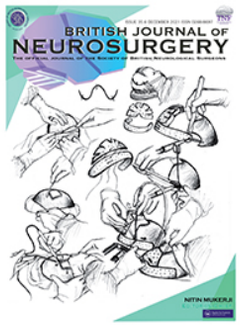

British Journal of Neurosurgery

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ibjn20

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To cite this article: Esra Aslan, Mehmet Gazi Boyacı, Hilal Güzel & Mehmet Bilgehan Pektaş (2021): Better neuroprotective profile of caffeic acid phenyl ester over resveratrol in non-traumatic ischemia-reperfusion injury of the spinal cord, British Journal of Neurosurgery, DOI: 10.1080/02688697.2021.1999391

To link to this article: https://doi.org/10.1080/02688697.2021.1999391

Published online: 09 Dec 2021.

BRITISH JOURNAL OF NEUROSURGERY
https://doi.org/10.1080/02688697.2021.1999391

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ORIGINAL ARTICLE

Better neuroprotective profile of caffeic acid phenyl ester over resveratrol in non-traumatic ischemia-reperfusion injury of the spinal cord

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ABSTRACT

Background: Spinal cord ischemia has serious sequelae. The aim of this study is to investigate the effects of resveratrol and caffeic acid phenyl ester (CAPE), a propolis derivative, on spinal cord injury induced by ischemia-reperfusion (IR).

Methods: In our research, 30 male Wistar albino rats, 200–250 gr, were used. Before the experiment, during a week of the process, the rats were fed with these two agents, and the experimental group rats were exposed to spinal cord IR injury. At the end of the experiment, spinal cord samples were taken from the sacrificed rats. Bax, p53, nNOS, and Beclin-1 immunoreactivity moreover TUNEL (+) cells were evaluated with immunohistochemically in the IR-induced damaged rats.

Results: It has been clearly determined that the TUNEL (+) apoptotic cell number and immunopositive cells of nNOS, Beclin-1, p53, Bax were raised in the IR group. However, these increments partially were restored in the resveratrol and CAPE-fed rats with IR-induced injury.

Conclusion: In light of our data, resveratrol, and CAPE could be beneficial in spinal cord IR injury. Although both agents provide beneficial effects, it can be said that CAPE is partially more effective in spinal cord injury caused by IR.

ARTICLE HISTORY
Received 6 April 2021
Revised 13 September 2021
Accepted 22 October 2021

KEYWORDS
CAPE; ischemia-reperfusion injury; resveratrol; spinal cord

Introduction

Spinal cord injury still maintains its importance due to the high prevalence in the population, the size of the damage it causes physically, psychosocially, and economically, and the lack of a universally accepted treatment protocol.[1] Spinal ischemia causing paraplegia can be of traumatic or non-traumatic origin. Thoracoabdominal aortic surgery and aortic cross-clamping have a risk of sudden or delayed paraplegia due to spinal cord ischemia-reperfusion (IR) injury rates being between 4% and 33%.[2] Apart from the mechanical problems caused primarily by spinal cord IR injury, secondary conditions such as inflammation, apoptosis, necrosis, and oxygen-derived free oxygen radicals cause the clinical picture to deteriorate.[3] The reason for the irreversibility of this process is the activation of cell death pathways characterized by necrosis or apoptosis that occurs in all neurons, especially motor neurons, and glial cells.[4] The control of secondary damage mechanisms may be a therapeutic approach, for example, p53 (Tumor protein 53), a tumor suppressor gene, regulates many signaling pathways triggered by various cell stresses, including DNA damage, abnormal oncogenic events, hypoxia, as well as some normal cell processes. Bax (Bcl-2-associated X protein) is a human protein that functions as a cofactor of p53. The Bax gene is a member of the Bcl-2 (B-cell lymphoma-2) gene family, which is induced by p53, accelerates the process of the cell where it is located to go into apoptosis. p53 is an important regulator for cell death and survival.[5] Beclin-1 is a key structure that plays a central role in autophagy via acts together with different cofactors to stimulate autophagy.[6] Nitric oxide synthase (NOS) catalyzes the formation of nitric oxide from L-citrulline and L-arginine. Neuronal NOS (nNOS) provides nitric oxide production in nervous tissues across the central and peripheral nervous system.[7] Resveratrol is a polyphenolic compound found in red grapes. It has extensive physiological and pharmacological functions, including anti-oxidative, antiplatelet, regulation of blood lipid metabolism, anti-inflammatory, and tumor growth inhibition. It is known that resveratrol has in vitro antioxidant and free radical scavenging effects.[8] Besides, resveratrol has neuroprotective properties.[9] It was reported to have protective effects on spinal cord IR injury.[10] Caffeic acid phenyl ester (CAPE), an active component of Propolis extract produced by honeybees, has anti-inflammatory, cytostatic, antiviral, antibacterial, and antifungal properties as well as antioxidant properties.[11,12]

Our aim in this study is to evaluate the prophylactic effects of resveratrol and CAPE in the IR spinal cord injury rat model at both histological and biochemical levels. In this study, we examined types of cell death such as apoptosis, autophagy, and necrosis separately unlike previous studies.

