

Efficiency of resveratrol and quercetin after experimental spinal cord injury

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ABSTRACT

BACKGROUND: The aim of this study was to investigate the effect of natural antioxidants resveratrol and quercetin on oxidative stress and secondary cell damage in rats with acute spinal cord injury.

METHODS: In this experimental study, 42 male Sprague-Dawley rats were used. Spinal cord injury was performed with clip compression method at level of T4-5. The study was conducted using 6 groups: control, trauma, trauma and solvent, trauma and resveratrol, trauma and quercetin, and trauma with combined resveratrol and quercetin. All rats were euthanized 48 hours after the procedure. Effects of resveratrol and quercetin on serum and tissue total antioxidant capacity and paraoxanase activity level were examined.

RESULTS: Compared to trauma group, there was a significant increase in total antioxidant capacity and paraoxanase activity level in resveratrol, quercetin, and combined treatment groups. There was no significant difference between resveratrol and quercetin groups with regard to total antioxidant capacity and paraoxanase activity level. Total antioxidant capacity and paraoxanase activity level were significantly higher in solvent group than trauma group. In histopathological evaluation, there was a decrease in polymorphonuclear leukocyte infiltration in solvent, resveratrol, quercetin, and combined treatment groups.

CONCLUSION: Biochemical and histological staining results of present study showed that resveratrol and quercetin may be effective in preventing secondary damage in spinal cord injury.

Keywords: Antioxidant; experimental spinal cord injury; quercetin; resveratrol.

INTRODUCTION

Spinal cord injury (SCI) is a clinical condition that often causes devastating outcomes such as permanent incapacity, especially in the younger population. It is well known that SCI is a type of trauma with no known effective therapeutic methods, and accordingly it is still difficult to estimate prog-

nosis. Poor prognosis and devastating outcomes of SCI justify scientific interest in the pathophysiology of SCI as well as in protective strategies and development of treatment options.^[1,2] Resveratrol and quercetin are naturally occurring antioxidative compounds that are referred to as polyphenols. Resveratrol reduces oxidation of low-density lipoprotein (LDL) by chelating copper (II) and phagocytizing oxygen free radicals. It also inhibits platelet aggregation and prevents thrombus formation and atherosclerosis, acting as a therapeutic agent to prevent potential cardiovascular disease.^[2-5] Quercetin has a broad range of pharmacological characteristics such as anticancer, antiviral, antihistaminic, antitumoral, immunomodulatory, antioxidant, and anti-inflammatory properties.^[5-10] Recently, there have been studies conducted on antioxidant effects of these agents. The current study explored therapeutic effects and antioxidant properties of post-SCI administered resveratrol and quercetin on secondary cell damage.

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MATERIALS AND METHODS

Male Sprague-Dawley rats (weight: 250 ± 50 g; $n=42$) were supplied for the study by the experimental research center of Eskisehir Osmangazi University. Animals had 2-week period of adaptation to surroundings before initiation of study. They were housed in polycarbonate cages in a temperature- and humidity-controlled room ($21\pm 10^{\circ}\text{C}$ and 45-55%, respectively) with 12/12 reversed light cycle. They were fed with standard rat chow and allowed to drink ad libitum. Procedures regarding animal care, surgery, and sample preparation were approved by the institutional animal care and experiments committee of Eskisehir Osmangazi University Faculty of Medicine. Regulations and guidelines for the care and use of laboratory animals of institutional animal care and experiments committee of Eskisehir Osmangazi University Faculty of Medicine (19.08.2009/130) were observed, as well as US National Institutes of Health guidelines regarding the care and use of animals for experimental procedures.

Surgical Procedure

The experimental SCI model was applied to 42 rats divided into 6 groups. Sedation was ensured with intramuscularly (i.m.) administered xylazine (Rompun; Bayer AG, Leverkusen, Germany) at dose of 10 mg/kg, followed by ketamine hydrochloride (Ketalar; Pfizer, New York, NY, USA) i.m. at dose of 50mg/kg for general anesthesia. Rivlin-Tator compression model (1978) was used: Surgical mark point was spinous process of T2 vertebrae, the most remarkable point of the rat spine. The area from T3 to T6 was shaved, and local surgery site antisepsis and environmental isolation were ensured. Midline skin incision was made through cutaneous and subcutaneous layers. Fascia was opened and paravertebral muscles were subperiostally lateralized. T4-T5 laminectomy was performed. Spinal cord was exposed and an aneurysm clip with extradural closing pressure of 50 g (Yaşargil clip FE 619K, Aesculap AG, Tuttlingen, Germany) was used to induce SCI. In SCI groups, the clip was in place for 1 minute followed by

immediate removal with clip holder. Post-SCI paraplegia was observed in all rats. Resveratrol and quercetin were dissolved in dimethyl sulfoxide (DMSO) and single dose 1 hour following SCI was administered. All rats were euthanized 48 hours after procedure. All procedures and group data are summarized in Table I.

