/1).

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