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Neuroprotective effect of carvacrol in an experimental cerebral ischemia and reperfusion rat model

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Abstract

The Neuroprotective effect of carvacrol, which has anti-inflammatory and antioxidant effects, on infarcted cerebral tissue is present in literature, but this contribution was not sufficiently clarified in terms of biochemistry. It is aimed to investigate the effect of orally administered carvacrol on plasma and intraparenchymal levels of TBARS, GSH, SOD, CAT, GSH-Px, IL-1β, IL-4, and TNF-α after the formation of global ischemia in cerebral tissue. Four groups were formed, each containing ten Wistar albino rats. After anesthesia and analgesia, bilateral carotid communis arteries of rats in the first two groups were clamped for 15 minutes with aneurysm clips. Oral 50 mg/kg/day carvacrol was administered for 15 days to the first group (I/R+CRV) of these two groups in which cerebral ischemia-reperfusion (I/R) was established. On the other hand, %0,01 carboxymethylcellulose (CMC), which is a solvent of carvacrol, at a same volume of first group was administered orally for the same duration to the other group in which also I/R was established (I/R+CMC). In the other two groups in which ischemia was not induced, only carotid artery dissections were made and sutured again. In these two groups, 50 mg/kg/day of carvacrol was administered to the first group (CRV). The Same dose of CMC was administered to the second group (control group). After all these treatments, plasma was collected, and brain tissue was dissected from all groups at the end of the 15th day. Carvacrol can be included in the possible treatment regimen of cerebral stroke with the help of other studies that can be supported on this topic.

Keywords: ischemia reperfusion, carvacrol, CMC, CAT, GSH-Px, GSH, IL-1β, IL-4, SOD, TBARS, TNF-α.

1. Introduction

Cerebrovascular accident (CVA) is a disease that is characterized by temporary or permanent occlusion of any of the vessels feeding the cerebral tissue or the disruption of the blood supply to the brain tissue due to extravasation of blood, which can cause serious neurological findings. The incidence of this pathology, which can cause mortality and morbidity, is expected to increase because of an elevated number of elderly populations in the future. Despite the increased diversity of treatment modalities and medical treatments, ideal treatment is still not present and a sufficient level of knowledge and experience has not been developed from the studies carried out (1, 2).

In recent years, the use of plant-derived drugs, which have attracted attention due to their antitumor and antioxidant effects, are becoming widespread in most diseases (2). Of these, carvacrol (CRV), which is obtained from thyme extract, is a popular research topic and there are studies in the literature showing that it may have a significant benefit in many diseases (3, 4). Phenolic compounds, present in the structure of CRV, are responsible from the antioxidant activity and they are strong neutralizing molecules against metabolites of oxidative stress (5, 6). Biochemical analysis in studies that were conducted to show the neuroprotective effect of CRV, which can be a glimmer of hope for CVA treatment, is not comprehensive enough. Our aim in this research is to investigate the effect of CRV, a powerful antioxidant, on an experimental I/R rat model.

2. Material and Methods

This study was planned in accordance with the Guide for the Care and Use of Laboratory Animals. This study was investigated by Malatya İnonu University Experimental Animals Ethics Committee and Ethics committee approval was obtained on 03.06.2016 with the decision number 2016/A-79. Wistar–albino rats were obtained from the University's Experimental Animal Production and Research Centre. Experimental procedure was conducted at the laboratory of the same center. A total number of 40 male Wistar Albino rats weighing approximately between 250-300 g were used in this

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study. Rats were randomly divided into 4 groups. Groups are as follows,

1st Group: Control group (n=10). In this group cerebral ischemia will not be induced, only 50/mg/kg/day carboxymethylcellulose (CMC), which is the solvent, will be administered for 15 days. 2nd Group: Ischemia-reperfusion (I/R) group (n=10). In this group, cerebral ischemia will be induced and only CMC will be administered for 15 days at a dose of 50mg/kg/day. Cerebral ischemia was induced in this group and only CMC was administered at a dose of 50mg/kg/day for 15 days. 3rd Group: I/R + CRV group (n=10). In this group, cerebral ischemia will be induced, and 50 mg/kg/day CRV was administered for 15 days. 4th Group: CRV group (n=10). 50 mg/kg/day CRV was administered for 15 days without inducing cerebral ischemia.