Materials and methods

Animal care

Prior to the study, approval was obtained from Afyon Kocatepe University Animal Experiments Local Ethics Committee numbered 49533702/60 regarding that the study complied with the AKUHADYEK regulations and universal ethical principles. In

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this project, 30 male Wistar-albino rats weighing 250–300 g were used. The rats were kept at a 12 hours night and 12 hours day cycle in an environment of 24–26 °C and 50–60% humidity before the experiment. The rats were cared for under the Experimental Animal Principles and Guidelines organized by the National Health and Medical Research Council and the Experimental Animal Care and Use Guidelines (NIH issue no. 85–23, 1985 revised) prepared by the National Institute of Health.

Surgical procedure

The rats fasted 12 hours before the surgical procedure and were anesthetized with an intramuscular administration of Ketamine HCL of 40 mg/kg (Katalar, Parke-Davis Eczacibasi, Istanbul, Turkey) 5 minutes after the intramuscular premedication of 5 mg/kg Xylazine (Rompin, Bayer, Istanbul, Turkey). Following the anesthesia, the abdomen was shaved, wiped with a povidoneiodine solution, then closed under sterile conditions and dissected with a median laparotomy. After going below, the abdominal aorta renal vein and above the iliac bifurcation, first distal and then proximal aortic non-traumatized vascular clamps were placed. After 30 minutes of clamping (Vascu-Statt® II, midi straight 1001-532; Scanlan Int., St. Paul, MN, USA), the clamps were removed and the perfusion of the visceral organs was provided for 24 hours.

Experimental groups

Group 1: Control group ($n = 6$); experimental group in which no procedure was applied.

Group 2: Sham group ($n = 6$); one week before the surgical procedure, a total of three doses of 5% ethyl alcohol were administered orally every other day, and the abdominal aorta was explored only by opening the abdomen on the day of the operation, no clamping was performed, and it was closed after 30 minutes.

Group 3: IR group ($n = 6$); one week before the surgery, a total of three doses of 5% ethyl alcohol were administered orally every other day and the IR procedure was performed on the day of the operation.

Group 4: Resveratrol protected group (R + IR) ($n = 6$); One week before the surgery, a total of three doses of 50 mg/kg resveratrol (Sigma, St. Louis, MO) were administered every other day, and the IR procedure was performed

Group 5: CAPE protected group ($n = 6$); A total of three doses of 10 µmol/kg CAPE (Sigma, St. Louis, MO) were administered every other day one week before the surgery, and the IR was performed.

Neurological evaluation

Motor function was evaluated with *Tarlov* criteria at the 1st, 12th and 24th hours of reperfusion.[13] *Tarlov* criteria grade 0: complete paralysis, grade 1: minimal movement, grade 2: active movement, able to stand up with assistance, grade 3: able to stand up alone, but not walk, grade 4: able to walk with difficulty, grade 5: able to walk normally, even running.[14]

TAS and TOS measurement

Total Oxidant Status (TOS) and Total Antioxidant Status (TAS) Assay Kit (Rel Assay Diagnostics, TR) were used for measurements. Measurements were made by the Manual method. The TAS results were offered as mmol Trolox Eq/mg for spinal cord tissue and mmol Trolox Eq/L for serum. However, TOS results were presented µmol 2HO Eq/mg for spinal cord tissue and µmol 2HO Eq/L for serum. The oxidative stress index (OSI) was calculated according to the formula;

$$\text{For tissues}: \quad \text{OSI} = [(\text{TOS},\ \mu\text{mol/mg}) / (\text{TAS},\ (\text{mmol Trolox Eq/mg}) \times 100)]$$

$$\text{For serums}: \quad \text{OSI} = [(\text{TOS},\ \mu\text{mol/L}) / (\text{TAS},\ (\text{mmol Trolox Eq/L}) \times 100]$$

Histopathological evaluations

Tissue samples taken from sacrificed rats were fixed in 10% formalin. Then histologically processed and embedded in paraffin. 5 µm sections were taken on polylyzed and classic slides. Slides were stained with Hematoxylin Eosin (HE) and toluidine blue for the general histomorphological appearance. Vascularization and glial cell infiltration were evaluated by HE. In toluidine blue-stained sections caryolitic, caryorectic nucleus and vacuolized cytoplasm, degenerate neurons were evaluated. Also, tissue samples were stained with primary antibodies p53, Bax, nNOS and Beclin-1 immunohistochemically. All the sections were evaluated under a light microscope (Eclipse E-600 Nikon, Japan) and Image analysis was made with Image Analysis Software (NIS Elements Nikon, Japan) for assessing the samples.

