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Effect of *Curcuma longa* on GPER-1 and Oxidative/Nitrosative Stress Biomarkers in Cardiac Ischemia-reperfusion Injury

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ABSTRACT

Background: The death rate caused by cardiovascular diseases is increasing all over the world. To investigate the cardioprotective effects of *Curcuma longa* on cardiac ischemia-reperfusion (I/R) injury, which was further examined through G protein-coupled estrogen receptor 1 (GPER-1) and oxidative/nitrosative stress biomarkers.

Methods: Twenty-four male Wistar-albino rats were divided into three groups (I/R group; n=8, sham group; n=8, treatment (*Curcuma longa*) group; n=8). 1 ml *Curcuma longa* 50 mg/kg was administered intraperitoneally in the treatment group. While catalase, superoxide dismutase and malondialdehyde values were measured by spectrophotometric method, the measurement of GPER-1 was performed by ELISA method. In the histopathological examination, the groups were evaluated in terms of hemorrhage, myocardial edema, myocytolysis and polymorphonuclear leukocyte (PMNL) infiltration.

Result: Statistically significant differences were observed between the treatment and I/R groups in terms of oxidative stress (malondialdehyde) level and antioxidant enzyme activities (catalase, superoxide dismutase) ($p<0.05$). The GPER-1 level was higher in the treatment group compared to the I/R group ($p<0.05$). Histopathologically, PMNL infiltration, hemorrhage, edema and myocytolysis were observed in the I/R and sham groups, whereas only hemorrhage was detected in the treatment group. This study showed that treatment with *Curcuma longa* inhibited lipid peroxidation by restoring existing antioxidant enzymes against cardiac ischemia-reperfusion injuries.

Key words: Cardiac ischemia-reperfusion injury, *Curcuma longa*, GPER-1, Oxidative/nitrosative stress.

INTRODUCTION

Cardiovascular diseases (CVD) are seen as the primary cause of death in humans (Oleg *et al*., 2023). Ischemic cardiac disease is the most common type of CVD. Coronary artery disease, which poses a risk in individuals over the age of 40, is seen in 50% of men and 32% of women (Roth *et al*., 2020). An inadequate supply of oxygen and nutrients to the heart due to ischemia leads to biochemical and metabolic changes in the myocardium. Oxygen deficiency or decreased levels terminate oxidative phosphorylation and lead to mitochondrial membrane depolarization, depletion of adenosine triphosphate (ATP) and inhibition of myocardial contraction (Piper *et al*., 2003). The provision of cellular metabolism by anaerobic glycolysis due to lack of oxygen leads to lactate accumulation, which reduces the intracellular pH (Demirhan and Kurutas, 2021). After ischemia, local inflammation that causes secondary damage with blood perfusion and production of reactive oxygen species (ROS) increases. Numerous cell-protective enzymes act against the potential damage of ROS and radical damage is tried to be limited with antioxidants. Cellular antioxidant enzymes, antioxidant substances and free radicals are in balance in the body (Barry *et al*., 2009). Antioxidants work rapidly and specifically (enzymatically) to reduce oxygen intermediate metabolites where oxygen is metabolized inside the cell. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) are intracellular enzymes that play an active role in antioxidant defense (Demirhan and Kurutas, 2023).

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There is a relationship between CVD and estrogen. Estrogen acts on estrogen receptors alpha and beta and a new G protein-coupled estrogen receptor 1 (GPER-1) has recently been identified. GPER-1 is also known as G protein-coupled receptor 30 (GPR30) (Oner, 2016). This protein is involved in non-genomic signaling commonly observed after stimulation of cells and tissues with estrogen and causes a decrease in cAMP production. It is named G protein since it binds to the Guanine nucleotide. It has been reported that GPER-1 levels are associated with patients' anxiety levels and that serum GPER-1 level is a predictor of the presence of anxiety regardless of gender. Moreover, it is suggested that GPER-1 has a cardioprotective effect against ischemia-

reperfusion (I/R) damage in the heart (Oner, 2016; Slater, 1984).