CMC, which was used as a solvent of CRV, was administered via gavage for 15 days to rats in control group (1st group). An ischemia model was created, as previously practiced in the literature (2), in rats in the I/R group (group 2). The muscle, ligament, and adventitia tissue around both common carotid arteries (CCA) were carefully stripped with the microsurgery method for ischemia induced groups (Fig. 1). Before starting drug therapies, the rats in the ischemia groups were exposed to ischemia for 15 minutes by clamping the bilateral common carotid arteries with Sugita® clips. Then reperfusion was applied for 24 hours. After 24 hours, same dose of CMC was given for the same duration (2). In the third group (I/R+CRV), CRV was administered via gavage for 15 days starting one day after I/R with a dose of 50 mg/kg/day. Finally in the 4th group (CRV); CRV dissolved in CMC at the same dose was administered via gavage for 15 days.

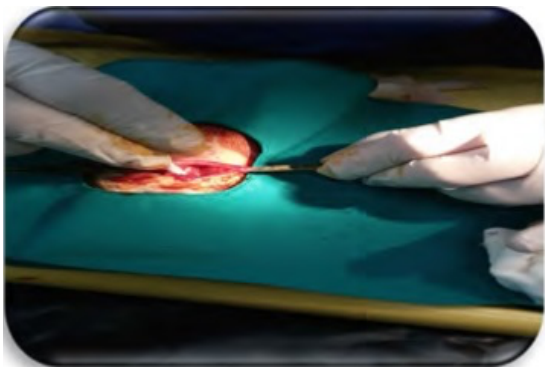

Fig. 1. Dissection of rat carotid arter

At the end of 15 days, brain tissue resection was performed under appropriate anesthesia (ketamine (60 mg/day) + xylazine (10 mg/kg)) and 4-6 cc of blood was collected from the left cardiac ventricle. After collection of blood, a craniectomy was performed to completely remove all components of the neuronal tissue, including the cerebrum, cerebellum, and brain stem. The collected tissue samples at the end of scarification were stored at -80°C until examination of TBARS levels and SOD, CAT, GSH-Pc, GSH and protein measurements. Tissue sampling and homogenate preparation were performed as stated in the literature (2). Cytokine levels in plasma were determined by the ELISA method using commercial kits in accordance with the procedures included in the kits. For TNF-a (cat#EK0526) and IL-1β (cat#EK0393), Boster® brand commercial kit was used and for IL-4 (lot#20161209) Relassay diagnostics® commercial kit was used. Results were read on a 450 nm microplate (CIOM Medical Co. Ltd, Changchun, China) reader.

2.1. Statistical Analysis

While evaluating the findings obtained in the study, SPSS (Statistical Package for Social Sciences) for Windows 11.5 program was used for statistical analysis. Study data were presented as mean±standard deviation and descriptive statistics were used. A Chi-square test was used to compare qualitative data. Kruskal Wallis test was used to compare the groups. Mann-Whitney U test was used for pairwise group comparisons for the parameters with a significant difference. A p-value of <0.05 was considered statistically significant.

3. Results

The values of TBARS, SOD, CAT, GSH, and GSH-Px levels taken from the brain tissue sample are given in Table 1. As a result of the evaluation, TBARS level, which is an indicator of oxidative damage, increased statistically significantly in I/R-induced rat brain tissue (IR group) compared to the control and all other groups. At the same time, it has been determined that CRV administration statistically significantly reduces the increase in TBARS caused by I/R and the level of TBARS in I/R+CRV group approached the values of the control group.

It was observed that no statistically significant changes are present between the group to which only CRV was administered and the control group. However, it was observed that I/R+CRV administration causes a statistically significant decrease in the levels of GSH, SOD, GSH-Px, and CAT, which are antioxidant defense system elements compare to the I/R+CMC group. However, it was observed that administration of CRV eliminated the changes in GSH, GSH-Px, CAT, and SOD levels due to I/R and a statistical difference was present when compared with I/R group. As shown in Table 2 and 3 of this experimental study in rats, the levels of TNF-α and IL-1β, which are proinflammatory parameters and important mediators of acute inflammation, in the blood and brain tissue supernatant were statistically significantly lower in I/R and I/R + CRV groups when compared with control and CRV groups. However, it has been determined that there was a significant difference between the I/R and I/R + CRV groups. In conclusion, it has been determined that the increase in both cytokine levels due to ischemia was partially eliminated by CRV as seen in figure 2, 3, 4, and 5. On the other hand, the level of IL-4, an anti-inflammatory cytokine, was significantly decreased in I/R group both in serum and brain supernatant, and this decrease was significantly improved with CRV treatment as shown in figure 6 and 7.