Biochemical Analyses

Thoracic cavity of rats was opened under general anesthesia in order to determine total antioxidant status (TAS) level and paraoxonase-I (PON-I) activity. A blood sample of 3 mL was drawn from heart, centrifuged at 1000 rpm for 10 minutes, and plasma was collected. Tissue samples and spinal cord samples of approximately 15 mm in length were taken from all groups and frozen in liquid nitrogen. Samples were transferred to tubes containing 1 mL 1x phosphate buffer saline and processed in ultrasonic homogenizer. The resultant homogenate was centrifuged at 3500 rpm for 15 minutes and supernatant was used for measurements. In plasma and tissue homogenates, TAS level was read using Shimadzu UV-1601 spectrophotometer (Shimadzu Corp., Kyoto, Japan) at absorbance level of 660 nm according to TAS assay kit (Rel Assay Diagnostics, Gaziantep, Turkey) procedures, while PON-I activity was measured using same device at absorbance level of 412 nm according to the PON-I kit (Rel Assay Diagnostics, Gaziantep, Turkey) procedures.

Histological Examinations

Approximately 15 mm-long samples of spinal cord taken from control and experiment groups for histological evaluation included white matter, grey matter, and trauma site. All specimens were carefully excised and fixed in neutral buffered formalin for histological analyses. After fixation, tissue was embedded in paraffin and serial sections ($4\ \mu\text{m}$) were prepared for each paraffin block; on average, 50 sections were collected per rat. Sections were stained with hematoxylin and eosin for assessment of SCI. Digital images were obtained using Olympus BX-61 microscope (Olympus Corp., Tokyo, Japan) with a

Table I. Procedures performed and substances used according to experiment group

Group	n	Procedure		Substance		
		T4 and T5 laminectomy	Spinal cord injury	DMSO	Resveratrol (dissolved in DMSO)	Quercetin (dissolved in DMSO)
C	7	√	–	–	–	–
SCI	7	√	√	–	–	–
SCI+DMSO	7	√	√	0.3 mL i.p.	–	–
SCI+R	7	√	√	–	100 mg/kg i.p.	–
SCI+Q	7	√	√	–	–	200 mg/kg i.p.
SCI+R+Q	7	√	√	–	100 mg/kg i.p.	200 mg/kg i.p.

C: Control group; DMSO: Dimethyl sulfoxide; i.p: Intraperitoneal; Q: Quercetin; R: Resveratrol; SCI: Spinal cord injury group; SCI+DMSO: Post-SCI DMSO group; SCI+Q: Post-SCI quercetin group; SCI+R: Post-SCI resveratrol group; SCI+R+Q: Post-SCI combined treatment group.

DP70 digital camera. Histological scoring of SCI was as follows: no damage (0), very mild (1), mild (2), moderate (3), and severe (4). Tissue samples were also examined for hemorrhage, edema, necrosis, axonal swelling, chromatolysis, polymorphonuclear leukocyte (PMNL) infiltration and microglia/macrophage/MNL (mononuclear leukocytes) infiltration (Dobrowolski et al. 2011; Hausmann 2003; İş et al. 2006; Oyinbo 2011).

Statistical Analyses

Statistical analyses were performed using Statistical Package for the Social Sciences for Windows, version 11.0 (SPSS Inc., Chicago, IL, USA) and SigmaStat 3.5 (Statcon Inc., B. Schäfer, Witzhausen, Germany) software packages. Kolmogorov-Smirnov normality test was used for data with normal distribution. Groups were compared using analysis of variance (ANOVA) for normally distributed data, and Tukey's HSD test for multiple comparisons; Kruskal-Wallis ANOVA test was used for non-normally distributed data. Results were expressed as mean±SD or median (25%–75% percentiles). A *p* value <0.05 was considered statistically significant.

RESULTS

Biochemical Results

Serum and tissue TAS and PON-I levels were lower in SCI group in comparison with the control group (*p*<0.05). Although post-SCI resveratrol and quercetin increased serum and tissue TAS and PON-I levels in comparison with SCI group, there was no statistically significant difference between the 2 groups. The most remarkable increase was observed in post-SCI combined treatment group (*p*<0.05) (Table 2).

Histological Results

When spinal cord was examined with regard to hemorrhage, a remarkable difference was found in white and gray mat-

ter of groups. When multiple comparison tests were examined in order to determine inter-group differences, significant increase was observed in hemorrhage in SCI and post-SCI DMSO groups compared with control group (*p*<0.05) (Figs. 1a, c, d, 2a, b). It was observed that resveratrol administered in post-SCI period was more effective than quercetin in terms of eliminating hemorrhage (Figs. 2c, 3c).

Remarkable differences were also observed in white and gray matter of groups with regard to parameter of edema. Significant increase was observed in SCI and post-SCI DMSO groups in comparison with control group (*p*<0.05). Particularly, a regression was observed in edema with post-SCI resveratrol and post-SCI combine treatment groups versus SCI group (Figs. 2c, 4c).

There were important differences in necrosis of white and gray matter, as well. Significant increase was observed in SCI and post-SCI DMSO groups compared with control group (*p*<0.05) (Fig. 1b, 2b). It was observed that post-SCI resveratrol was effective achieving in regression of necrosis (Fig. 2d, 3a).

