DOI: https://doi.org/10.53350/pjmhs202317197

ORIGINAL ARTICLE

Antioxidant effects of Citric Acid on Myocardium

WARDAH SIDDIQUE[1], HAFIZ MUHAMMAD IMRAN AZIZ[2], SIDRA MUSHTAQ[3], AMINA ZUBAIR[4], OBAID ANWAR[5]
[1]*Assistant Professor Pharmacology, Lahore Medical & Dental College Lahore.*
[2]*Assistant Professor Pharmacology, ABWA Medical College Faisalabad.*
[3]*Associate Professor Pharmacology, Independent Medical College Faisalabad.*
[4]*Demonstrator Pharmacology, Lahore Medical & Dental College Lahore.*
[5] *Assistant Professor Pharmacology, ABWA Medical College Faisalabad.*
Correspondence to Dr. Wardah Siddique, Email: dr4humanities@gmail.com, Cell No. 03364138407.

ABSTRACT

Background: Myocardial ischemia is associated with myocardial damage and necrosis. The pathogenesis of myocardial damage includes increased oxidative stress and diminished antioxidant defense.
Aim: To assess levels of oxidative stress and scavenger enzyme system in experimental model of ischemia.
Study d**esign:** This was a randomized experimental study.
Place and duration: King Edward Medical University and University of Veterinary & Animal Sciences, Lahore for six months.
Methodology: Thirty male rabbits were randomly divided into three groups. **Control**--**group**: animals got normal saline 1ml per oral for 14 days. **ISO--group:** animals got normal saline 1ml per oral for 14 days. **CA+ISO--group:** animals got citric acid 500 mg/kg body weight per oral for 14 days. Myocardial infarction was induced on 15th day in CA-group and CA+ISO-group by two doses of isoproterenol, administered subcutaneously at the interval of 24 hours. (isoproterenol dose= 85mg/kg body weight). To measure cTn-I (serum cardiac troponin I), tissue SOD (Super -oxide dismutase), CAT(catalase), GPx (glutathione peroxidase), GR (glutathione reductase), MDA (Malondialdehyde) and GSH (total reduced glutathione) animal serum was obtained from blood sample. SPSS was utilized to analyze the data. Quantitative data was shown as mean ± standard-deviation. One way analysis of variance and multiple comparison test LSD was applied. p value ≤0.05 was considered as statistically significant.
Result: ISO--group showed significant decline in the level of SOD, CAT, GPx, GR and GSH as compare to control—group. ISO-group showed significant rise in the level of cTn-I and MDA. CA-group showed significantly recovery in SOD, CAT, GPx, GR and GSH levels in comparison to ISO-group. CA-group also showed significant decline in levels of cTn-I and MDA.
Practical implication :Consumption of citric acid or diet rich in citric acid enhances the scavenger enzymes capability thus protects from acute myocardial infarction.
Conclusion: It is concluded that citric acid possess strong anti-oxidant potential. It improves the anti-oxidant capability of myocardium in term of GSH content and level of scavenger enzymes in ischemic myocardium that help myocardium to battle against free radical injury and survive during ischemic condition.
Keywords: Antioxidant, citric acid, SOD, CAT, GPx, GR, GSH

INTRODUCTION

Cardiac ischemia generates oxidative stress by augmenting the production of reactive oxygen species (ROS). ROS is highly reactive variety of oxygen that damages the cellular-proteins and nucleic acids[1]. MDA: a product of lipid peroxidation, produced by disintegration of polyunsaturated fatty acids under the action of reactive species of oxygen[2]. MDA is a renowned marker of oxidative stress in myocardium and drastic spike has been observed in MDA levels during ischemia. Additionally, researchers reported double and triple rise in MDA levels during acute myocardial infarction (AMI)[3].