Table 1. The level of TBARS, GSH, CAT, SOD and GSH-Px in ischemia-induced (I/R) and CRV administered (with therapeutic purpose) rats (mean±SD n=10)

	TBARS nmol/gr	GSH µmol/gr	SOD U/mg	CAT µmol/gr	GSH-Px µmol/gr
Control	14.28±1.27[a]	255.8±28.1[a]	14.97±1.56[a]	0.014±0.0002[a]	231.4±21.1[a]
CRV	13.37±1.16[a]	280.1±32.5[a]	15.31±1.70[a]	0.013±0.0004[a]	225.9±19.2[a]
I/R	19.10±2.03[b]	163.9±24.6[b]	9.57±0.95[b]	0.006±0.0003[b]	152.1±14.2[b]
I/R+CRV	15.19±2.91[a]	215.7±28.9[c]	12.98±1.25[c]	0.010±0.0005[c]	198.7±18.7[c]

The letters a, b and c in the same column indicate the statistical difference (p≤0.05) between the groups.

Table 2. IL-1β, TNF- α and IL-4 levels in rat serum

Serum	IL-1β pg/ml	TNF- α pg/ml	IL-4 ng/L
Control	360.9±50.6[c]	81.5±10.4[c]	43.75±1.01[c]
I/R	693.2±25.2[a]	167.8±23.2[a]	27.43±2.97[a]
CRV	391.5±46.6[c]	80.3±7.48[c]	43.18±1.14[bc]
I/R+CRV	482.9±60.2[b]	109.0±13.7[b]	40.65±2.76[b]

The letters a, b and c in the same column indicate the statistical difference (p≤0.05) between the groups

Table 3. IL-1β, TNF-α and IL-4 levels in rat brain supernatant

Serum	IL-1β pg/ml	TNF- α pg/ml	IL-4 ng/L
Control	44.4±5.08[d]	45.33±9.92[c]	19.98±1.85[c]
I/R	94.4±9.77[a]	102.4±7.35[a]	11.17±0.74[a]
CRV	35.0±7.73[c]	50.75±11.6[c]	21.86±2.56[c]
I/R+ CRV	63.9±4.56[b]	86.16±9.27[b]	15.61±1.70[b]

The letters a, b and c in the same column indicate the statistical difference (p≤0.05) between the groups

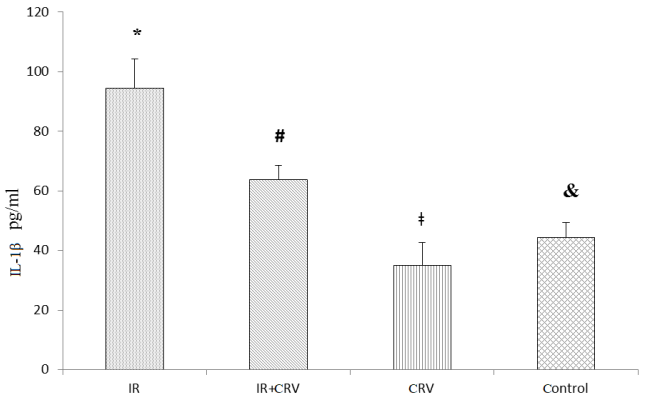

Fig. 2. Rat serum IL-1β levels (pg/ml±SD); * # ǂ and & show the difference between groups (p<0.05)

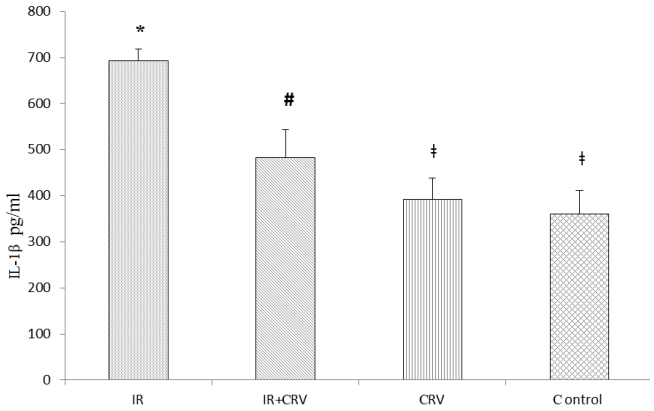

Fig. 3. Rat brain IL-1β levels (pg/ml ±SD); * # ǂ and & show the difference between groups (p<0.05)

