

Investigation of Oxidative/Antioxidant Effects of Turmeric (*Curcuma longa L.*) Supplementation on Heart Tissue in Adult Rats

Erişkin Ratlarda Zerdaçal (*Curcuma longa L.*) Takviyesinin Kalp Dokusundaki Oksidan/Antioksidan Etkilerinin İncelenmesi

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Abstract: In present study, the effects of curcumin supplementation on oxidative stress and antioxidant defense system in heart tissue in adult rats were investigated. Sixteen rats (8-weeks-old) were selected and divided into two groups. The first group is the experimental group and these rats (n=8) were gavaged with curcumin, dissolved in corn oil, at a dose of 300 mg/kg per day for 28 days. The second group is the control group, rats in this group were given curcumin and equal amount of corn oil to eliminate the porter effect. Euthanasia was performed and total antioxidant capacity (TAS), total oxidant capacity (TOS), malondialdehyde (MDA) and glutathione (GSH) levels were analyzed from heart tissues. Curcumin supplementation resulted in significantly increased GSH levels (P<0,05). However, curcumin supplementation decreased MDA levels although it was not statistically significant (P>0,05). The total antioxidant capacity (TAS) and total oxidant capacity (TOS) ratios were found to be statistically significant. In the other group supplemented with curcumin, MDA levels tended to decrease compared to the control group, and there was no statistically significant difference between the two groups. Curcumin supplementation protects the heart tissue against oxidative damage and strengthens the antioxidant defense system in adult rats.

Keywords: Curcumin, Antioxidant, Oxidative Stress, Heart.

Öz: Bu çalışmada, erişkin ratlarda zerdaçal (kurkumin) takviyesinin kalp dokusundaki oksidatif stres ve antioksidan savunma sistemi üzerinde meydana getirdiği etkileri incelendi. Çalışmada 8 haftalık 16 adet rat kullanılmış ve bu ratlar iki gruba ayrılmıştır. İlk grup olan deney grubundaki ratlara (n=8) 28 gün süresince günde 300 mg/kg dozunda kurkumin (C1386; Sigma Chemical, St. Louis, MO) mısır yağı içinde çözündürülerek ağızdan gavaj yöntemi ile verildi. İkinci grup olan kontrol grubundaki ratlara ise portör etkisini yok etmek için kurkumin ile eşit miktarda mısır yağı verildi. Ötenazi gerçekleştirildikten sonra kalp dokuları alınarak total antioksidan kapasiteleri (TAS), total oksidan kapasiteleri (TOS), malondialdehit (MDA) ve glutatyon (GSH) seviyeleri analiz edildi. Kurkumin takviyesi GSH seviyelerini önemli ölçüde artırmıştır (P<0,05). Bununla birlikte, kurkumin takviyesi istatistiksel olarak anlamlı olamamasına rağmen MDA seviyelerini azaltmıştır (P>0,05). Total antioksidan kapasitesi (TAS) ve total oksidan kapasitesi (TOS) oranları istatistiksel olarak anlamlı bulundu. Kurkumin takviyeli diğer grupta MDA seviyeleri kontrol grubuna oranla azalma eğilimi göstermiş olup iki grup arasında anlamlı istatistiksel bir fark bulunmamıştır. Kurkumin takviyesi erişkin ratlarda kalp dokusunu oksidatif hasara karşı korumakta ve antioksidan savunma sistemini güçlendirmektedir.

Anahtar Kelimeler: Kurkumin, Antioksidan, Oksidatif Stres, Kalp.

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Introduction

Curcumin *longa* is a plant from the Zingiberaceae family, mostly found in India and China. Curcumin, obtained from the tubers of the plant, has been used as a spice, medicinal drug and cosmetic product for many years. Curcumin (diferuloylmethane) is a yellow pigmented product of *Curcumin* *longa* (turmeric). Turmeric, which is used as a colorant in foods, contains tetrahydrocurcumin, a heat-resistant, odorless, antioxidant compound. Curcumin has a wide spectrum of effects including anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antidiabetic, antibacterial, antiviral and neuroprotective effects (Choudhuri et al., 2002; Naik et al., 2011).