Plants are generally rich sources of exogenous antioxidants, containing phenolic compounds. For this reason, plants have been commonly used in many diseases in alternative medicine from the past to the present (Gulcin, 2012). *Curcuma longa* shows antioxidant activity since it contains tetrahydro curcumin, a phenolic compound, in its structure. It has even been reported that *Curcuma longa* is more effective than vitamin E in preventing lipid peroxidation (Demirhan and Kurutas, 2021). *Curcuma longa* exhibits multiple pharmacological activities, including antioxidant, anticarcinogenic and anti-inflammatory, in different models of rodents (Zhao *et al*., 2010).

However, some studies investigating the cardioprotective effects of *Curcuma longa* are recorded in the literature and no studies have been conducted on GPER-1 in heart I/R injury. GPER-1 levels were investigated for the first time in this present study. This study aimed to examine the possible effects of *Curcuma longa* against I/R-related heart damage using histological and biochemical parameters of oxidative/nitrosative injury.

MATERIALS AND METHODS

This research was carried out in the medicinal biochemistry laboratory of Kahramanmaras Sutcu Imam University between February and August 2022.

Animals

The study was conducted with the approval of the Experimental Animals Ethics Committee of Kahramanmaras Sutcu Imam University dated 01.06.2020. Wistar rats (N=24) weighing 250-300 grams were acquired from Kahramanmaras Sutcu Imam University Laboratory Animal Breeding and Experimental Research Center.

The procedure of the guide care and use for the rights of international experimental animals was followed in this study. This study was conducted by the Department of Medical Biochemistry, Experimental Research Laboratory of KSÜ Faculty of Medicine with the approval of Kahramanmaraþ Sütçü İmam University Faculty of Medicine Ethics Committee, protocol no. 031.

Study groups

The research was started by dividing the rats into three groups.

Positive control group (n = 8): Only IR was applied to this group. This group After the surgical procedure, 10 minutes of ischemia and 10 minutes reperfusion were applied to the heart.

Negative control group (n = 8): Two days before the procedure, 1 ml of intraperitoneal saline (0.9% NaCl) was started in this group, with 10 minutes of ischemia and 10 minutes reperfusion applied. Following the reperfusion, 1 mL of saline (single dose) was performed again.

Treatment group (n = 8): 1 ml *Curcuma longa* 50 mg/kg was administered intraperitoneally two days before the intervention and surgical procedure and 10 minutes of ischemia and 10 minutes reperfusion were applied in the heart after the surgical procedure. Following the reperfusion, 1 ml (single dose) of *Curcuma longa* was performed again.

Surgical method

The rib cages of 250-300 g adult Wistar-Albino rats anesthetized with ketamine HCl (50 mg/kg) were surgically opened. The pericardium around the heart was stripped, the aorta and pulmonary vessels were cut and the heart was rapidly removed. Hearts placed in an ice-cold Petri dish containing Krebs-Henseleit were rapidly cannulated through the aorta and connected to the Langendorff system. It was perfused with Krebs-Henseleit (KH) solution at 37°C (Altunkaynak *et al*., 2009; Minasian *et al*., 2013). At the end of the experiment, heart tissues were divided into two parts for biochemical and histopathological analysis.

Preparation of the heart tissue homogenates

The tissues were 1/5 (weight/volume) in cold 1.15% KCl (potassium chloride) at 13500xrpm with a homogenizer (ultra turrax) on ice. Then the homogenates were centrifuged at 14000xrpm in a cooled centrifuge at +4°C for 30 minutes. GPER-1 measurement was made in the obtained supernatants. The antioxidant enzyme activities (CAT, SOD, MDA, NO, 3-NT and GPER-1 levels in supernatant samples were measured after centrifugation at 14.000 rpm.

Biochemical analysis

The SOD activity in tissues was determined using the Fridovich method and reported as U/mg protein (Fridovich, 1974). Additionally, the CAT activity in tissues was determined using the Beutler method and reported as U/mg protein (Beutler, 1984). This method expressed the decrease of hydrogen peroxide concentration at 230 nm. GSH-PX activity was determined in the supernatant using the Beutler method (14)-1M Tris-HCl, 5 mM EDTA Buffer, 0.1 M GSH (glutathione), 10 U/ml GR (glutathione reductase), 2 mM NADPH (nicotinamide adenine dinucleotide phosphate), 7 mM t-butyl hydroperoxide reagents were added to the tubes at specified ratios and the tubes were incubated at 37°C for 10 minutes (Beutler, 1984).