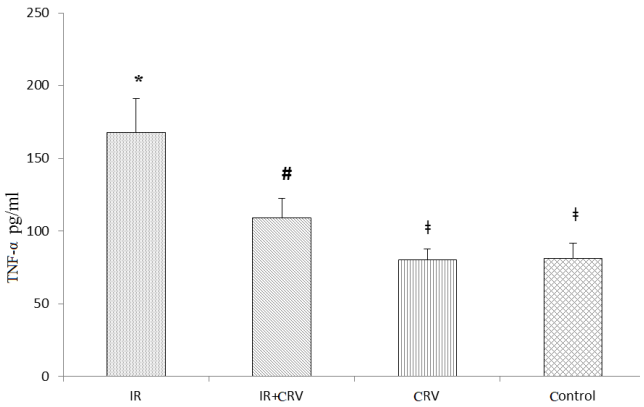

Fig. 4. Rat brain TNF-α levels (pg/ml ±SD); * # ǂ and & show the difference between groups (p<0.05)

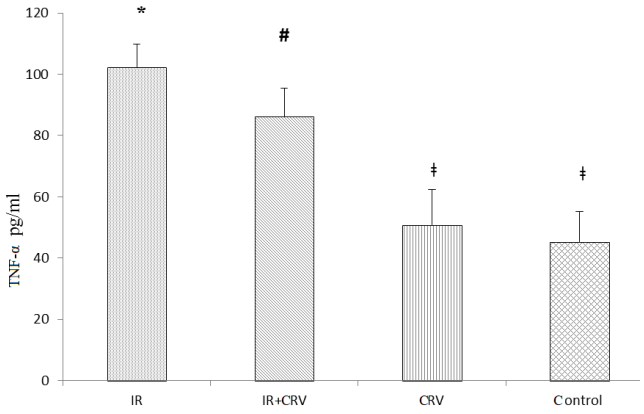

Fig. 5. Rat serum TNF-α levels (pg/ml ±SD); * # ǂ and & show the difference between groups (p<0.05)

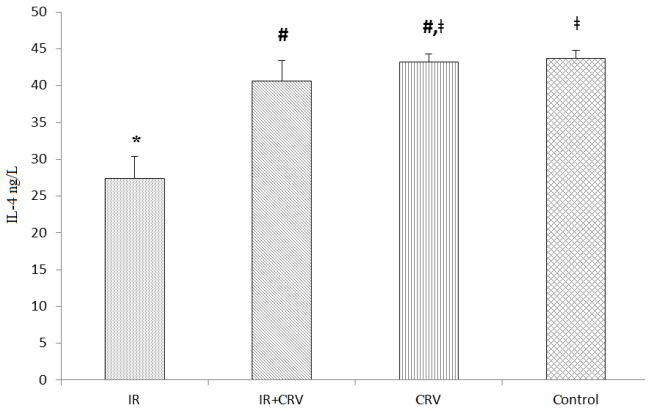

Fig. 6. Rat brain IL-4 levels (ng/L ±SD); * # ǂ and & show the difference between groups (p<0.05)

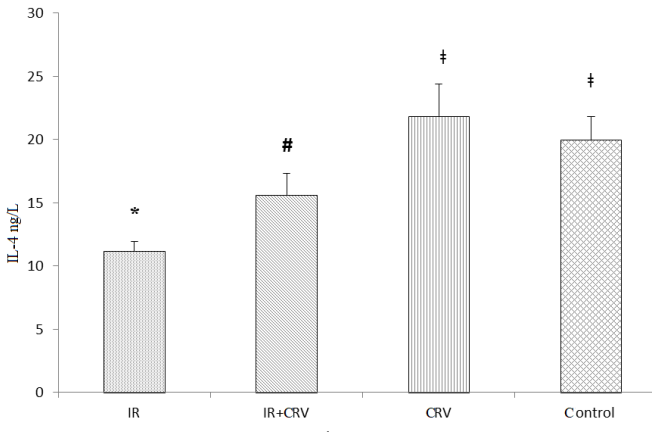

Fig. 7. Rat serum IL-4 levels (ng/L ±SD); * # ǂ and & show the difference between groups ($p<0.05$)