During oxidative stress there is imbalance between reactive free radicals and antioxidant defensive/ free radical scavenger system that leads to pathogenesis of AMI (acute myocardial infarction)[4]. Anti-oxidants/ free radical scavenging enzymes system comprises of SOD, CAT, GPx and GR[5]. These are scavengers that protect cells from reactive variety of oxygen. SOD is of two types, cytosolic and mitochondrial. Mitochondrial variety is meganaise dependent[6]. SOD normalizes highly reactive superoxide radicals by converting them into hydrogen peroxide (H_2O_2)[7,8]. Later on hydrogen peroxide (H_2O_2) is neutralizes into water and oxygen by GSH [9]. While detoxifying the Free radicals GSH_{Red} converts to GSH_{Oxi}. GSH_{Oxi} is inactive one[10]. Here comes the enzyme GR. The GR shifts GSH_{Oxi} to GSH_{Red} form[11]. Whenever there is ischemic condition like AMI the free radicals production is augmented and the levels of SOD, CAT, GPx and GR diminish severely[12,13].

Therefore, maintaining adequate levels of free scavenger enzymes and improving the endogenous antioxidant capacity could be beneficial during AMI. It may prolong the ability of myocardium to with stand against ischemic phase and reduce the damage to myocardium done by free radicals.

Received on 27-08-2022
Accepted on 16-12-2022

Citric acid: a weak acid comes from organic group with strong anti-oxidant potential. Citrus fruits are abundant with citric acid like orange, lemon and wolfberry. Researchers also reported hepatoprotective, neuroprotective, hypolipidemic, anti-platelet, antimicrobial and Anti-inflammatory properties of citric acid[14,15] .
Research gap; Anti-oxidant effects of CA were not studied yet.
Significance of study ; This study showed consumption of citric acid or citrus fruits enhance the anti-oxidant capacity of myocardium and protect from acute myocardial infarction.

METHODOLOGY

This randomized experiment study was conducted at King Edward Medical University and University of Veterinary and Animal Sciences, Lahore for a period of six months. Simple random sampling was done by lottery method. Only healthy rabbits of male gender weighing 1.0 to 1.5 kg were included in this study. Any rabbit of female gender, sick of weighing less than 1.0 kg was excluded from study.

Thirty male rabbits were arranged for this study and randomly divided into three groups. **Control**--**group**: animals got normal saline 1ml per oral for 14 days. **ISO--group:** animals got normal saline 1ml per oral for 14 days. **CA+ISO--group:** animals got citric acid 500 mg/kg body weight per oral for 14 days. Myocardial infarction was induced on 15th day in ISO-group and CA+ISO-group by two doses of isoproterenol, administered subcutaneously at the interval of 24 hours. (isoproterenol dose= 85mg/kg body weight). Animals were anesthetized with intra-peritoneal administration of thiopental sodium, 50 mg/kg b.w. and then sacrificed. Rabbit heart was removed and washed in ice-cold saline and shifted to lab to measure tissue MDA, SOD, CAT, GPx, GR and GSH. Blood sample was collected for to measure cTn I[16].

Statistical Analysis: SPSS was applied to analyze the data. Quantitative data was shown as mean±standard deviation. One way analysis of variance and multiple comparison test LSD was applied to compare the mean difference in cTn I, MDA, SOD, CAT, GPx, GR, GSH. The p value ≤ 0.05 was considered as statistically significant.

RESULT

Effect of citric acid on cT-I: The control group showed normal levels of cTn-I (216±11.20 pg/ml). While ISO-group developed significant rise in the acute myocardial injury biomarker, cTn-I (1000±13.6 pg/ml). Citric acid treatment significantly reduces the ischemic injury thus decrease the cTn-I levels to 482±9.34 pg/ml. Comparing the difference between ISO--group vs CA--group shows significant decline ($p < 0.05$) as shown in table-1.

Effect of citric acid on MDA: The MDA value observed in control group was 63 ± 8.09 mmol/g tissue. ISO--group developed significant oxidative stress indicated by surge in MDA levels 217 ± 10.2mmol/g tissue. Citric acid treatment drastically reduces the oxidative stress on myocardium evident by decrease **MDA** levels 148±7.5mmol/g tissue. The $p < 0.05$ verifies the significance of difference among ISO--group vs CA—group. As shown in table-2.