GPER-1 levels in tissue samples were determined using enzyme-linked immunosorbent assay (ELISA) kits (MyBioSource, catalog number: MBS095620, USA).

The levels of protein in tissue samples were measured by the Folin Cioacalteu method. It is based on the spectrophotometric method of absorbance measurement at 750 nm of the color reaction of the tyrosine and tryptophan residues contained in the proteins in the extract with phosphotungstic-phosphomolybdic acid (Lowry *et al*., 1951).

NO level was measured spectrophotometrically using the Cortas and Wakid method (Cortas and Wakid, 1990). Cadmium granules, glycine-NaOH buffer, sulfanilamide, zinc

sulfate ($ZnSO_4$), copper sulfate ($CuSO_4$), sodium hydroxide (NaOH) and the standards were used. Nitrotyrosine (3-NT) level was measured with the ELISA technique using a commercial kit (MyBioSource, catalog number: MBS023877, USA).

Histopathological evaluation

Cardiac muscle tissues were fixed in 10% formalin solution at room temperature for three days and underwent routine histological procedures. Sections of 4-5 µm thickness taken from the paraffin blocks obtained were stained with Hematoxylin-eosin and Masson-trichrome dyes and viewed under a Leica DM-4000B microscope and evaluated. Groups include myocardial edema, myositis, hemorrhage and polymorphonuclear leukocyte infiltration. Pathological scoring; 0-no, 1-mild, 2-moderate and 3-severe.

Statistical analysis

The SPSS (Statistical Package for Social Sciences) 15.0 program was used for statistical analysis. The results were given as mean ± standard deviation. In the evaluation of biochemical data, the non-parametric Kruskal-Wallis test was used to determine the differences between the groups and the Mann-Whitney U test was used to evaluate the difference between the two groups at P<0.05.

RESULTS AND DISCUSSION

Biochemical results

The present study showed that there were significant differences in SOD, CAT and MDA levels between the positive control, negative control and treatment groups (P: 0.001) (Fig 1, 2 and 3). Decreased antioxidant activities (SOD and CAT) were seen in the positive control group compared to the other groups, however, the MDA level increased in this group (P: 0.001) (Fig 1, 2 and 3).

According to our study, there was no difference between the negative control and treatment groups in terms of GPER-1 levels (p:0.518). When the GPER-1 levels were compared between the groups, there were significant differences between the positive control group and the negative control and treatment groups (p: 0.011) (Fig 4). GSH-PX activity decreased significantly in the positive control group compared to the negative control and treatment groups (p:0,001). In our study, there was no statistical difference between the negative control and treatment groups in terms of GSH-PX activity (p:0.734) (Fig 5).

There were significant differences between the positive control group and the negative control and treatment groups in terms of NO (p: 0.001) and 3-NT levels (p:0.023). No statistical differences were between the negative control and treatment groups in terms of NO levels (p:0.244) and 3-NT levels (p: 0.023) (Fig 6 and 7).

Histopathological results

Myocardial edema, myocytolysis, hemorrhage and polymorphonuclear leukocytes (PMNL) infiltration were shown in the positive control and negative control groups. A statistically significant difference was found only in PMNL infiltration between the groups (p <0.05). There were significant differences between the treatment group and positive control and negative control groups in terms of hemorrhage (p=0.516), myocardial edema (p=0.025), myocytolysis (p=0.039) and PMNL infiltration (p=0.008). The histopathological grade of the groups is shown in Table 1 and Fig 8A-D.

In this study, *Curcuma longa* was evaluated biochemically and histopathologically and was found to be protective against cardiac I/R injuries. GPER-1 is a newly identified estrogen receptor analog. GPER-1 is stimulated in the adenylate cyclase/cAMP pathway (Korthuis and Granger, 1993). In our study, while the GPER-1 level decreased significantly in I/R injury, it increased considerably in the treatment group. The decrease in GPER-1 due to I/R injury may be due to the extension in estrogen level. We think that estrogen levels decrease when GPER-1 is suppressed. Studies that are compatible