With regard to PMNL infiltration, there were important inter-group differences in white matter. Significant increase was observed in PMNL infiltration in SCI group in comparison with control group (*p*<0.05) (Fig. 1d). There was significant decrease in post-SCI combined treatment group in comparison with SCI group, and regression was observed in white matter of all groups (*p*<0.05). There was significant difference between groups with regard to PMNL infiltration to the vessel wall and subarachnoid space. A significant increase was observed in PMNL infiltration to the vessel wall in the SCI group in comparison with control group (*p*<0.05). There was a significant decrease in PMNL infiltration in post-SCI resve-

Table 2. Serum and tissue TAS and PON-I values of all groups (mean±SD or median 25%–75% percentiles)

	Serum TAS Level (mmol Trolox Eq/L)	Tissue TAS Level (mmol Trolox Eq/L)	Serum PON-I Level (U/L)	Tissue PON-I Level (U/L)
	Median (25%–75% per.)	Mean±SD	Mean±SD	Median (25%–75% per.)
C	1.65 (1.62–1.66)	0.75±0.007	146.28±2.61	82.91 (80.06–84.37)
SCI	0.63 (0.62–0.64)	0.15±0.02	67.11±2.89	35.75 (34.46–39.61)
SCI+DMSO	0.89 (0.89–0.96)	0.29±0.01	81.18±4.05	43.98 (40.24–48.50)
SCI+R	1.24 (1.23–1.27)	0.44±0.03	104.71±1.81	61.58 (53.95–62.64)
SCI+Q	1.23 (1.10–1.24)	0.42±0.03	100.93±2.14	58.75 (52.95–60.74)
SCI+R+Q	1.39 (1.36–1.46)	0.61±0.02	118.41±1.69	70.01 (66.05–71.35)
Statistics	H=38.916 ^a	F=655.156 ^a	F=772.748 ^a	H=38.380 ^a
Statistics, <i>p</i>	<0.001	<0.001	<0.001	<0.001

a: All groups different from each other (*p*<0.05) except SCI+R and SCI+Q (*p*>0.05); C: Control group; DMSO: Dimethyl sulfoxide; i.p: Intraperitoneal; PON-I: Paraoxonase-I; Q: Quercetin; R: Resveratrol; SCI: Spinal cord injury group; SCI+DMSO: Post-SCI DMSO group; SCI+Q: Post-SCI quercetin group; SCI+R: Post-SCI resveratrol group; SCI+R+Q: Post-SCI combined treatment group; TAS: Total antioxidant status.

ratrol, quercetin and combined treatment groups compared with SCI group ($p < 0.05$).

When microglia/macrophage/MNL infiltration was examined, important differences were observed in white and gray matter. Microglia/macrophage/MNL infiltration was not observed in white matter of control and SCI groups or post-SCI resveratrol group. Significant increase in microglia/macrophage/MNL infiltration was observed in white and gray matter of

quercetin and combined treatment groups in comparison with SCI group ($p < 0.05$) (Fig. 3b, 4c). Therefore, it was determined that quercetin was more effective than resveratrol against increased microglia/macrophage/MNL infiltration.

It was found that resveratrol and quercetin had no beneficial effect on axonal swelling induced with SCI.