Effect of citric acid on GR: The normal value of GR is observed in control group was 89± 2.3 U/mg protein. While dropped value of GR in ISO-group (26± 2.0 U/mg protein) shows significant collapse in scavenger enzyme system. The rise in value of GR in citric acid treated group (53 ± 3.1 U/mg protein) shows considerable recovery of scavenger enzyme. System. Significant p value ($p< 0.05$) present effect recovery of GR in citric acid treated animals vs. ISO-group animals. As shown in table-3.

Effect of citric acid on GPx: GPx at normal level (245± 13.5 U/mg protein) can be observed in control group.

The ISO-group shows sharp fall in GPx level (13 ± 3.8 U/mg protein) indicating significant drop in scavenger enzyme system. While an upsurge in value of GR (226± 8.6 U/mg protein) in citric acid treated group demonstrated noticeable recovery of scavenger enzyme system. The $p < 0.05$ indicate significant difference among the results of citric acid treated group and ISO-groups. As shown in table-3.

Effect of citric acid on CAT: The control group has normal levels of **CAT i.e.** 22+2.7 U/mg protein**.**

ISO-group developed significant decline (9.4+ 3.5 U/mg protein) in scavenger enzyme system predicted by significant low levels of CAT. Citric acid treated group developed significantly retrieval shown by spike in value of CAT (18+2.3 U/mg protein). As shown in table-3.

Effect of citric acid on SOD: The control group shows normal levels of SOD (24±5.2 U/mg protein). ISO-group showed low levels of SOD (13±3.8 U/mg protein) because of significant weakening of scavenger enzyme system. Citric acid treated group showed SOD levels near normal (21±4.4 U/mg protein) leads to significant recovery of scavenger enzyme system. Comparison of groups gives significant p value. ($p < 0.05$). As shown in table-3.

Effect of citric acid on GSH: Normal GSH levels (215±13.01 pmol/mg tissue) can be seen in control group. ISO-group showed low levels of GSH (167±7.9 pmol/mg tissue) because of severe oxidative stress on myocardium because of ischemia. Citric acid treated group showed improved GSH levels (181±6.3 pmol/mg tissue) because of significantly recovery of scavenger enzyme system. Significant p value ($p < 0.05$) was seen on comparison among experimental groups. As shown in table-3.

Table 1. Effect of citric acid on Cardiac Troponin -I

	Control group	ISO-group	CA+ISO-group
cTn-I (pg/ml)	216 ±11.20	1000±13.6	482± 9.34

P <0.001 between CA+ISO-group vs ISO-group , Control group vs ISO-group

Table 2. Effect of citric acid on Malondialdehyde.

	Control group	ISO-group	CA+ISO-group
MDA mmol/g tissue	63 ± 8.09	217 ± 10.2	148 ± 7.5

P <0.001 between CA+ISO-group vs ISO-group , Control group vs ISO-group

Table 3. Effect of citric acid on Scavenger enzyme system.

	Control group	ISO-group	CA+ISO-group
GR U/mg protein	89± 2.3	26± 2.0	53 ± 3.1
GPX U/mg protein	245± 13.5	128± 11.0	226± 8.6
CAT U/mg protein	22+2.7	9.4+ 3.5	18+2.3
SOD U/mg protein	24 ± 5.2	13 ± 3.8	21 ± 4.4
GSH pmol/mg tissue	215 ± 13.01	167 ± 7.9	181 ± 6.3

P <0.001 between CA+ISO-group vs ISO-group, Control group vs ISO-group

DISCUSSION

cTn-I is the most sensitive diagnostic marker for acute myocardial infarction. It is the 1st cardiac marker that appears just 4 to 8 hours after the onset of AMI, reaches to maximum after 12 to 18 hours and return to normal in 5 to 10 days. It is used in clinical settings to confirm the AMI findings on ECG. Its quantitative value is directly proportional to extent of damage produced to myocardium. Higher value is associated with more severe damage and vice versa. The animals treated with citric acid showed a significant decline in cTn-I levels as compare to ISO-group, indicating that the lesser damage of myocardium due to anti-oxidative effect of citric acid. Many researchers support these findings [17,18].