Terminal deoxynucleotidyl transferase dUTP Nick-end labeling (TUNEL) assay

Apoptosis of spinal cord neurons was detected by TUNEL assay according to the instructions of ApopTag Plus Peroxidase *In Situ* Apoptosis Detection Kit (Chemicon, Millipore, Billerica, MA, USA). 5 µm sections were taken from paraffin blocks and stained by manufacturer's protocol. TUNEL-positive cells were counted in 6 randomly chosen areas (400×).

Immunohistochemistry

The slides were labeled with nNOS, p53, Bax and Beclin-1 primary antibodies for the immunohistochemical evaluation. The sections were first deparaffinized and rehydrated. Then, antigen retrieval was made by citrate buffer (pH = 6.0) (Thermo Scientific Labvision Corp, Fremont, CA) in a microwave for 20 minutes. For blocking endogenous peroxidase activity, 3% hydrogen peroxide was used for 12 minutes. Blocking solution was applied for 5 minutes to block nonspecific background staining and then the slides were incubated with nNOS (PAI-033, Thermofisher Scientific, 1/50), p53 (sc-6243, Santa Cruz Biotechnology, 1/100), Bax (sc 526, Santa Cruz Biotechnology, 1/100), Beclin-1 (sc 11427, Santa Cruz Biotechnology, 1/100) overnight at 4 °C. After incubation, HRP secondary antibody kit (Anti-polyvalent HRP, Labvision Corp, Fremont, CA) was used as a secondary antibody and sections were visualized with an

Table 1. Effects of resveratrol and CAPE treatment with IR-induced injuries on TAS, TOS, and OSI levels in the plasma or spinal cord tissues of rats.

Groups		Control	Sham	IR	R + IR	CAPE + IR
Spinal cord	TAS (mmol Trolox Eq/mg)	2.1 ± 0.2	1.85 ± 0.3	1.52 ± 0.2	1.80 ± 0.2	2.03 ± 0.3
	TOS (μmol 2HO Eq/mg)	1.92 ± 0.2	1.97 ± 0.27	2.47 ± 0.1$^{*\#}$	2.21 ± 0.1	1.68 ± 0.2$^{\delta}$
	OSI	97.88 ± 7.7	100.2 ± 17.5	173.8 ± 20.3$^{*\#}$	124.1 ± 4.8$^{\delta}$	113.2 ± 9.1$^{\delta}$
Serum	TAS (mmol Trolox Eq/L)	13.3 ± 0.24	13.03 ± 0.63	11.83 ± 0.18	12.42 ± 0.33	13.19 ± 0.42
	TOS (μmol 2HO Eq/L)	7.07 ± 0.42	7.26 ± 0.45	8.70 ± 0.56	8.60 ± 1.20	7.34 ± 0.41
	OSI	53.25 ± 3.14	56.73 ± 5.10	73.77 ± 5.43	69.78 ± 10.11	56.06 ± 4.02
Tarlov scoring		5 ± 0.0	5 ± 0.0	3 ± 0.2$^{*\#}$	3.83 ± 0.3	3.40 ± 0.2
Degenerated neurons		6.17 ± 1	9.8 ± 1.5	54 ± 5$^{*\#}$	37.5 ± 1.7$^{\delta}$	35.6 ± 1.38$^{\delta}$

Values are expressed as mean ± SEM, $n = 6$; $^{*}p < 0.05$, significantly different from control; $^{\#}p < 0.05$, significantly different from sham; $^{\delta}p < 0.05$, significantly different from IR group.

AEC kit (Labvision Corp, Fremont, CA). For counterstaining, Mayers hematoxylin was used and slides were mounted with a water-based mounting medium. In each sample, immunopositive cells were counted in six different randomly chosen areas under ×20 objective magnification.

Statistical analysis

The resource equation method' was used to determine the number of animals to be used in the study.[15,16] The results are given as mean ± standard error of the mean (SEM); n is the number of rats. Statistical analyses were performed by Student's t-test for unpaired data or one-way ANOVA followed by the Bonferroni *post hoc* analysis where appropriate. For *Tarlov* scoring, analyzes were made using the Chi-Square test. $p < 0.05$ was considered statistically significant.