The antioxidant property of curcumin prevents damage caused by exposure to harmful factors such as alcohol, drugs, radiation and heavy metals. Because it is a good free radical scavenger and hydrogen donor. It is especially bonded to metals such as iron and copper and acts as an iron clamp. Curcumin is not very toxic and has limited bioavailability. Curcumin is a strong hydroxyl radical scavenger and also scavenges superoxide radicals. Protects DNA (Deoxyribo Nucleic Acid) from oxidative damage due to its ability to capture free radicals (Reddy and Lokesh, 1994; Sreejayan, 1997).

Due to these properties, the protective effects of curcumin on the cardiovascular system have recently attracted attention (Dkhar and Sharma, 2010; Duan et al., 2012; Miriyala, 2007; Naik et al., 2011). It has been reported in the literature that curcumin protects smooth and endothelial muscle cells from damage, prevents cardiac toxicity and damage, protects the heart against ischemic damage, and accelerates cardiac and vascular regeneration (Duan et al., 2012; Morimoto et al., 2008; Nirmala et al., 1999; Srivastava and Mehta, 2009; Venkatesan, 1998; Yeh et al., 2005). Although it is known that curcumin supplementation improves oxidative damage, there are not many studies examining its effects

on oxidative damage and antioxidant defense in heart tissue. Our aim in this study was to investigate the oxidant/antioxidant effects of curcumin supplementation on the heart tissue of adult rats and to contribute to the literature on this subject.

Material and Method

Approval was obtained from Burdur Mehmet Akif Ersoy University Experimental Animals Local Ethics Committee and the study was carried out in the same center. In the study, 16 adult rats (no gender priority) of about 8 weeks old and weighing 300-450 g, obtained from Burdur Mehmet Akif Ersoy University Experimental Animal Production and Experimental Research Laboratory, were used. During the study, the rats were kept in rooms with a 12-hour dark/light cycle at 21 ± 2 °C room temperature, 50% humidity.

Experimental animals were fed *ad libitum* with standard feed and tap water with 4 animals in each cage. Rats were randomly selected and divided into 2 experimental groups (n=8 in each group).

1. Control Group (K1): The group fed only corn oil throughout the study.
2. Experimental Group (D1): The group fed with curcumin at a dose of 300 mg/kg/day in the study.

Curcumin supplementation: In accordance with the literature, the dose amount was calculated by making a preliminary study. The rats in group D1 (n=8) were given 300 mg/kg/day curcumin (C1386; Sigma Chemical, St. Louis, MO) dissolved in corn oil and injected by oral gavage for twelve days. The rats in the K1 group (n=8) were given equal amount of curcumin and corn oil to eliminate the carrier effect. Rats were weighed daily for twelve days before curcumin or carrier supplementation. Taking tissue samples: One day after curcumin or carrier feeding on the last day, blood samples were taken from the hearts of rats

under 50 mg/kg ketamine + 10 mg/kg xylazine anesthesia. After the samples were taken, euthanasia was performed by cervical dislocation method. Heart tissues were rapidly removed after euthanasia. Tissues were washed with cold saline and placed in liquid nitrogen. Tissue samples were stored at -80 °C until analysis.

Biochemical analysis: To perform the analysis, the tissues were thawed by removing them from the – 80°C freezer. The dissected tissue samples were homogenized in ice by adding 10 times their weight in phosphate buffer (50 mmol/L, pH 7.4). After the homogenized sample was taken in sufficient amount for TAS, TOS, MDA, PC and GSH studies, the remaining homogenate was vortexed with an equal volume of 3/5 prepared chloroform/ethanol mixture and centrifuged at 3200 rpm +4 °C for 30 minutes. Protein determination was made from the upper ethanol phase. TAS and TOS were measured with commercially available kits and results are shown as mmol Trolox equivalent/L. MDA measurement was performed using a commercial kit (Bioxytech MDA-586 Assay Kit, Oxis Research, Poland). GSH levels were determined by the enzymatic colorimetric method using commercial kits (Cat. #703002, Cayman Chemical, Ann Arbor, MI). Results are shown as $\mu\text{mol}/\text{mg}$ protein. Tissue protein amount was determined according to the method of Lowry et al. (Lowry et al., 1951).