The high levels of MDA in ISO-group is suggestive of oxidative stress developed during cardiac ischemia. The results showed that ischemic condition may double the production of super oxide, hydrogen peroxide and MDA or slow down the destruction/ neutralization of super oxide, hydrogen peroxide and MDA. On the other hand reduced levels of MDA in myocardium in citric acid treated animals indicate citric acid may arrest the pathway of MDA synthesis in myocardium. These results are in line with earlier reported data[19,20].

The comparison of scavenger enzymes SOD, CAT, GR and GPx among different study groups demonstrated that the citric acid treatment enhance the levels of these anti-oxidant enzymes within ischemic myocardium and successfully reduce the cardiac injury by providing protection to myocardium against ROS during ischemia. While untreated groups revealed low levels of scavenger enzymes within ischemic myocardium associated with severe injury to myocardium by ROS during ischemia. Many researchers along with their teams also reported similar alteration during studying the cardioprotective effect on animal model [21,22].

Also the ischemic myocardium suffers from deficiency of GSH. But citric acid treatment elevated the level of GSH in ischemic myocardium significantly, that render the extent of injury to myocardium. This data also validate the previous study on melatonin for cardioprotection [23].

CONCLUSION

So it is concluded that citric acid possess strong anti-oxidant potential. It improves the anti-oxidant capability of myocardium in term of GSH content and level of scavenger enzymes in ischemic myocardium that help myocardium to battle against free radical injury and survive during ischemic condition.

Conflict of interest: No competing interest.

Ethical approval: This study was carried out after ethical review committee's approval at King Edward medical university.

Financial Disclosure: None

REFERENCES

1. Filonenko M, Zhuravlyova L, Sokolnikova N. Correlation of cardiac biomarkers with the levels of selenium and antioxidant enzymes in patients with acute myocardial infarction and a history of hypertension. Wiadomości Lekarskie. 2022 Jan 1;75(2):362-5.
2. Sack MN, Fyhrquist FY, Saijonmaa OJ, Fuster V, Kovacic JC. Basic biology of oxidative stress and the cardiovascular system: part 1 of a