Results

Evaluation of neural functions

The control and sham groups were evaluated as Grade 5 according to the *Tarlov* scoring. These data revealed that there was no neuronal dysfunction in the control and sham groups. As shown in Table 1, it was determined that the values in the IR, R + IR, and CAPE + IR groups were lower when compared with the control and sham groups, and this decrease was statistically significant. In the IR group, it was observed that four of the six rats were at Grade 3 and one of Grade 2, thereby neuronal dysfunction occurred. The motor functions of the animals in the R + IR and CAPE + IR groups were found to be better than the IR group, but it was determined that the change between the values of these groups was not statistically significant (Table 1).

Biochemical results

As shown in Table 1, there was no significant difference between the groups in terms of TAS, TOS, and OSI values in the serum. However, although not statistically significant, spinal cord tissue TAS, TOS, and OSI values were between control and sham groups. On the other hand, there was no change between all groups in the TAS levels of spinal cord tissue. It was found that higher tissue TOS levels in IR compared to control and sham groups. Moreover, CAPE-treatment reduced TOS levels of spinal cord tissue, but unchanged with resveratrol. Thereby, OSI rates were found similar to tissue TOS values of IR and CAPE + IR groups. Interestingly, resveratrol-feeding decreased tissue OSI rates regardless of TOS levels with IR-induced damaged rats.

HE and toluidine blue results

As shown in Figure 1, the general histological appearance and vascularization in the control and sham groups were within normal limits in the HE staining. It was observed that the cells in the IR had a shrunken appearance and both vascularization and glial cell infiltration in the spinal cord tissue increased compared to control and sham groups. These changes were normalized with both resveratrol and CAPE treatment.

The neurons in the control and sham groups were found to have normal morphology in the evaluation made on toluidine blue-stained sections (Figure 1). The degenerated neurons in all ischemia-generated groups were the motor neurons in the gray matter and had features of morphologically shrunken and cytoplasmic vacuolization (Figure 1). It was found that the number of damaged neurons increased in the IR and there was a difference between the control and sham groups. However, the numbers of degenerated neurons were reduced with resveratrol and CAPE treatment in the spinal cord tissues of IR rats (Table 1).

Immunohistochemical and TUNEL staining results

The evaluations showed no significant difference between the control and sham groups in terms of TUNEL *assay* (Figures 2 and 3), p53 (Figure 4), Bax (Figure 5), nNOS (Figure 6), and Beclin-1 (Figure 7) +cell numbers. On the other hand, there was an increase in the number of all these parameters' +cells in IR compared to control and sham groups (Figures 3–7). Both resveratrol and CAPE treatments were reduced TUNEL, Bax, nNOS, and Beclin-1 +cell numbers significantly by comparison to IR (Figures 3 and 5–7). However, compared to IR, while the p53 +cells reduced significantly by giving CAPE, but unchanged with resveratrol-feeding (Figure 4).

Discussion

Our study suggests that resveratrol and CAPE treatment have a protective effect on the IR-with aortic occlusion model of rats. In line with the results obtained, we demonstrated that both resveratrol and CAPE caused a decrease in oxidant substances and the number of apoptotic cells in the spinal cord tissues. Therewithal, we determined in our study that CAPE was partially superior to resveratrol. Specifically, we have demonstrated that it is more effective in cell death during this injury process in addition to apoptotic cell death. In light of these data, we suggest that resveratrol and CAPE could be beneficial in the spinal cord.

IR-injury is a common secondary injury of the spinal cord. Complications of patients undergoing thoracoabdominal aortic surgery may result in immediate or delayed paraplegia, which causes long-term morbidity and major medical costs. In recent years, new or complementary medicinal products, especially