It was also determined that chromatolysis had non-significant

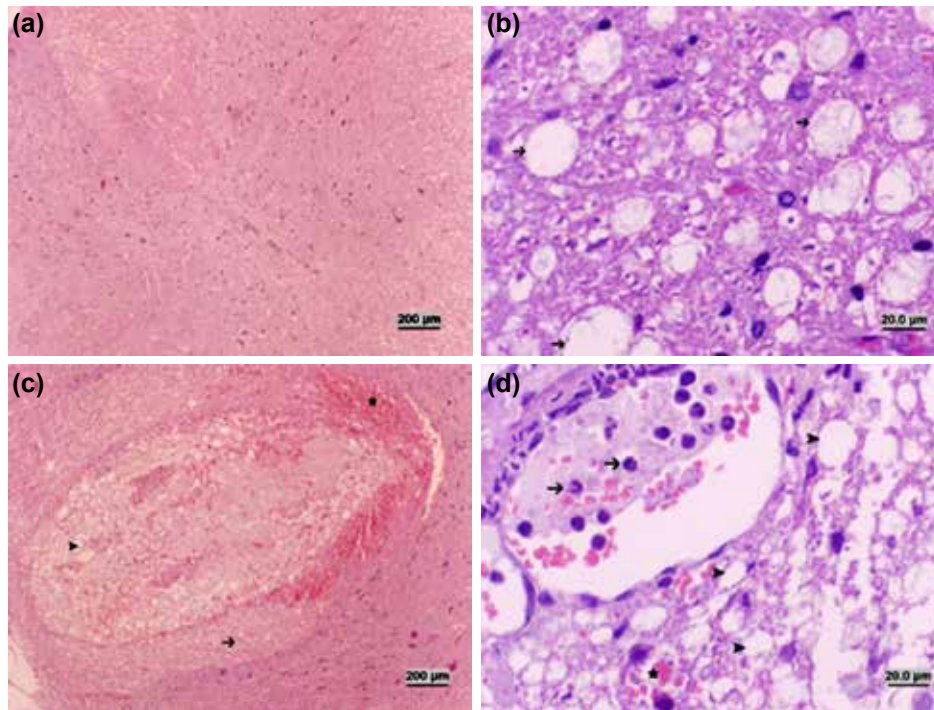


Figure 1. Spinal cord tissue morphology indicated by hematoxylin-eosin staining in control and SCI groups. **(a)** Control group; normal view. H&E, Scale Bar 200 μ m. **(b)** SCI group; moderate axonal swelling (arrow) and necrosis in the white matter. H&E, Scale Bar 20 μ m. **(c)** SCI group; severe axonal swelling (arrow), hemorrhage (asterisk), edema (arrow head) and necrosis in the white matter. H&E, Scale Bar 200 μ m. **(d)** SCI group; moderate PMNL infiltration (arrow) in vascular wall, severe axonal swelling (arrow head), hemorrhage (asterisk), edema and necrosis in the white matter. H&E, Scale Bar 20 μ m.

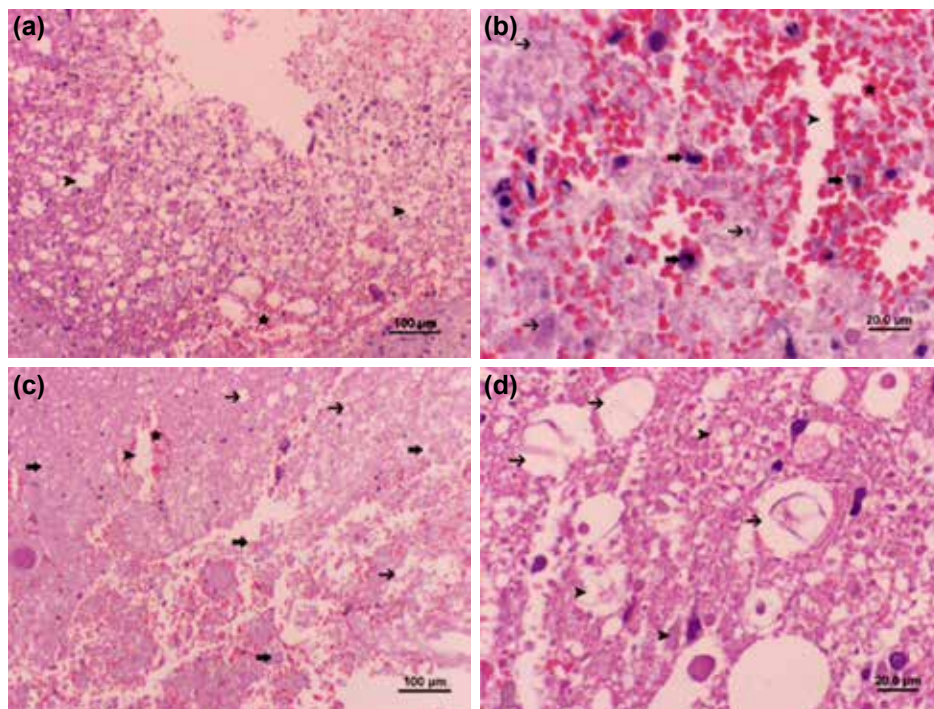


Figure 2. Spinal cord tissue morphology indicated by hematoxylin-eosin staining in post-SCI DMSO and post-SCI Resveratrol groups. **(a)** Post-SCI DMSO group; moderate hemorrhage (asterisk), edema, axonal swelling (arrow head), and severe necrosis in the white matter. H&E, Scale Bar 100 μ m. **(b)** Post-SCI DMSO group; mild microglia/macrophage/MNL infiltration (thick arrow) and severe hemorrhage (asterisk), edema (arrow head) and necrosis (thin arrow) in the white matter. H&E, Scale Bar 20 μ m. **(c)** Post-SCI Resveratrol group; severe hemorrhage (asterisk), edema (arrow head), necrosis, axonal swelling (thin arrow) and chromatolysis (thick arrow) in the White and gray matter. H&E, Scale Bar 100 μ m. **(d)** Post-SCI Resveratrol group; severe axonal swelling (arrow) and necrosis (arrow head) in the white matter. H&E, Scale Bar 20 μ m.