3-part series. Journal of the American College of Cardiology. 2017 Jul 11;70(2):196-211.
3. Carretero A, Gomez-Cabrera MC, Rios-Navarro C, Salvador-Pascual A, Bodi V, Vina J. Early reductive stress and late onset overexpression of antioxidant enzymes in experimental myocardial infarction. Free Radical Research. 2020 Mar 3;54(2-3):173-84.
4. Zhou T, Prather ER, Garrison DE, Zuo L. Interplay between ROS and antioxidants during ischemia-reperfusion injuries in cardiac and skeletal muscle. International journal of molecular sciences. 2018 Jan 31;19(2):417.
5. D'Oria R, Schipani R, Leonardini A, Natalicchio A, Perrini S, Cignarelli A, Laviola L, Giorgino F. The role of oxidative stress in cardiac disease: from physiological response to injury factor. Oxidative medicine and cellular longevity. 2020 May 14;2020.
6. Incalza MA, D'Oria R, Natalicchio A, Perrini S, Laviola L, Giorgino F. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. Vascular pharmacology. 2018 Jan 1;100:1-9.
7. Meshkibaf MH, Maleknia M, Noroozi S. Effect of curcumin on gene expression and protein level of methionine sulfoxide reductase A (MSRA), SOD, CAT and GPx in Freund's adjuvant inflammation-induced male rats. Journal of Inflammation Research. 2019;12:241.
8. Gusti AM, Qusti SY, Alshammari EM, Toraih EA, Fawzy MS. Antioxidants-Related Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPX), Glutathione-S-Transferase (GST), and Nitric Oxide Synthase (NOS) Gene Variants Analysis in an Obese Population: A Preliminary Case-Control Study. Antioxidants. 2021 Apr 13;10(4):595.
9. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, De Ferranti SD, Floyd J, Fornage M, Gillespie C, Isasi CR. Heart disease and stroke statistics—2017 update: a report from the American Heart Association. circulation. 2017 Mar 7;135(10):e146-603.
10. Papas M, Arroyo L, Bassols A, Catalán J, Bonilla-Correal S, Gacem S, Yeste M, Miró J. Activities of antioxidant seminal plasma enzymes (SOD, CAT, GPX and GSR) are higher in jackasses than in stallions and are correlated with sperm motility in jackasses. Theriogenology. 2019 Dec 1;140:180-7.
11. Cecerska-Heryć E, Surowska O, Heryć R, Serwin N, Napiontek-Balińska S, Dołęgowska B. Are antioxidant enzymes essential markers in the diagnosis and monitoring of cancer patients–a review. Clinical Biochemistry. 2021 Jul 1;93:1-8.
12. Fan Y. Cardioprotective effect of rhapontigenin in isoproterenol-induced myocardial infarction in a rat model. Pharmacology. 2019;103(5-6):291-302.
13. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria journal of medicine. 2018;54(4):287-93.
14. Gil A, van der Pol A, van der Meer P, Bischoff R. LC-MS analysis of key components of the glutathione cycle in tissues and body fluids from mice with myocardial infarction. Journal of Pharmaceutical and Biomedical Analysis. 2018 Oct 25;160:289-96.
15. van der Pol A, van Gilst WH, Voors AA, van der Meer P. Treating oxidative stress in heart failure: past, present and future. European Journal of Heart Failure. 2019 Apr;21(4):425-35.
16. Giam, B., Chu, P.Y., Kuruppu, S., Smith, A.I., Horlock, D., Kiriazis, H., Du, X.J., Kaye, D.M. and Rajapakse, N.W., 2016. N-acetylcysteine attenuates the development of cardiac fibrosis and remodeling in a mouse model of heart failure. *Physiological Reports*, *4*(7), p.e12757.
17. Hayıroğlu Mİ, Keskin M, Uzun AO, Yıldırım Dİ, Kaya A, Çinier G, Bozbeyoğlu E, Yıldırımtürk Ö, Kozan Ö, Pehlivanoğlu S. Predictors of in-hospital mortality in patients with ST-segment elevation myocardial infarction complicated with cardiogenic shock. Heart, Lung and Circulation. 2019 Feb 1;28(2):237-44.
18. Myocardial infarction and oxidative damage in animal models: objective and expectations from the application of cysteine derivatives. Toxicology Mechanisms and Methods. 2022 May 6:1-7.
19. Verma VK, Malik S, Narayanan SP, Mutneja E, Sahu AK, Bhatia J, Arya DS. Role of MAPK/NF-κB pathway in cardioprotective effect of Morin in isoproterenol induced myocardial injury in rats. Molecular biology reports. 2019 Feb;46(1):1139-48.
20. Godugu C, Kumari P, Khurana A. Nanoyttria attenuates isoproterenol-induced cardiac injury. Nanomedicine. 2018 Dec;13(23):2961-80.
21. Sun L, Hu Y, Mishra A, Sreeharsha N, Moktan JB, Kumar P, Wang L. Protective role of poly (lactic-co-glycolic) acid nanoparticle loaded with resveratrol against isoproterenol-induced myocardial infarction. Biofactors. 2020 May;46(3):421-31.
22. Van Der Pol A, Gil A, Silljé HH, Tromp J, Ovchinnikova ES, Vreeswijk-Baudoin I, Hoes M, Domian IJ, Van De Sluis B, Van Deursen JM, Voors AA. Accumulation of 5-oxoproline in myocardial dysfunction and the protective effects of OPLAH. Science translational medicine. 2017 Nov 8;9(415):eaam8574.
23. Hayıroğlu Mİ, Bozbeyoglu E, Yıldırımtürk Ö, Tekkeşin Aİ, Pehlivanoğlu S. Effect of acute kidney injury on long-term mortality in patients with ST-segment elevation myocardial infarction complicated by cardiogenic shock who underwent primary percutaneous coronary intervention in a high-volume tertiary center. Turk Kardiyoloji Dernegi Arsivi. 2020;48(1):1.