Statistical Analyses

Obtained results are given as mean \pm standard deviation. One of the nonparametric tests, Kruskal-Wallis analysis of variance test was applied. Comparisons were made using the Mann Whitney-U test for the parameters that were statistically different. Calculations were made using the Windows compatible SPSS 15.0 statistical program.

Results

The MDA levels of the heart tissue of rats fed with curcumin supplementation are shown in Figure 1. MDA levels decreased in the curcumin supplemented group compared to the control group, and there was no statistically significant difference between the two groups (1.97 ± 0.24 and 1.81 ± 0.48 in the K1 and D1 groups, respectively) ($P > 0.05$).

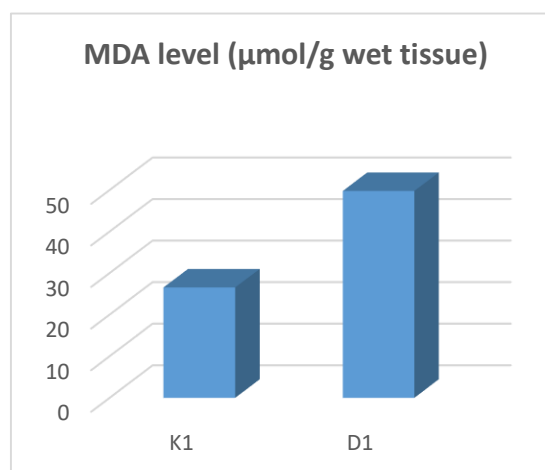


Figure 1. Effect of curcumin on MDA levels in heart tissue. K₁: Control, D₁: Curcumin

GSH levels of heart tissue of rats fed with curcumin supplement are given in Figure 2. GSH levels in the curcumin supplemented group were statistically significantly higher than the control group (28.44 ± 6.97 and 59.76 ± 39.88 in K1 and D1 groups, respectively) ($P < 0.05$).

Total antioxidant capacity (TAS) values between K1 and D1 groups showed that the experimental group showed a statistically significant increase compared to the control group (1.44 ± 6.97 and 1.56 ± 39.88 in K1 and D1 groups, respectively) ($p < 0.05$).

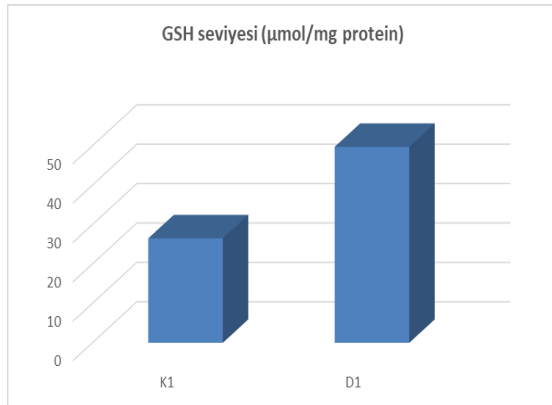


Figure 2. Effect of curcumin on GSH levels in heart tissue. K1: Control, D1: Curcumin. *KON according to $P < 0,05$

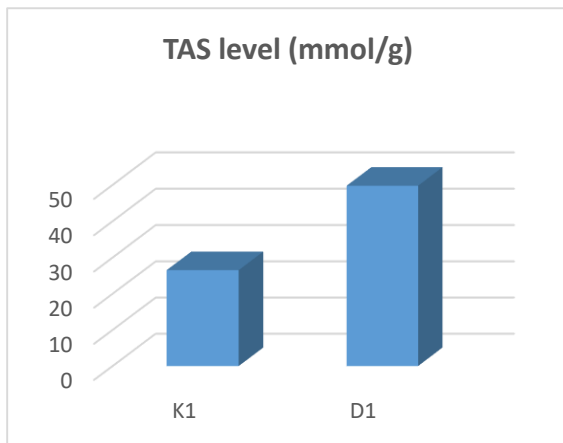


Figure 3. Effect of curcumin on TAS levels in heart tissue. K1: Control, D1: Curcumin