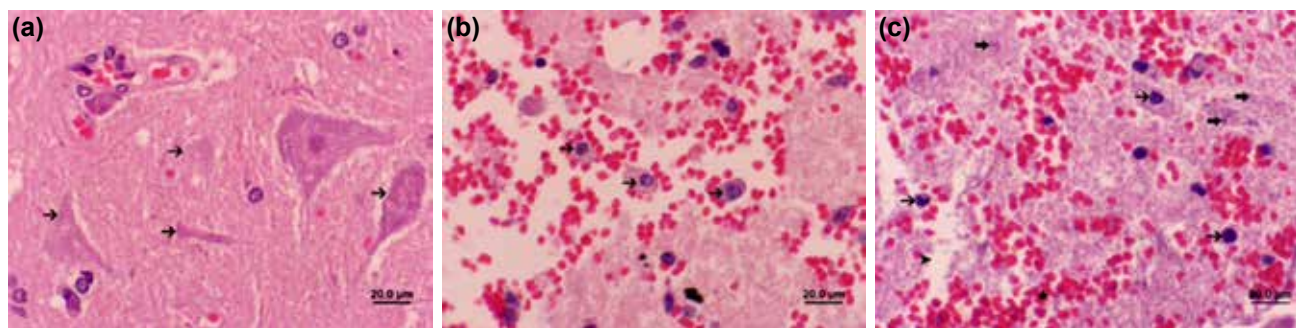


Figure 3. Spinal cord tissue morphology indicated by hematoxylin-eosin staining in post-SCI Quercetin group. (a) Post-SCI Quercetin group; mild chromatolysis (arrow) and necrosis in the gray matter. H&E, Scale Bar 20 µm. (b) Post-SCI Quercetin group; severe microglia/macrophage/MNL infiltration (arrow) in the white matter. H&E, Scale Bar 20 µm. (c) Post-SCI Quercetin group; severe hemorrhage (asterisk), edema (arrow head), necrosis (thick arrow) and mild microglia/macrophage/MNL infiltration (thin arrow) in the white matter. H&E, Scale Bar 20 µm.

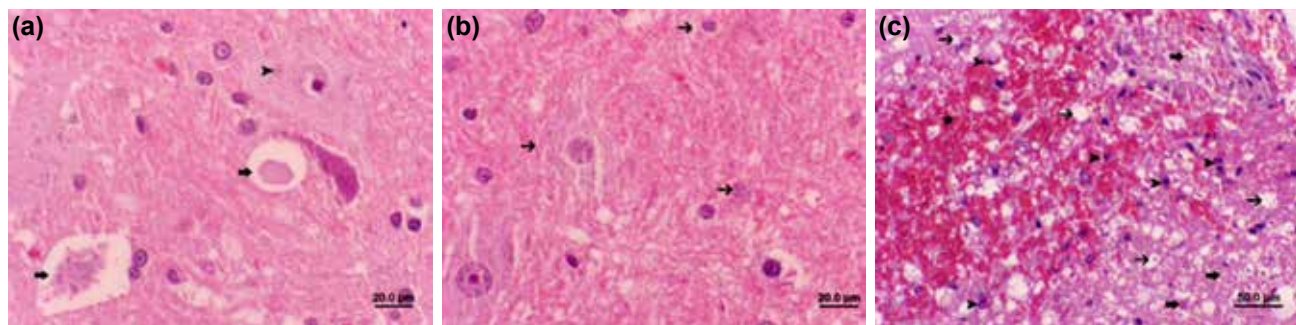


Figure 4. Spinal cord tissue morphology indicated by hematoxylin-eosin staining in Post-SCI combined treatment group. (a) Post-SCI combined treatment group; mild chromatolysis (arrow) and necrosis (arrow head) in the gray matter. H&E, Scale Bar 20 µm. (b) Post-SCI combined treatment group; severe chromatolysis (arrow) in the gray matter. H&E, Scale Bar 20 µm. (c) Post-SCI combined treatment group; moderate hemorrhage (asterisk), edema, necrosis (thick arrow), axonal swelling (thin arrows) and microglia/macrophage/MNL infiltration (arrow head) in the white matter. H&E, Scale Bar 50 µm.

decrease trend in post-SCI combined treatment group in comparison with SCI group (Fig. 4a, b).

Considering all available histological findings, the notable common finding was that PMNL infiltration, an indicator of primary response, had statistically significant decrease in post-SCI resveratrol, quercetin, and combined treatment groups ($p < 0.05$), and it had tendency to completely disappear. It was observed that indicator of secondary response, microglia/macrophage/MNL infiltration, significantly increased in post-SCI quercetin and combined treatment groups ($p < 0.05$). When all available results were examined, it was seen that resveratrol is more efficient in primary response and quercetin is more efficient in secondary response, and most effective result was obtained in post-SCI combined treatment group (Table 3).

DISCUSSION

Resveratrol produces anti-inflammatory and anti-anaphylactic effects by influencing arachidonic acid metabolism. It possesses antibacterial, anti-cancerous and anti-mutation effects, and inhibits protein kinase activity. All those properties indicate that resveratrol could affect pathophysiological processes of inflammatory or traumatic reactions.^[2,3,5]

Yang and Piao demonstrated that resveratrol and methylprednisolone (MP) had protective effect against secondary damage that occurred following SCI.^[2] Moreover, they determined that resveratrol had effect on post-SCI energy metabolism, inhibition of lipid peroxidation was more effective, and resveratrol had potent therapeutic effect on SCI. Recent investigations indicate that resveratrol is potential antioxidant, since it prevents formation of free radicals in tissue such as red cell membrane, heart, liver, brain, and kidney; prevents functional injury and improves nerve function; and promotes restoration after trauma. Resveratrol is a candidate as therapeutic agent to ameliorate neurodegenerative processes. Kızıltepe et al. demonstrated that resveratrol protects spinal cord against ischemia-reperfusion damage by reducing oxidative stress and increasing free nitric oxide (NO).^[11] In s study conducted by Cemil et al., post-SCI effects of curcumin and MP were compared in biochemical and histopathological terms.^[12] Curcumin, another polyphenol, has antioxidative, anti-cancerous, and anti-inflammatory effects. In curcumin group, tissue malondialdehyde (MDA) level decreased and levels of antioxidant enzymes copper-zinc superoxide dismutase, catalase, and phospholipid hydroperoxide glutathione peroxidase increased. Moreover, it was demonstrated that curcumin histopathologically protected tissue integrity. In rats with in-