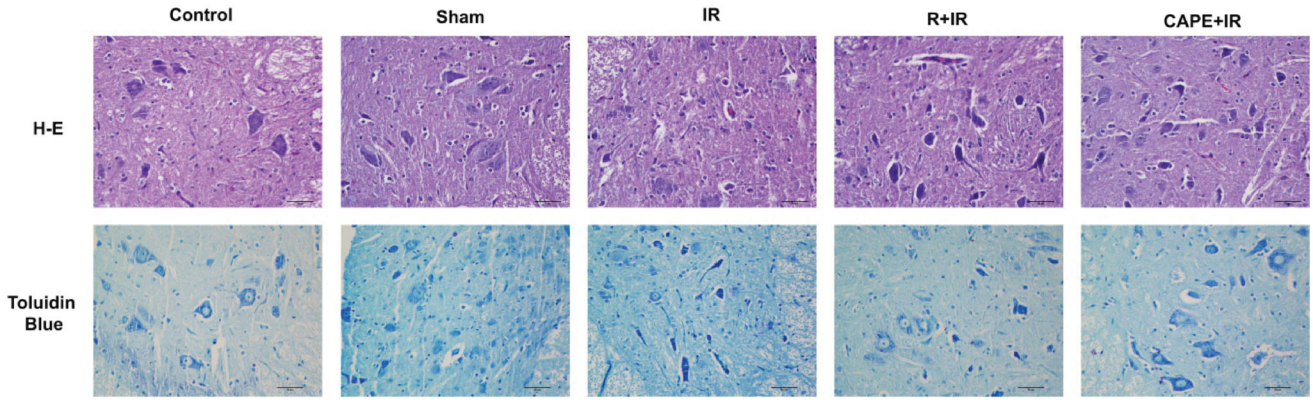

Figure 1. Histopathological features of the spinal cord sections from control, sham, IR, R + IR or CAPE + IR groups. HE staining shows more vascularization and infiltration in IR group. Toluidine blue-stained shows number of damaged neurons increased in the IR and there was a difference between the control and sham groups. Resveratrol and CAPE application improved vascularization, infiltration, and reduced number of damaged neurons in spinal cord tissues of rats with IR. Bar = 50 μm.

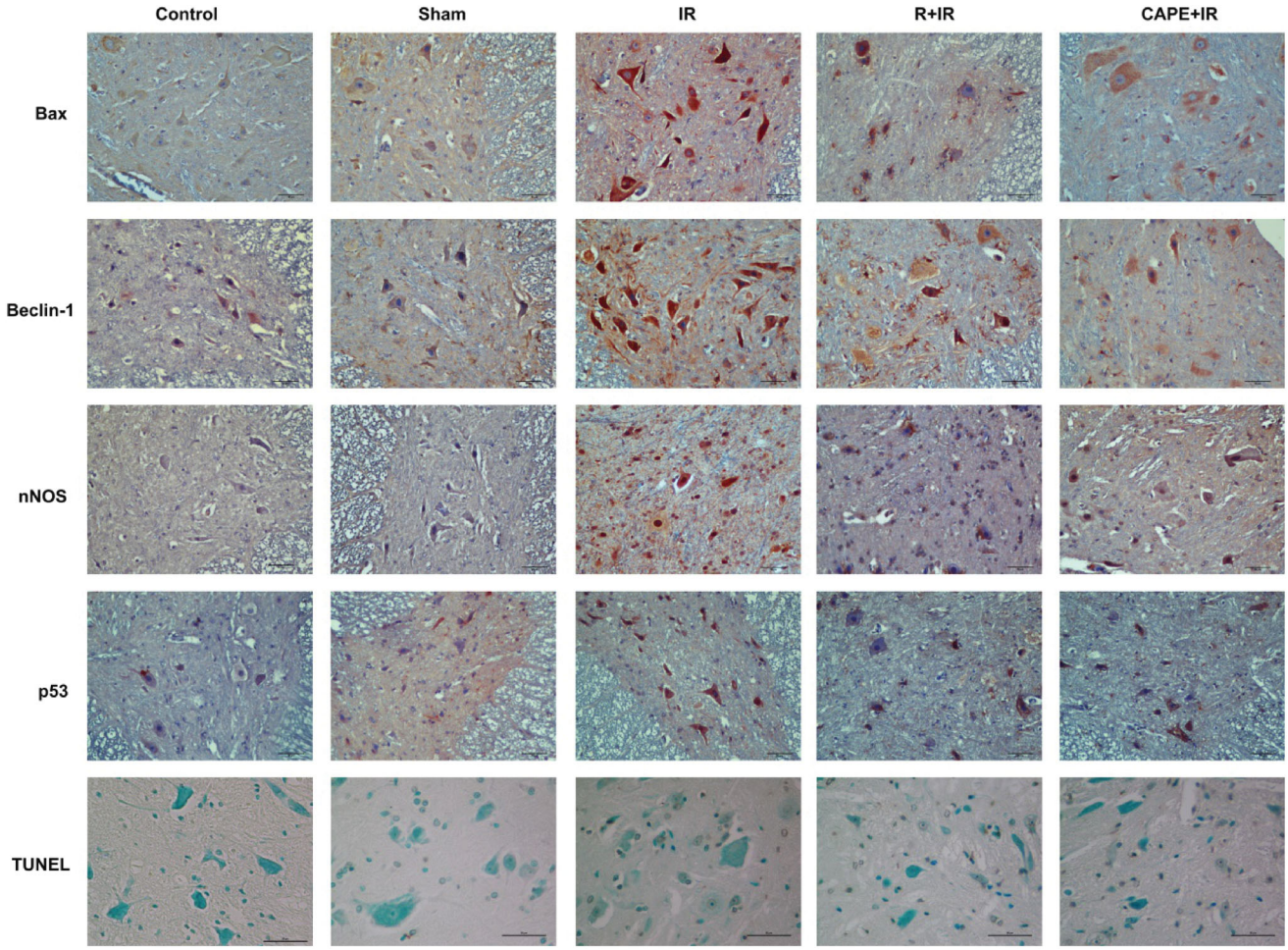

Figure 2. Spinal cord neuronal apoptosis detected via TUNEL *assay* (×400 magnifications) and proteins p53, Bax, nNOS, and Beclin-1 included + cell expressions in control, sham, IR, R + IR or CAPE + IR groups (×200 magnifications).