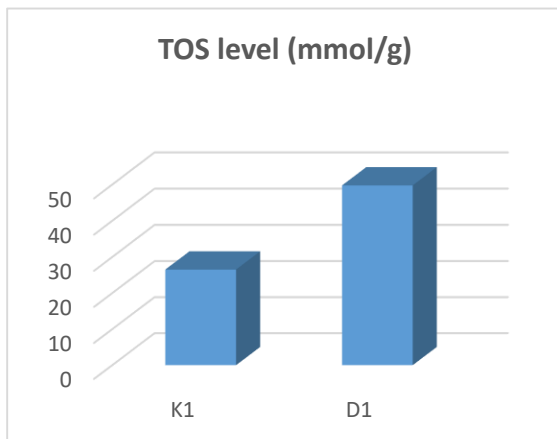


Figure 4. Effect of curcumin on MDA levels in heart tissue. K₁: Control, D₁: Curcumin

Total oxidant capacity (TOS) values between the two groups revealed that the experimental group showed a statistically significant increase compared to the control group (3.16 ± 0.97 and 3.66 ± 0.88 in K₁ and D₁ groups, respectively) ($p < 0.05$).

Conclusion

The aim of this study was to investigate the effects of curcumin supplementation on oxidative stress and antioxidant defense in heart tissue in adult rats. The results revealed that 12-day curcumin supplementation reduces oxidative damage in heart tissue and strengthens the antioxidant defense system.

The heart is the only organ in our body that is in continuous operation (Nakao et al., 2000). The constant working of the heart is one of the causes of high oxygen consumption. This causes it to be exposed to more oxidative stress than other organs (Jenkins, 1993). As a result of increased oxidative stress, DNA, protein and lipid damage and a decrease in antioxidant levels occur (Parildar et al., 2008).

The protective effect of curcumin on the cardiovascular system is due to its inhibition of lipid peroxidation as a result of scavenging free radicals. It is known that it provides a cardioprotective effect on cardiac damage caused by free oxygen radicals by inhibiting free oxygen radicals. It also increases the formation of sulfhydryl groups. It also protects the heart against damage by preserving the integrity of the membrane. Studies have shown that the antioxidant effect of curcumin has a protective role on the heart and the 300 mg/kg dose applied has an antioxidant effect (Wongcharoen and Phrommintikul, 2009).

In this study, TAS and TOS levels were found to be significantly increased ($P < 0.05$) in the

curcumin given group compared to the control group, and there was no statistically significant difference between the two groups. According to this result, curcumin strengthens the antioxidant defense system and this feature is due to the phenolic component curcumin. GSH levels were higher in the group that took the curcumin supplement. GSH is an important component of the antioxidant defense system and ensures the protection of cell membrane integrity (Kakarla et al., 2005). The literature supports that increased GSH level has a cardioprotective effect (Naik et al., 2011).

Although there was no statistically significant difference between the groups ($p > 0.05$), MDA levels in the curcumin given group showed a tendency to decrease compared to the control group. These data show that curcumin, which has antioxidant properties, has a protective effect on the heart (Nazam et al., 2007; Thiyagarajan and Sharma, 2004; Zhao et al., 2008).

In conclusion, our findings from this study show that 12 days of curcumin supplementation protects the heart tissue of aged female rats against oxidative damage and strengthens the antioxidant defense system. However, since there are not enough studies on this subject in the literature, more detailed studies are needed, especially considering the mechanism of action of curcumin.

Conflicts of interest

There are no conflicts of interest to declare.

References

Choudhuri, T., Pal, S., Agwarwal, ML., Das, T., Sa, G., 2002. Curcumin induces apoptosis in human breast cancer cells through p53-dependent Bax induction. *FEBS Lett*; 512(1-3):334-40.

Dkhar, P., Sharma, R., 2010. Effect of dimethylsulphoxide and curcumin on protein carbonyls and reactive oxygen species of cerebral hemispheres of mice as a function of age. *Int J Dev Neurosci*. 28:351-7.