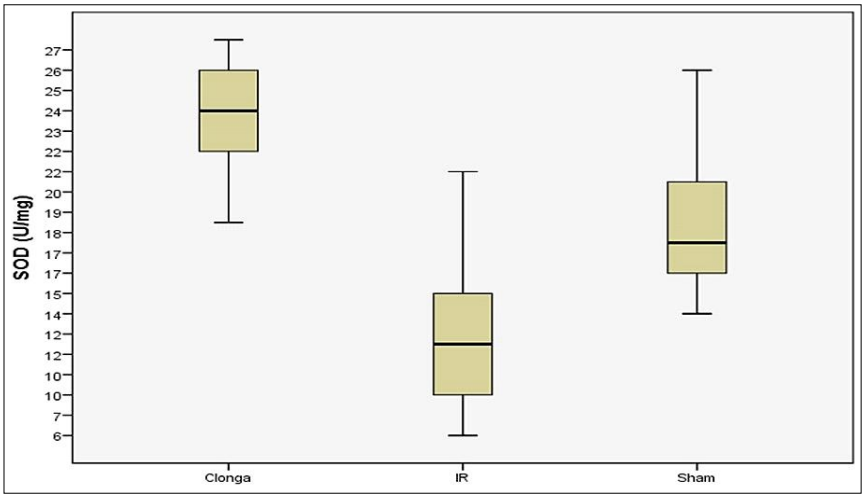


Fig 1: SOD levels of groups.

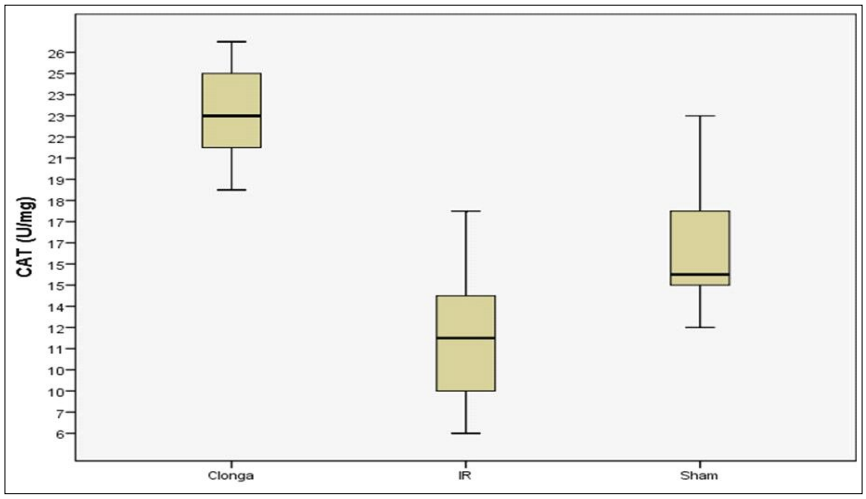


Fig 2: CAT levels of groups.

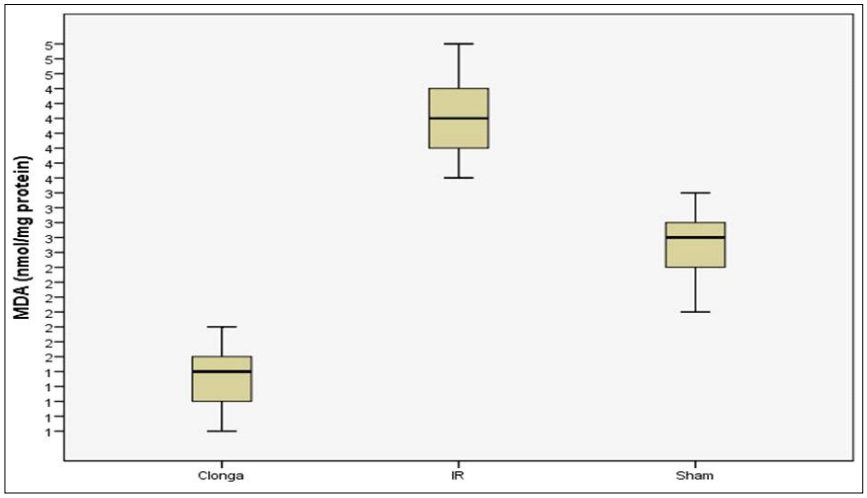


Fig 3: MDA levels of the groups.