medical food homology products, have shown potential for therapeutic applications worldwide due to their minimal side effects.[17] Although medical and social treatment is so necessary, satisfactory results of the drugs used after paraplegia are not seen.[18] This suggests the need for a combination of drugs. We believe that resveratrol and CAPE should be investigated in more detail as a good choice if drug combinations that are effective in many steps but with the least number of drugs will be a strategy in the early stages of secondary injury after trauma or in pre-and post-thoracoabdominal surgery applications.

We used *Tarlov* scoring to evaluate neurological functions. In our study, similar to previous studies, an expected significant difference was observed between the results of the IR group and the results of the control and sham groups. Again, similar to other

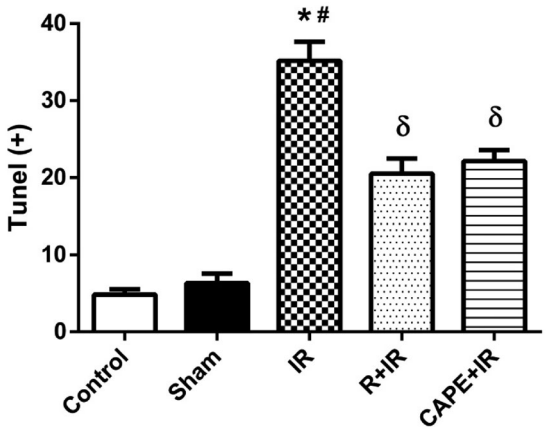

Figure 3. TUNEL *assay* apoptotic + cells. Values are expressed as mean ± SEM, $n = 6$; $^{*}p < 0.05$, significantly different from control; $^{\#}p < 0.05$, significantly different from sham; $^{\delta}p < 0.05$, significantly different from IR group.

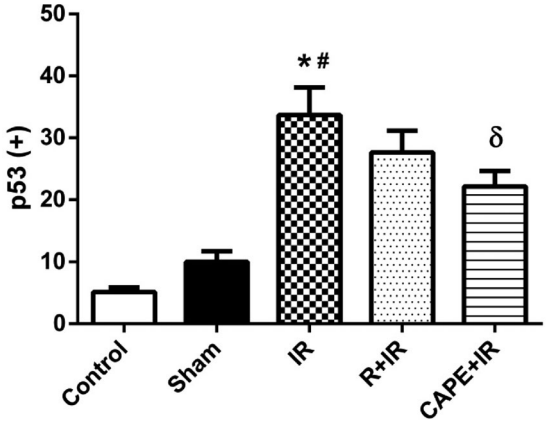

Figure 4. Expression of p53 included + cells. Values are expressed as mean ± SEM, $n = 6$; $^{*}p < 0.05$, significantly different from control; $^{\#}p < 0.05$, significantly different from sham; $^{\delta}p < 0.05$, significantly different from IR group.

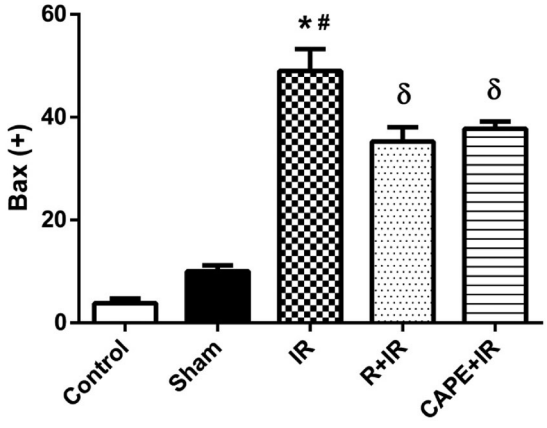

Figure 5. Expression of Bax included + cells. Values are expressed as mean ± SEM, $n = 6$; $^{*}p < 0.05$, significantly different from control; $^{\#}p < 0.05$, significantly different from sham; $^{\delta}p < 0.05$, significantly different from IR group.

studies, there was no difference between the results of the IR group and the treatment groups.[2,19] However, the neurological evaluations of the treatment groups were found to be better than the IR group.

