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ORIGINAL ARTICLE

Histopathological Evaluation of Ameliorated Effect of Citric Acid on Infarcted Myocardium

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ABSTRACT

The estimated disease burden of ischemic heart disease in Pakistan is in millions and discovery of cardioprotective agents of organic origin has great importance to decrease the financial burden on society.

Objective: The study was designed to analyze and grade histopathological changes in experimental model of myocardial infarction.

Study Design: This was an experimental randomized controlled trial.

Place and Duration: The study was organized at King Edward Medical University and University of Veterinary and Animal Sciences, Lahore for a period of 180 days.

Methodology: During initial 14 days, cont-group and Isopro-group were treated with 1 ml normal saline, while Citric-250 and Citric-500-group were treated with citric acid at 250 mg and 500 mg per-kg-body-weight respectively. Isoproterenol (85mg per-kg-body-weight) was injected subcutaneously for induction of acute myocardial infarction in all rabbits except for cont-group. Histopathological analysis was done, serum-C.K-M.B (creatine-kinase-M.B), serum-L.D.H (lactate-dehydrogenase) and cTn-I (cardiac.troponin-I) were recorded at the end of experiment. S.P.S.S software (statistical package for social sciences) was applied for statistically analyzed of data.

Results: Isopro-group showed augmented odema, infiltration and necrosis in myocardium on histopathological study along with elevated level of C.K-M.B, L.D.H and cTn-I as compare to other group. Citric-250 and citric-500-group showed significant recovery with reduced odema, infiltration and necrosis in myocardium on histopathological analysis along with lesser values of C.K-M.B, L.D.H and cTn-I as compare to Isopro-group.

Practical Implication: Consumption of citric acid protects from acute myocardial infarction.

Conclusion: It is concluded that citric acid possesses strong cardioprotective potential. It reduces the myocardial injury during infarction in term of edema, infiltration and necrosis that help myocardium to survive during ischemic condition.

Keywords: Citric acid, Isoproterenol, Formalin, cTn-I, Infiltration and Myocardial Infarction.

INTRODUCTION

Nowadays ischemic heart disease (I.H.D) is the major problem throughout the globe. World Health Organization (WHO) reports indicate that, ischemic conditions of heart have become the major killer globally[1]. The life lost per annum from I.H.D is more than from any other illness[2]. Also I.H.D is the most dominant cause of disability world-wide. The rising incidence of I.H.D placed a great financial burden on the general public[3]. The estimated disease burden of I.H.D in Pakistan is 5.09375 million[4].

Citric acid belongs to organic group of weak acids [5]. All the fruits with citrus flavor contain citric acid in them like, minneola, orange, wolfberry strawberry and cherry [6]. Scientists have observed and reported various qualities of citric acid like neuroprotective potential, hepatoprotective effect, hypolipidemic properties and bactericidal activity [7,8]. Even citric acid provide promising results against ophthalmic and nephrotic complications of diabetes mellitus like cataract and proteinuria [9,10].

Research Gap: Protective role of citric acid in myocardial infarction was not established yet. It is the first study of its kind designed to evaluate protective role of citric acid in myocardial infarction on histopathological basis.

Significance of Study: This study signifies that consumption of citric acid may provide protection from acute attack of myocardial infarction.

METHODOLOGY

Study Design. This was an experimental randomized controlled trial.

Place and Duration: The study was organized at King Edward Medical University and University of Veterinary and Animal Sciences, Lahore for a period of 180 days.

Sampling Technique: Technique of simple random sampling was applied via lottery method.

Sample Size Calculation Method: Following method was adopted to calculate sample size.

$$n = \frac{(Z_{1-\beta} + Z_{1-\alpha/2})^2 + (\delta_1^2 + \delta_2^2)}{(\mu_1 - \mu_2)^2}$$

Inclusion Criteria: Male healthy rabbit having body weight more than 1.5 kg was included in this study.

Exclusion Criteria: Any rabbit that is sick, belong to female gender or having body weight less than 1.5 kg was excluded from study.

Rabbits were kept in animal house with controlled environment like temperature range 18 to 29 °C, humidity between 30 to 70% and 12 hour light-dark cycle. Rabbits were acclimatized to laboratory environment before starting the experiment for seven days and were fed on standard diet and water ad libitum.

Sample size was thirty two rabbits. Four experimental groups were designed with eight rabbit in each. Cont-group: experimental-animals received normal saline 1ml per oral for 14-days. Isopro-group: experimental-animals received normal saline 1ml per oral for 14-days and later on myocardial infarction was introduced on 15th day. Citric-250-group: experimental-animals received citric acid 250 mg per kg weight of rabbbit per oral for 14-days and later on myocardial infarction was introduced on 15th day. Citric-500-group: experimental-animals received citric acid 500 mg per kg weight of rabbbit per oral for 14-days and later on myocardial infarction was introduced on 15th day. Myocardial infarction was introduced by subcutaneous administration of isoproterenol on day 15 and 16. (Isoproterenol dose= 85 mg per kg weight of rabbbit).