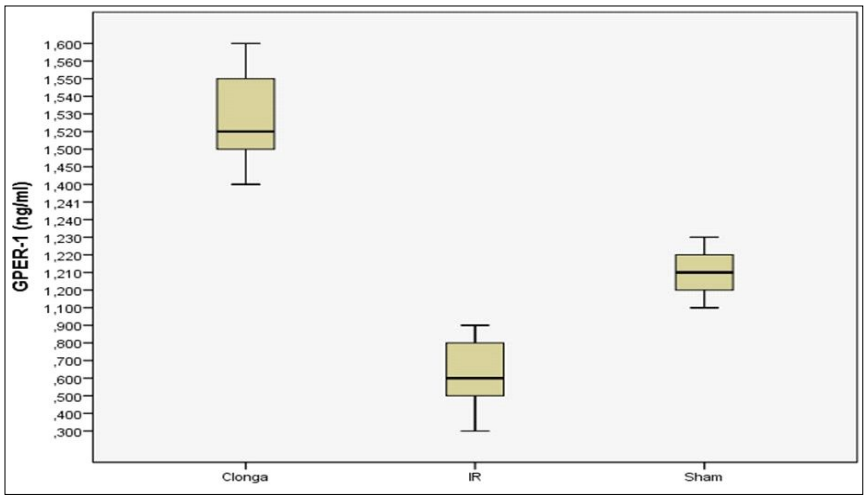


Fig 4: GPER-1 (ng/ml) levels of the study groups.

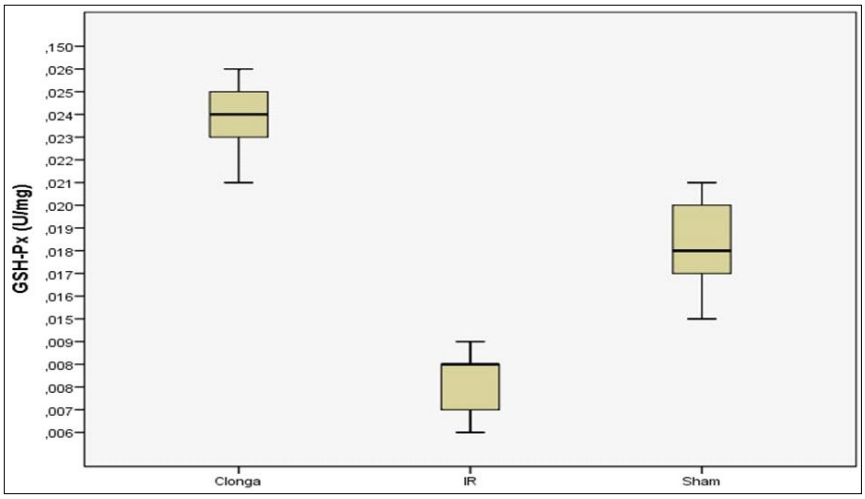


Fig 5: GSH-Px (U/mg) levels of the study groups.

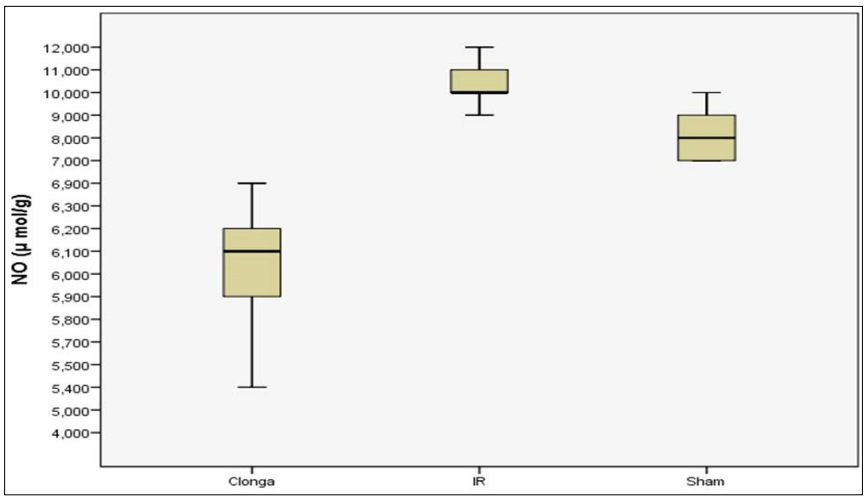


Fig 6: NO (µ mol/g) levels of the study groups.