The fact that IR injury promotes neuronal death is a complex process. Early reperfusion limits the extent of necrosis and may show potentially harmful effects, which is called reperfusion injury. However, the flow of intracellular events during

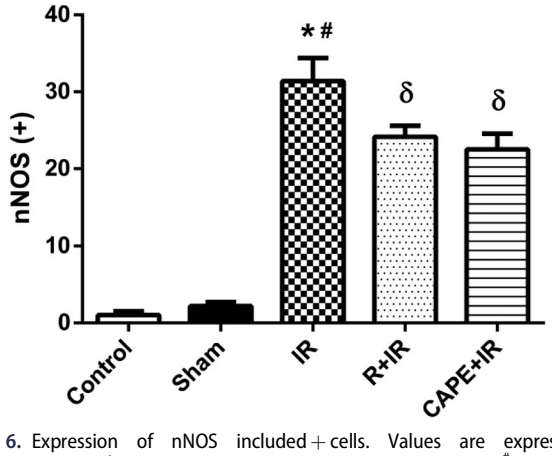

Figure 6. Expression of nNOS included + cells. Values are expressed as mean ± SEM, $n = 6$; $^{*}p < 0.05$, significantly different from control; $^{\#}p < 0.05$, significantly different from sham; $^{\delta}p < 0.05$, significantly different from IR group.

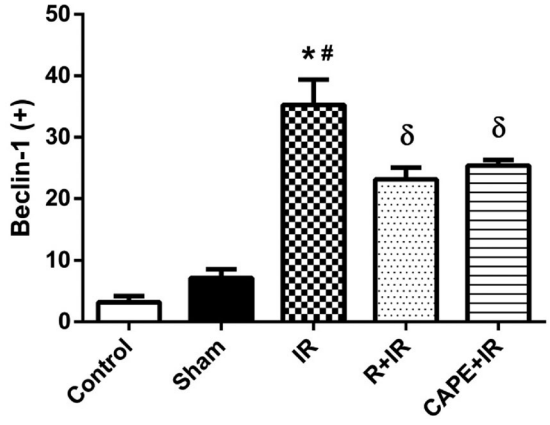

Figure 7. Expression of Beclin-1 included + cells. Values are expressed as mean ± SEM, $n = 6$; $^{*}p < 0.05$, significantly different from control; $^{\#}p < 0.05$, significantly different from sham; $^{\delta}p < 0.05$, significantly different from IR group.

reperfusion is still not fully understood. A common belief is that cells that lack energy and are metabolically re-oxygenated trigger reactive oxygen radicals.[20] It has been reported in many studies that intravenous methylprednisolone is neuroprotective in spinal cord ischemia injury. The protective effects of steroids have been attributed to their ability to stabilize membranes, modulate the immune system, and scavenge free radicals.[21] However, today, the use of high-dose methylprednisolone in treatment is controversial due to the high risk of morbidity.[22] New pharmacological agents that may be effective in treatment are still under investigation. In our study, TAS and TOS measurements were made in both spinal cord tissue and serum to determine oxidant and antioxidant levels, and OSI values were also calculated. It was observed that the OSI, which increased in the IR group, decreased in the groups treated with these two natural agents. This finding was evaluated as data supporting the antioxidant properties of resveratrol and CAPE.[11,23] The fact that the OSI decrease was more pronounced in the IR group treated with CAPE suggested that its efficiency was higher than R + IR. While the damage initiated by primary spinal cord injury is irreversible, secondary injury is an active process that occurs at the molecular and cellular level, and can be reversed and modified. This turns the irreversibility of spinal cord injuries into reality.[24] Although studies are conducted on the mechanisms in the IR process, possible treatment methods, and the presence of protective agents, if any, the available data are still insufficient.[25] Especially in recent

years, the trend towards naturally derived antioxidant substances has increased considerably.[20] Therefore, in our study, we preferred to investigate and compare the effects of two important and easily available natural substances, resveratrol and CAPE, instead of classical chemicals or drugs.

Resveratrol has broad physiological and pharmacological functions, including anti-oxidative, antiplatelet, regulation of blood lipid metabolism, anti-inflammatory, and tumor growth inhibition properties.[26,27] CAPE, on the other hand, has both antioxidant properties and anti-inflammatory, cytostatic, antiviral, antibacterial, and antifungal properties.[11,12] It has been reported to completely block the production of reactive oxygen radicals in human neutrophils and the xanthine/xanthine oxidase system at a concentration of 10 mM.[28] Some studies have shown that resveratrol has a neuroprotective property against IR injury to the nervous system.[10,27,29,30] However, the protective effect of resveratrol against IR-induced injury appears to be multifactorial,[31] but the mechanism underlying its neuroprotective effects remains unclear. We know that the antioxidant effect may also be neuroprotective against IR-induced injury by suppressing the apoptosis cascade.[32] Liu C *et al.*[33] demonstrated that resveratrol treatment after spinal cord injury has a neuroprotective effect with its antioxidant, anti-inflammatory, and anti-apoptotic effects. Fu S. *et al.*[30] showed in their study that its antioxidant effects have a good therapeutic potential with higher doses and longer use. However, CAPE also exhibits neuroprotective properties against oxidative stress.[34,35] Studies are showing that CAPE protects different tissues, such as the brain and heart, by inhibiting IR-induced injury.[35,36] In the current study, we evaluated the nNOS expression to determine the distribution of free radicals at the histological level in IR-induced injury. Similarly, an increase in the amount of nNOS was found as a result of IR-induced injury, and a decrease was observed in the level of nNOS after both resveratrol and CAPE-feeding