Table 3. Histological analysis results of all groups (median 25%-75% percentiles)

		CG	SCI	SCI+DMSO	SCI+R	SCI+Q	SCI+R+Q	Analysis (p)
Hemorrhage	WM	0 (0–0)	4 (2.25–4)	3 (2.25–3.75)	2 (2–2.50)	3.50 (3–4)	3 (2–3)	24.906 (<0.001) ^{a,b,d}
	GM	0 (0–0)	3 (1.25–4)	3 (2–3)	0 (0–0.50)	2 (1–4)	1.50 (0–2)	23.722 (<0.001) ^{a,b,d}
Edema	WM	0 (0–0)	4 (2.50–4)	3 (3–4)	3 (3–4)	4 (4–4)	3.50 (2–4)	24.405 (<0.001) ^{a,b,d}
	GM	0 (0–0)	4 (1.25–4)	2 (2–2.75)	2 (1–2)	2.50 (2–3)	2 (1–2)	22.033 (<0.001) ^{a,b,d}
Necrosis	WM	0 (0–0)	4 (3.25–4)	4 (3–4)	2 (2–3)	4 (3–4)	3 (2–3.50)	27.532 (<0.001) ^{a,b,d}
	GM	0 (0–0)	3 (2.25–3)	3 (3–3)	2.50 (2–3)	3 (3–4)	2 (2–3)	24.518 (<0.001) ^{a,b,d}
PMNL infiltration	WM	0 (0–0)	3 (2–4)	0 (0–0)	0.50 (0–1)	1 (0–2)	0 (0–0.50)	28.831 (<0.001) ^{a,f,h}
	GM	0 (0–0)	2 (0–2)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	16.671 (>0.05)
	VW	0 (0–0)	3 (3–4)	2 (0–4)	0 (0–0.50)	0 (0–0)	0 (0–0)	26.928 (<0.001) ^{a,g,h}
	SAS	0 (0–0)	3 (3–4)	3 (0–4)	0 (0–0.50)	0 (0–1)	0 (0–0)	21.086 (<0.001) ^{a,h}
Microglia/macrophage/MNL Infiltration	WM	0 (0–0)	0 (0–0)	2 (2–2.75)	1 (1–2)	3 (3–4)	3 (3–3)	39.506 (<0.001) ^{d,l}
	GM	0 (0–0)	0 (0–0)	2 (2–2)	0 (0–1.50)	2 (2–4)	3 (2–3)	37.203 (<0.001) ^{d,e,g,k,l}
Axonal swelling		0 (0–0)	4 (2.50–4)	3 (2.25–4)	3 (3–4)	4 (3–4)	3 (2.50–4)	22.100 (<0.001) ^{a,c,d}
Chromatolysis		0 (0–0)	3 (2–3)	3 (3–4)	3 (3–3)	3.50 (3–4)	2 (1.50–3)	27.109 (<0.001) ^{b,c,d}

0: No damage; 1: Very mild; 2: Mild; 3: Moderate; 4: Severe.

a: p<0.05 for control group and SCI group; b: p<0.05 for control group and SCI+DMSO group; c: p<0.05 for control group and SCI+R group; CG: Control group; D: p<0.05 for control group and SCI+Q group; DMSO: Dimethyl sulfoxide; E: p<0.05 for control group and SCI+R+Q group; F: p<0.05 for SCI group and SCI+DMSO group; G: p<0.05 for SCI group and SCI+Q group; GM: Gray matter; H: p<0.05 for SCI group and SCI+R+Q group; K: p<0.05 for SCI+R group and SCI+Q group; L: p<0.05 for SCI+R group; MNL: Mononuclear leukocytes; PMNL: Polymorphonuclear leukocyte; Q: Quercetin; R: Resveratrol; SAS: Subarachnoid space; SCI: Spinal cord injury group; SCI+DMSO: Post-SCI DMSO group; SCI+Q: Post-SCI quercetin group; SCI+R: Post-SCI resveratrol group; SCI+R+Q: Post-SCI combined treatment group; VW: Vessel wall; WM: White matter.

duced SCI, it was found that curcumin was as biochemically and histopathologically effective as MP in functional improvement. Ates et al. investigated the effects of resveratrol and MP on induced SCI and found that MDA, NO and xanthine oxidase levels were lower and glutathione groups were higher in resveratrol and resveratrol+MP (combination) groups in comparison with just MP group.^[13] It was observed that neurological improvement was better in resveratrol and combined treatment group relative to MP alone group. No significant histological difference was found in the treated groups. It was found in the current study that resveratrol was as effective as MP on secondary damage that occurs in post-SCI period and combined treatment offered no extra protection in chronic phase of SCI. It was determined that resveratrol and quercetin combination ameliorated post-traumatic oxidative stress and led to regression of edema. Therapeutic value and mechanisms of resveratrol on SCI should be studied further.