4. Discussion

I/R injury is one of the types of injuries that occurs in cerebral stroke. The most important mechanism for I/R injury is an accumulation of oxygen free radicals (OFR) and subsequent cell damage (2, 6). OFR have a feature that can disrupt the integrity of cell membrane by forming lipid peroxidation. The injury of the parenchyma might increase because of the increased release of OFR from leukocytes that migrate to the area during re-oxygenation. Leukocytes have an affinity to accumulate at reperfused tissues during I/R. Cell damage further increases with the presence and involvement of cytokines which were released afterwards. In order to prevent this, the main goal is to prevent the destructive effect of OFR in cerebral I/R injury (7, 8).

Experimental and clinical studies are present about reducing OFR in I/R injury (9, 10, 11). Antioxidants (vitamin E, ascorbic acid, glutathione etc.) and various enzymes (catalase, superoxide dismutase, glutathione peroxidase etc.) were tested in these studies (10, 11, 12). The results of the drugs, which stand out with their antioxidant properties, are promising especially in experimental studies. A drug with high therapeutic potential in this regard is CRV. Although there are various studies in the literature about the effect of CRV on the neural injury model (3, 4), we detect that this issue is not sufficiently analyzed. In our study, we aimed to demonstrate the effect of CRV on cerebral I/R injury, especially with biochemical analysis. Suo et al., showed in their experimental study that, CRV decreases I/R injury in liver tissue by regulating thePI3K-Akt pathway. As a result of that study, it has been reported that CRV significantly reduces ALT and AST serum levels, histological changes, and apoptosis of liver cells in rats after liver I/R. In addition to that, it has been stated that it protects against tissue damage due to I/R, significantly reduces SOD activity and increase MDA content. Also, CRV displays an antioxidant activity by restoring the CAT activity and GSH content (13). There are also other studies that demonstrate the anti-oxidative feature of CRV at the molecular level. There are several studies that showed that CRV decreases the expression of Bax and increases the expression of Bcl-2 (11, 14, 15). When our results were compared with the literature, it can be seen that similar results were obtained in terms of antioxidant parameters and CRV causes an improvement of ischemic parameters in I/R.

Wanget al., showed that CRV prevents ethanol-induced hippocampal neuron damage. They reported that the level of SOD, GSH, GSH-PX, and CAT were significantly changed with 50 and 100 mg/kg CRV treatment in rats exposed to ethanol. In addition to that, it was seen in the same study that the level of MDA, which is an important indicator of lipid peroxidation, was repressed (3). Our results about the activities of SOD, GSH, GSH-PX, and CAT enzymes between I/R and I/R + CRV groups were similar to the study of Wanget al.

Yuet al., also investigated the effect of CRV after I/R like our study. They reported that 50mg/kg CRV treatment given after 75 minutes of ischemia and 24 hours of reperfusion injury significantly reduced the infarct volume and improved neurological deficits. They showed that the neuroprotective characteristic of CRV is also dose-dependent (16). In our study, a similar experiment was conducted on I/R induced rats. It has been observed that CRV provides a decrease in mediators that can increase ischemia through oxidative damage, and an increase in protective enzymes. Both studies are similar in this manner, and the efficacy of CRV, which is a protective agent against ischemic injury, was reinforced both in our study and in this study.

Another study on the neuroprotective properties of CRV was conducted with a trauma model. It has been shown in mice given CRV after traumatic brain injury that brain injury was significantly recovered, and a marked improvement was observed (17). Additionally, it has been shown in the literature that the addition of TRPC1 elimination to CRV treatment causes a significant improvement (18). Jiang et al., also emphasized that CRV has a neuroprotective and therapeutic feature in traumatic spinal cord injury (14). Guan at al. reported that CRV protects hippocampal neurons against I/R in rats by inhibiting ferroptosis by increasing the glutathione peroxidase 4 expression (19). Also, CRV reduces neuronal damage after Cerebral I/R via attenuation of transient receptor potential melastatin (20). All these studies in the literature support the data in our study and show that CRV may be a potential treatment for cerebral I/R injury.

Our study showed that CRV significantly prevents I/R damage due to its antioxidant and anti-inflammatory properties. CRV is a promising molecule not only for I/R but also for many neurological diseases, and this effect should be demonstrated with similar studies.

Conflict of interest

The author(s) confirm that this article content has no conflicts of interest.

Acknowledgments

None to declare.

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