Sample Collection: Twenty hours after the last isoproterenol injection, blood sample was collected and serum was separated via centrifugation to determine markers of acute myocardial infarction (C.K-M.B, L.D.H and cTn-I). After that rabbits were slaughtered, hearts were removed, washed in saline (ice-cold), weighted and then preserved.

Preparation of Slides: Formalin (10% concentrated) was preferred to preserve the heart tissue. Then it is further processed and embedded within paraffin wax. Sections of 5-6 µ-meter thickness were prepared; stained by H&E (Hematoxyline and Eosin) and observed under light microscope to evaluate myocardial injury. All slides were coded. Histopathologist studied all slides for odema, necrosis, infiltration and graded from 0 to 4. Grade 0 stands for no change, +1 for slight, +2 for mild, +3 for moderate and +4 for severe histopathological changes.
Data Collection Procedure: Specialized proforma for each animal was used for data collection purpose.
Statistical Analysis: A software application S.P.S.S was applied for statistical analysis of research data. Research data was presented as mean ± standard deviation and evaluated by A-N-O-V-A (one-way-ANOVA) to compare the mean difference of various parameters in study groups. The mean difference showed p-value less than 0.05 was labeled as significant.

RESULTS

Effect of citric acid on C.K-M.B: The mean value of serum-C.K-M.B in rabbit of Cont-group with healthy heart was 771.12± 49.27. Myocardial infarction of extensive type developed in Isopro-group supported by the highest value of serum-C.K-M.B (1636.13± 147.87). Ameliorated effect of citric acid observed in Citric-250 and Citric-500 groups with down-fall in level of C.K-M.B (1198± 93.89, 1032.63± 103.30). The p value for mean difference amid Isopro-group, Citric-250 and Citric-500 groups was below 0.05 proving significance of results.

Table-1: Effect of citric acid on CK-MB (U/L) in rabbits (n=8).

Group	Cont	Isopro	Citric-250	Citric-500
Mean ± SD	771.12± 49.27	1636.13± 147.87	1198± 93.89	1032.63± 103.30

Data is expressed as Mean ± SD P<0.05 = Citric-250 as compared to Isopro-group. Citric-500 as compared to Isopro-group

Table-2: Effect of citric acid on L.D.H (U/L) in rabbits (n=8).

Group	Cont	Isopro	Citric-250	Citric-500
Mean ± SD	473.42± 16.00	1011± 59.17	698± 13.6	602.23± 17.21

Data is expressed as Mean ± SD P<0.05 = Citric-250 as compared to Isopro-group. Citric-500 as compared to Isopro-group.

Table-3: Effect of citric acid on Cardiac Troponin -I **(**pg/ml) in rabbits (n=8)

Group	Cont	Isopro	Citric-250	Citric-500
Mean ± SD	216 ± 11.20	1000± 13.6	482± 9.34	405.05 ± 0.21

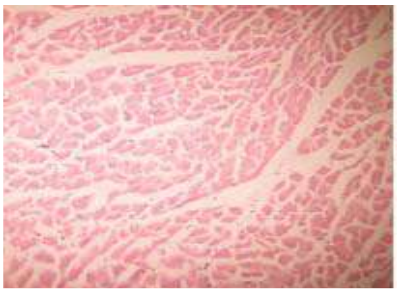
Data is expressed as Mean ± SD P<0.05 = Citric-250 as compared to Isopro-group. Citric-500 as compared to Isopro-group.

Table-4: Comparison of histopathological changes between experimental groups. (n=8)

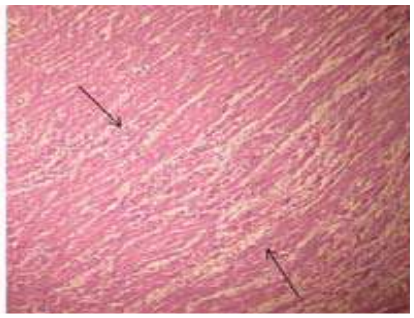
Group	Odema	Infiltration	Necrosis
Cont	0+0	0+0	0+0
Isopro	7.4+ 0.65	7.03+ 0.41	6.06+ 0.54
Citric-250	4.92+ 0.22	5.03+ 0.29	4.19+ 0.56
Citric-500	3.46+ 0.05	3.63+ 0.17	3.31+ 0.32

Data is expressed as Mean ± SD P<0.05 = Citric-250 as compared to Isopro-group. Citric-500 as compared to Isopro-group.