One means of antioxidant action exerted by quercetin involves scavenging free radicals, such as superoxide radicals generated by xanthine and xanthine oxidase. The antioxidant capacity of this molecule may be a very significant function and responsible for many of the beneficial effects. Quercetin interferes with the production of reactive oxygen species and reduces tissue damage through chelating effect and elimination of lipid peroxidation.^[5–7,10]

It has been reported that quercetin is effective in prevention

of oxidative damage to DNA or to cell membrane. One possible mechanism is that quercetin stabilizes lipid membranes and protects lipid peroxidation by exerting free radical scavenging effects. Quercetin is characterized by strong oxygen radical scavenging property and good metal chelating. Quercetin diffuses into membranes at high concentrations, resulting in antioxidative effect, and thus, it may scavenge oxyradicals at several sites through lipid bilayer. Pentahydroxyflavone structure possibly allows it to chelate metal ions via orthodihydroxy phenolic structure and thereby scavenge lipid alkoxyl and peroxy radicals. It has also been suggested that quercetin acts as an antioxidant by inhibiting oxidative enzymes such as xanthine oxidase, lipoxigenase, and nicotinamide adenine dinucleotide phosphate oxidase. Inhibition of these enzymes is also responsible for attenuation of oxidative stress, as they play key role in initial process of free radical-induced cellular damage. Further, it has been reported that quercetin metabolites can also inhibit peroxynitrite-mediated oxidation, similar to free quercetin. In addition to direct hydrogen-donating properties, attention has focused on influence of quercetin on signaling pathways and its indirect interaction with endogenous antioxidant defense system.^[5,10]

Epidemiological studies have indicated beneficial effects of quercetin in neural protection.^[14] Cerebral neuroprotective effect of quercetin derives from anti-oxidative effect and free radical scavenging property.^[15,16] In a study conducted by Genovese et al., the authors investigated effect of Hyper-

cum perforatum extract on neuroprotection and neuronal improvement in rats after SCI was induced.^[17] Hypericum perforatum extract comes from an herb that is a member of polyphenol group and contains flavonoid and phenolic acid. It was determined that Hypericum perforatum extract improved spinal cord inflammation, tissue damage, and PMNL infiltration. It was also found to improve neuronal function. In an experimental study conducted by Schültke et al. (2010), it was demonstrated that the extract protected tissue integrity in the area of SCI and success rate of the treatment was dependent on frequency of administration and total dose.^[18] Schültke et al. (2003) demonstrated in other experimental study that amelioration of secondary damage via chelation of iron is an important mechanism in functional improvement after SCI develops that is supported by quercetin.^[19]

Although primary physiological role of PON-I is not still completely known, recent studies have demonstrated that it is associated with high-density lipoprotein (HDL) cholesterol and prevents lipid peroxidation by playing protective role against oxidative modification of LDL cholesterol in addition to having antioxidant and anti-inflammatory activities.^[20-22] In patients with diseases characterized by increased oxidative damage, the higher tendency of HDL for peroxidation is considered to be related to decreased PON-I activity.^[23] It has been observed that PON-I offers protection against lipid peroxidation damage caused by free radicals on cell membranes and lipoproteins.^[24]

In a study conducted by Rael et al., plasma oxidation reduction potential, PON-I and arylesterase activity were measured in 39 patients with multiple traumas and results were compared with those of 10 healthy volunteers.^[25] It was determined that PON-I and AE activity were significantly lower in patients with multiple traumas, and those parameters may be beneficial when severity of trauma and potential efficiency of treatment are examined. In the current study, significantly higher serum and tissue TAS and PON-I activity levels found in post-SCI resveratrol group relative to SCI and post-SCI DMSO groups are consistent with findings of Ates et al (2006; 2007) indicating increased glutathione peroxidase level in rats given resveratrol after SCI and head trauma.^[13,25] Elmali et al. demonstrated that resveratrol significantly reduced MDA level in ischemia/reperfusion damage of skeletal muscle.^[26] It was also observed in the current study that TAS level, which indicates total antioxidative effect, increased in resveratrol treatment group. It can be speculated that effect derives from anti-oxidative effect and free radical scavenging property of resveratrol as determined in the literature, particularly studies conducted by Ates et al. (2006; 2007).^[13,26] The increased level of TAS indicates that resveratrol offers better protection against oxidative damage of the spinal cord. In addition, in the current study it was seen that PON-I activity increased in rats given resveratrol. As indicated in studies conducted by Elmali et al. and Ray et al., resveratrol may inhibit lipid peroxidation and alleviate LDL oxidation by binding free radicals.

^[27,28] We observed in the current study that serum and tissue TAS levels and PON-I activities were significantly higher in quercetin group relative to SCI and SCI+DMSO groups. Increase may be due to antioxidative effects deriving from free radical scavenging properties.