Duan, W., Yang, Y., Yan, J., Yu, S., Liu, J., Zhou, J., et al., 2012. The effects of curcumin post-treatment against myocardial ischemia and reperfusion by activation of the JAK2/STAT3 signaling pathway. *Basic Res Cardiol*. 107:263.

Jenkins, RR., 1993. Exercise, oxidative stress, and antioxidants: A review. *Int J Sport Nutr*. 3:356-75.

Kakarla, P., Vadluri, G., Reddy, KS., 2005. Leeuwenburgh C. Vulnerability of the mid aged rat myocardium to the age- induced oxidative stress: influence of exercise training on antioxidant defense system. *Free Radic Res*. 39:1211-7.

Lowry, OH., Rosebrough, NJ., Farr, AL., Randall, RJ., 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem*. 193:265-75.

Miriyala, S., 2007. Panchatcharam M, Rengarajulu P. Cardioprotective effects of curcumin. *Adv Exp Med Biol*. 595:359-77.

Morimoto, T., Sunagawa, Y., Kawamura, T., Takaya, T., Wada, H., Nagasawa, A., et al., 2008. The dietary compound curcumin inhibits p300 histone acetyltransferase activity and prevents heart failure in rats. *J Clin Invest*. 118:868-78.

Naik, SR., Thakare, VN., Patil, SR., 2011. Protective effect of curcumin on experimentally induced inflammation, hepatotoxicity and cardiotoxicity in rats: evidence of its antioxidant property. *Exp Toxicol Pathol*. 63:419-31.

Nakao, C., Ookawara, T., Kizaki, T., Oh-Ishi, S., Miyazaki, H., Haga, S., et al., 2000. Effects of swimming training on three superoxide dismutase isoenzymes in mouse tissues. *J Appl Physiol*. 88:649-54.

Nazam Ansari, M., Bhandari, U., Pillai, KK., 2007. Protective role of curcumin in myocardial oxidative damage induced by isoproterenol in rats. *Hum Exp Toxicol*. 26:933-8.

Nirmala, C., Anand, S., Puvanakrishnan, R., 1999. Curcumin treatment modulates collagen metabolism in isoproterenol induced myocardial necrosis in rats. *Mol Cell Biochem*. 197:31- 7.

Parildar, H., Dogru-Abbasoglu, S., Mehmetçik, G., Ozdemirler, G., Koçak-Toker, N., Uysal, M., 2008. Lipid peroxidation potential and antioxidants in the heart tissue of beta-alanine- or taurine- treated old rats. *J Nutr Sci Vitaminol (Tokyo)*. 54:61-5.

Reddy, AC., Lokesh, BR., 1994. Studies on the inhibitory effects of curcumin and eugenol on the formation of reactive oxygen species and the oxidation of ferrous iron. *Mol Cell Biochem.* 137:1-8.

Sreejayan, Rao MN., 1997. Nitric oxide scavenging by curcuminoids. *J Pharm Pharmacol.* 49:105-7.

Srivastava, G., Mehta, JL., 2009. Currying the heart: curcumin and cardioprotection. *J Cardiovasc Pharmacol Ther.* 14:22-7.

Thiyagarajan, M., Sharma, SS., 2004. Neuroprotective effect of curcumin in middle cerebral artery occlusion induced focal cerebral ischemia in rats. *Life Sci.* 74:969-85.

Venkatesan, N., 1998. Curcumin attenuation of acute adriamycin myocardial toxicity in rats. *Br J Pharmacol.* 124:425-7.

Wongcharoen, W., Phrommintikul, A., 2009. The protective role of curcumin in cardiovascular diseases. *Int J Cardiol.* 133:145-51.

Yeh, CH., Chen, TP., Wu, YC., Lin, YM., 2005. Jing Lin P. Inhibition of NFkappaB activation with curcumin attenuates plasma inflammatory cytokines surge and cardiomyocytic apoptosis following cardiac ischemia/reperfusion. *J Surg Res.* 125:109-16.

Zhao, J., Zhao, Y., Zheng, W., Lu, Y., Feng, G., Yu, S., 2008. Neuroprotective effect of curcumin on transient focal cerebral ischemia in rats. *Brain Res.* 1229:224-32.