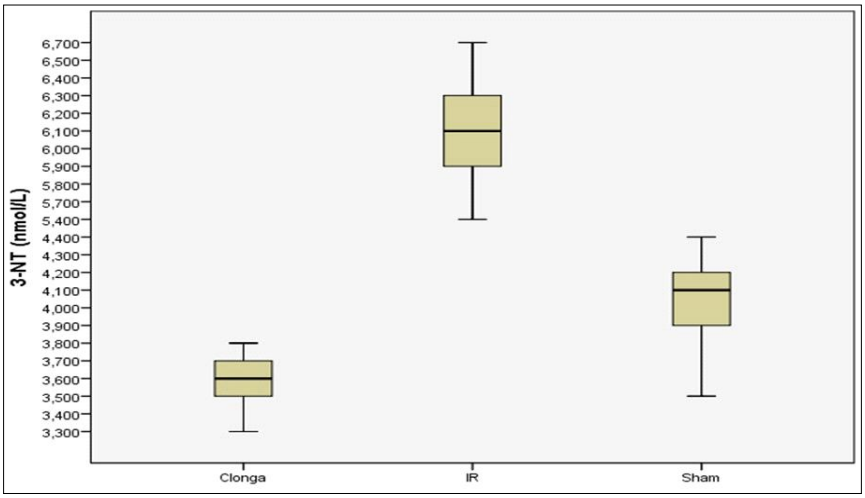
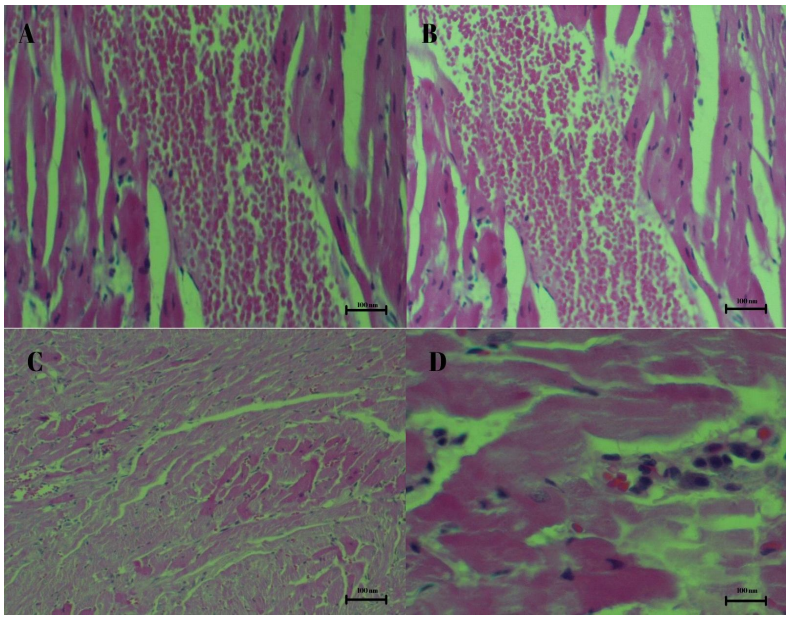


Fig 7: 3-NT (nmol/L) levels of the study groups.



A: Normal heart muscle. B: *Curcuma longa ethanol extract* treatment group (Myocardial edema, myocytolysis and Inflammation decreased). C: Sham group (Myocardial Edema, Degeneration in muscle bundles). D: I / R group (Polymorphonuclear Leukocyte (PMNL) Infiltration) (Scale bar: 0.019 µm). A: Normal heart muscle. B: *Curcuma longa ethanol extract* treatment group (Myocardial edema, myocytolysis and Inflammation decreased). C: Sham group (Myocardial Edema, Degeneration in muscle bundles). D: I / R group (Polymorphonuclear Leukocyte (PMNL) Infiltration) (Scale bar: 0.019 µm).

Fig 8: The histopathological results on the effect of *Curcuma longa ethanol extract* in experimental heart ischemia.

Table 1: Histopathological grade of the groups.