In the present study it was found that serum and tissue TAS levels and PON-I activities in DMSO group were significantly higher than SCI group and lower than control group. DMSO, which was used as solvent, is a free oxygen radical scavenger, largely specific to hydroxyl radicals. Turan et al. demonstrated substantial neuroprotective effect of DMSO in rabbits with induced spinal cord ischemia/reperfusion damage.^[29] In a study conducted by Chiueh et al., it was also demonstrated that DMSO suppressed generation of free radicals in cranial trauma.^[30] In the current study, PMNL infiltration was eliminated from white matter of group given DMSO. It was observed that TAS level was significantly higher in comparison with SCI group. As noted by Albin et al. (1986) and Chiueh et al. (1994) with studies published in the literature, it is believed that the increase was secondary to free radical scavenging effects of DMSO.^[30,31] Moreover, increased PON-I activity may be related to effect of DMSO on lipid metabolism.

In the present study, serum and tissue TAS levels and PON-I activity were significantly higher in post-SCI combined treatment group relative to both SCI and resveratrol monotherapy and quercetin monotherapy groups. In a study conducted by Carvalho et al., ganglioside, MP, and ganglioside+MP combination were administered to rats after SCI was induced.^[32] It was demonstrated that results obtained in combined treatment group were better than MP monotherapy group. Ates et al. demonstrated that neurological improvement was better in combined treatment group relative to MP monotherapy group.^[26] In comparison with monotherapy groups and solvent groups, the significantly higher serum and tissue TAS levels and PON activity in combined treatment group as well as serum and tissue levels almost equal to control group may be due to antioxidative and anti-inflammatory potential of both therapeutic agents and the DMSO. In concordance with these findings, the better results obtained in combined treatment group in the post-SCI period relative to monotherapy groups support idea that those two agents and solvent DMSO have synergistic effect.

Elmali et al. demonstrated that resveratrol significantly reduced PMNL infiltration and edema in muscular tissue, changes in length of muscle fibers, and segmented necrosis relative to control group in ischemia-reperfusion injury.^[27] As a remarkable common finding, it was observed in the current study that in comparison with SCI group, PMNL infiltration was reduced in the white matter of post-SCI DMSO and combined treatment groups, in the vessel wall of post-SCI quercetin and combined treatment groups, and in the subarachnoid space of the post-SCI combined treatment group, the reduction of the infiltration tended to completely

disappear. It was observed that microglia/macrophage/MNL infiltration significantly increased in the white matter of post-SCI quercetin and combined treatment groups in comparison with SCI group, in the gray matter of post-SCI quercetin group in comparison with the SCI and post-SCI resveratrol group, and in gray matter of post-SCI combined treatment group in comparison with post-SCI resveratrol group. This effect may derive from anti-inflammatory effects of two drugs and DMSO.

Conclusions

In summary, our results indicate that resveratrol and quercetin combination alleviated post-SCI oxidative stress. Resveratrol was more effective on primary response, while quercetin was more effective on secondary response, and most effective results were obtained in post-SCI combined treatment group. The 2 compounds may have protected the spinal cord against secondary damage due to possible anti-oxidative effects. We believe that more extensive future studies are warranted to include resveratrol and quercetin in the treatment protocol of SCI.

Conflict of interest: None declared.

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DENEYSSEL ÇALIŞMA - ÖZET

Deneyssel spinal kord travması sonrası resveratrol ve kuersetin'in etkinliğinin araştırılması

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AMAÇ: Bu çalışmada, doğal antioksidanlardan olan resveratrol ve kuersetin'in, deneyssel spinal kord travması oluşturulmuş sıçanlarda, oksidatif stres ve sekonder doku hasarı üzerine olan etkilerini araştırmak amaçlandı.

GEREÇ VE YÖNTEM: Bu deneyssel çalışmada, 42 adet Sprague-Dawley cinsi erkek sıçan kullanıldı. Spinal kord hasarı klip kompresyon metodu kullanılarak T4-T5 seviyesinden yapıldı. Çalışma kontrol, travma, travma+çözücü, travma+resveratrol, travma+kuersetin ve travma+kombine (resveratrol ve kuersetin) olmak üzere 6 grup üzerinden yürütüldü. İşlemden 48 saat sonra tüm sıçanlar sakrafiye edildi. Resveratrol ve kuersetin'in serum ve dokuda total antioksidan kapasite düzeyi ve paraoksonaz enzim aktivitesine ve histopatolojik bulgulara olan etkisi araştırıldı.

BULGULAR: Sonuç olarak resveratrol, kuersetin ve kombine tedavi verilen gruplarda TAOK düzeyi ve PON aktivitesi değerlerinde travma grubuna göre anlamlı bir artma saptandı. Resveratrol ve kuersetin grupları arasında TAOK düzeyi ve PON aktivitesi açısından anlamlı bir farklılık yoktu. Çözücü grubunda TAOK düzeyi ile PON aktivitesi travma grubuna göre anlamlı derecede yüksekti. Histopatolojik incelemede ise sadece çözücü, resveratrol, kuersetin ve kombine tedavi gruplarında PMNL infiltrasyonunun azaldığı görüldü.

TARTIŞMA: Çalışmamızdaki biyokimyasal ve histolojik değerlendirmede resveratrol ve kuersetin uygulamasının spinal kord travmasında görülen ikincil hasardan, spinal kordu korumak için kullanılabileceği sonucuna varılmıştır.

Anahtar sözcükler: Antioksidan; deneyssel spinal kord hasarı; kuersetin; resveratrol.

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