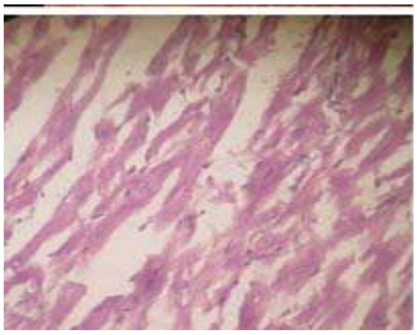
Effect of citric acid on L.D.H: Rabbits with healthy heart appeared with L.D.H value 473.42± 16.00 in Cont-group. While marked infarcted death of cardiac myocytes in Isopro-group showed the highest value of L.D.H (1011± 59.17) among all groups. On other hand L.D.H values of Citric-250 and Citric-500 groups (698± 13.6, 602.23± 17.21) were much lesser than Isopro-group, most probably due to ameliorated effect of citric acid. p value <0.05 proved significance of result among groups for L.D.H.
Effect of citric acid on cT-I: The standard value of cTn-I (216 +11.20) was recorded in Cont-group having animal without any cardiac injury. Isopro-group touched the peak level for cTn-I (1000+13.6) manifesting the highest level of infarction among all groups. Citric acid bring cTn-I levels near normal as seen in Citric-250 and Citric-500 groups (482± 9.34, 405.05 ± 0.21), suggesting ameliorated impact of citric acid intervention. The p value for mean difference amid Isopro-group, Citric-250 and Citric-500 groups was below 0.05 proving significance of results.



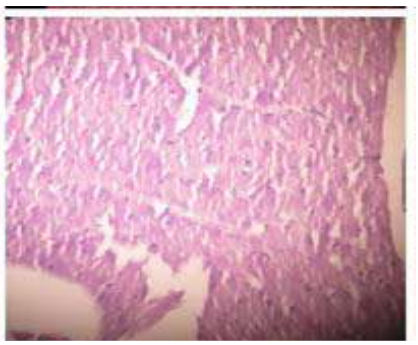
Cont- group



Isopro-group



Citric-250 group.



Citric-500 group.

Figure 1: Histopathological slides of experimental groups.

Comparison of histopathological changes between experimental groups: The Cont-group scored zero for all three indicators such as odema, infiltration and necrosis that is a sign of healthy myocardium free from injury. The Isopro-group scored 7.4+

0.65 for odema, 7.03+ 0.41 for infiltration and 6.06+ 0.54 for necrosis, the highest score among all groups. It showed severe injury to myocardium of Isopro-group rabbits. Numerical indicators for edema, infiltration and necrosis significantly reduced among Citric-250 and Citric-500-groups showing reduced cardiac injury by intervention of citric acid. Citric-250 and Citric-500-group showed significant decline in values of edema, infiltration and necrosis as compare to Isopro-group.

DISCUSSION

Isoproterenol is a catecholamine of synthetic group with long duration of acting. It is a nonselective β-receptor agonist and is frequently used in intervention trails to induce ischemia and infarction of myocardium[11]. Being β-receptor agonist, it is positive ionotropic as well as chronotropic on myocardium that increase workload thus oxygen demand of myocardium[12,13]. This ultimately leads to ischemia and infarction. Ischemia of myocardium is also associated with free oxygen radical generation and oxidative stress[14]. In addition to these, altered permeability of myocardium due to lipid peroxidation, interruption of mitochondrial oxidative phosphorylation and disturbed electrolyte contents are also involved in myocardial injury[15].

C.K-M.B, L.D.H and cTn-I are marker of acute Myocardial infarction. These are used in clinics and medical emergencies to ratify the Myocardial infarction. Quantitatively these are directly proportional to extent of infarction in myocardium. These are the diagnostic biomarker for acute myocardial infarction with much higher sensitivity.

Highest values of biomarkers for acute myocardial infarction in Isopro-group are indicative of extensive damage to cardiac tissue in the absence of citric acid treatment. Animals intervened with citric acid come up with prominent decline in levels of biomarkers of acute myocardial infarction as compare to untreated groups. These findings are suggestive of lesser damage of myocardium in the presence of citric acid. Many researchers support these findings [16,17].

Presence of citric acid may halts or at least minimize the production of free oxygen radicals thus reduce oxidative stress on myocardium. Citric acid may enhance the activity of scavenger enzymes SOD, CAT, GR and GPx thus strengthen myocardium to stand against ischemic condition. It may also inhibit lipid per oxidation and successfully maintain cellular permeability of cardiac myocytes.

Histopathological analysis and grading of myocardial tissue revealed that addition of citric acid to rabbits produced dramatic change in results with major decline in severity of edema, infiltration of cells and extent of necrotic tissue of myocardium in comparison to untreated groups. Similar protective role of wogonin loaded nanoparticles and Andrographolide were observed by scholars[18,19].

CONCLUSION

So it is concluded that citric acid administration provides protection from myocardial infarction that is substantiated by histopathological analysis of myocardium tissue.

Conflict of Interest: None.

Ethical Approval: Ethical Review Committee's approval got from King Edward Medical University.

Financial Disclosure: None

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