	Myocardial edema	Myocytolysis	Hemorrhage	PMNL infiltration
I/R	1	1	2	5
Sham	1	1	1	2
Treatment	0	0	1	0

with our research results are registered in the literature (Herson *et al.*, 2013; Wang *et al.*, 2019; Li *et al.*, 2022). Therefore, increased GPER-1 level in the treatment group suggests that *Curcuma longa* may have estrogenic activity.

With the re-initiation of blood flow in the ischemic tissue, ROS released into the environment by PMNL, which migrates and settles in the tissue, has an increased destructive effect on the tissue. Various studies have revealed that ROS performs an essential role in I/R injury by initiating lipid peroxidation (Avnioglu *et al.*, 2021). At the same time, ROS increases tissue damage in the body by reacting with biomolecules. In this study, CAT, SOD, GSH-PX enzyme activities and the lipid peroxidation biomarker MDA were studied to determine the degree of oxidant damage. The literature has reported that SOD activity decreases in renal injury induced by intestinal, kidney and liver I/R and aortic (Gulmen *et al.*, 2011). This study showed that was a significant decrease only in the I/R group in terms of SOD activity. This suggests that I/R injury increases superoxide anion radicals in the cell and inhibits the SOD enzyme. We also observed that SOD enzyme activity increased with *Curcuma longa* against I/R injury in the treatment group. We think that *Curcuma longa* increases SOD activity by decreasing the level of superoxide anion radicals in the cell.

The CAT enzyme converts hydrogen peroxide (H_2O_2) formed by SOD into water and oxygen in peroxisomes. CAT is a metalloprotein that is produced naturally in the body and acts in combination with SOD *in vivo*. Our study demonstrated that the CAT enzyme activity decreased crucially only in the I/R group. It is thought that H_2O_2, which is highly produced in the I/R cell, reduces CAT activity. Therefore, the combined effect of SOD and CAT draws attention. CAT activity is thought to increase in the treatment group.

This study showed that MDA levels were seriously higher in the I/R group compared to the sham and treatment groups. It has been shown that MDA levels, an indicator of lipid peroxidation, significantly increased in rats that underwent cardiac I/R and that cardiac I/R resulted in cardiac necrosis in rats due to increased MDA levels (Demirhan *et al.,* 2018). The results of literature studies showed that pretreatment with curcumin could decrease MDA concentration (Yuan *et al.*, 2015; Ghoneim *et al.*, 2002; Shen *et al.,* 2007).

We can attribute the decreased GSH-PX enzyme activity found in our study to the decrease in the amount of selenium (Se) element in its content, which may occur due to I/R. It is thought that low Se levels may have affected GSH-PX enzyme activity before macromolecules. Some studies also support this view (Bozkurt *et al.,* 2012).

NO and 3-NT are the biomarkers of nitrosative stress. We thought that the increase in the NO level might be related to the increase in the formation of iNOS during the reperfusion change in the heart during I/R and therefore, the histopathological injury may be due to peroxynitrite that is formed when NO combines with O_2^- formed during reperfusion. Furthermore, in our study, the increase in 3-NT levels in cardiac I/R indicates that peroxynitrite is formed in cardiac I/R injury. However, this study showed that the level of NO and 3-NT decreased significantly in the treatment group. These can be attributed to the complete blockage of ROS (like O_2^-) by *Curcuma longa* in human neutrophils, thus preventing the release of NO and 3-NT from being used by these radicals.

PMNLs have an essential role in cardiac injury after I/R, the reduction of PMNL protects from this injury (Gulmen *et al.*, 2011). In our study, PMNL infiltration, edema, hemorrhage and myocytolysis were observed histopathologically in the I/R and sham groups who underwent cardiac I/R, suggesting that ROS is formed with the disruption of intracellular Ca_+^2 balance and activation of the inflammatory cascade. On the other hand, the absence of injury other than hemorrhage in the histopathological examination of the treatment group indicated that *Curcuma longa* is an essential agent in preventing cardiac I/R injury (Gulmen *et al.*, 2011; Suzuki *et al.*, 1991). The results of this study are consistent with previous studies conducted with different antioxidants (Junqueira *et al.*, 2012).

CONCLUSION

In summary, we found that Curcuma longa is effective on oxidative stress damage. In addition, we think that the level of GPER-1 may be a determining marker in heart damage but needs to be proven by large-scale clinical studies.

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Conflict of interest

All authors declared that there is no conflict of interest.

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