It has been shown in many studies that mild or severe IR-induced injury also triggers apoptotic cell death.[32,37–39] In a previous study, an increase in apoptotic cell death was observed with TUNEL staining in rabbit motor neurons after 15 minutes of ischemia, and it was revealed that apoptosis had an important role in delayed paraplegia.[38] In our study too, we used the TUNEL method to show apoptotic cell death and to make our study more specific, various types of cell death such as necrosis, autophagy, and necroptosis were also investigated. Therefore, we examined the autophagic cell death pathway activation by demonstrating Beclin-1 expression. In addition, we separately examined possible activation pathways by determining the levels of Bax and p53 expressions in cell death pathways. According to the results obtained, it was determined that both the number of apoptotic cells and the expressions of Bax, p53, and Beclin-1 were at the highest level in the IR group and that this increase was significantly higher than the values of the control and sham groups. Additionally, there was a decrease in values due to IR in the treatment groups, these decreases were found to be statistically significant in Bax and Beclin-1 expressions. Moreover, CAPE-treated rats gained significantly lower p53 values than the IR group, whereas the p53 levels of resveratrol-treated rats showed a tendency towards a decrease compared to IR, but was not found significant. Liu C. *et al.*[33] examined Bax, Bcl-2, and Caspase 3 levels in their similar study on rats and found a decrease in the expression of these genes and molecules in the resveratrol group compared to the IR-induced injury group. They reported that different genes such as Bcl-2, Bax, p53, c-myc, and Fas played a role in apoptosis. They were shown to be highly correlated factors especially in the regulation of Bcl-2 and Bax apoptosis.[40] The data obtained in our study showed a significant decrease in Bax and p53 expressions in the CAPE + IR group, but resveratrol treatment only showed a diminishment of Bax + cell numbers with IR. The positive results of CAPE on spinal cord injury have been shown by a limited number of studies.[41–43] Akgun B *et al.*[42] examined micro-hemorrhages and edema to compare the histopathological effects of CAPE with methylprednisolone after spinal cord injury, demonstrated a significant decrease in micro-hemorrhages, and positive effects on inflammatory response were also observed in their studies. Their findings were also supported by another similar study.[44] In our study, we obtained unique positive results on necrosis, autophagy, and necroptosis for CAPE. Beclin-1 plays a central role in autophagy. It acts together with different cofactors to stimulate autophagy.[45] It was found in our study that Beclin-1 expressions increased in the IR group. The increase of this parameter, which has been studied very little in the literature, the IR group reveals that it has a role in this process in autophagic cell death, and this original data will shed light on further studies to be conducted in the future. It is furthermore provided the deceleration of apoptosis via resveratrol and CAPE in IR-induced damage with Beclin-1 dependent anti-autophagic effect, will contribute to the literature as new data.

Although no histopathological difference was found in the control and sham groups in our study, we determined that vascularization and glial cell infiltration significantly increased in the spinal cords of rats in the IR group. Although in the groups administered resveratrol and CAPE compared to the IR group, we found a significant decrease in these parameters. In parallel with other parameters in the studies, it was observed that the number of degenerated neurons increased significantly in the IR group, while in the IR-induced damaged groups under resveratrol and CAPE treatment, it was significantly diminished. In our study, the fact that we did not examine the markers of the inflammatory response, which are one of the steps in the secondary spinal cord injury, and the response of resveratrol and CAPE in combined therapy can be considered as limitations.

Conclusion

In conclusion, we demonstrated that both resveratrol and CAPE caused a decrease in oxidant substances in tissues and a decrease in the number of apoptotic cells, but CAPE is partially more effective compared to resveratrol. In the present study, we showed that it was effective in cell death as well as apoptotic cell death during the injury process. In light of these current data, it is suggested that resveratrol and CAPE have a protective effect against spinal cord injury after IR.

Author contribution

EA, HG, and MGB performed the research. EA, MBP, and MGB helped during the experimental work, statistical analysis and in writing the manuscript. MGB and MBP drafted the manuscript. EA and MBP conceived and designed the study and critically revised the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was funded by the Afyon Kocatepe University Research Foundation, Afyonkarahisar, TURKEY [Grant number: 14-TIP-06].

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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