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Original Article

Evaluation of cardioprotective effect of aqueous extract of *Allium cepa* Linn. bulb on isoprenaline-induced myocardial injury in Wistar albino rats

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Abstract

To investigate the cardioprotective potential of the aqueous extract of *Allium cepa* Linn. bulb in isoprenaline-induced myocardial injury in Wistar albino rats. *In vitro* total phenolic, total flavonoid content and 2, 2'-diphenyl-1-picrylhydrazyl hydrate radical scavenging activity was measured. Isoprenaline-induced myocardial injury model was used to evaluate *in vivo* effect of aqueous extract of *A. cepa* in Wistar albino rats. Seventy two rats were randomly divided in 6 groups. Rats were treated with *A. cepa* 400 mg/kg and 800 mg/kg doses for 30 days and myocardial injury was produced by subcutaneous injection of isoprenaline (ISO) 85 mg/kg on day 28 and 29. Carvedilol 1 mg/kg for 30 days served as active control. Electrocardiogram parameters, cardiac injury markers, oxidative stress markers and histopathological changes were evaluated in each group and compared using appropriate statistical tests. *In vitro* evaluation of aqueous extract of *A. cepa* showed significant antioxidant property. ISO produced significant myocardial injury as compared to normal control group ($P < 0.05$). Administration of *A. cepa* in the dose of 400 mg/kg significantly recovered the altered parameters (Troponin-I, Creatine kinase-MB, glutamate-pyruvate transaminase, HR, R-R interval, and oxidative stress markers) compared to disease control group ($P < 0.05$) while *A. cepa* in the dose 800 mg/kg recovered the altered parameters (HR, heart weight/body weight ratio, and superoxide dismutase level) compared to disease control group. Histopathological parameters did not recover in the doses of 400 and 800 mg/kg ($P > 0.05$). The aqueous extract of *A. cepa* 400 mg/kg was found to be cardioprotective against myocardial injury while *A. cepa* 800 mg/kg did not show significant cardioprotective activity. So, we presume that *A. cepa* might be effective within certain dose range only.

Keywords: *Allium cepa*; Cardioprotective; Isoprenaline; Carvedilol; Myocardial infarction

INTRODUCTION

Ischemic heart disease is a major non communicable disease and it has become an important problem worldwide. According to World Health Organization (WHO), it will be a leading cause of mortality worldwide by 2020 (1). Among ischemic heart disease, acute myocardial infarction (MI) is the most alarming one and it occurs due to imbalance between coronary blood supply and myocardial demand. Free radical mediated myocardial damage is an important etiological mechanism that is associated with increased level of reactive oxygen species and/or inadequate antioxidant defense system (2). Isoprenaline (ISO) is a potent non selective beta-adrenergic agonist with low affinity for alpha adrenergic receptors which produces infarct like necrosis of myocardium in high dose (3). ISO-induced MI is the most commonly used model for evaluation of cardioprotective effect of various drugs (4). Administration of ISO in high doses produces myocardial lesions same as those produced in human MI. Modern medicines are effective in preventing heart diseases but their use is limited due to various adverse effects (5). Many dietary plants and food antioxidants are increasingly being recognized as health promoter in heart diseases (6).

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Allium cepa Linn (Onion) is a member of the family Liliaceae (7). Many studies suggest that *A. cepa* helps against many chronic diseases. That's probably because *A. cepa* contains generous amounts of the flavonoid quercetin which protects against cardiovascular disease. Organo sulphur compounds of the *A. cepa* are beneficial to lower cholesterol and blood pressure level. It also has been found to help in heart disease, inhibit strokes, and stimulate the immune system (8). Allium family, including garlic and onion, are known to have a potential to reduce the toxic effect of cardiovascular risk factors, and their beneficial roles are suggested to be associated with antioxidant mechanism. Compared to garlic, very few publications have been reported about the myocardial beneficial effect of onion. Based on these considerations, the present study was carried out to evaluate whether onion extract has cardioprotective effect against ischemic injury myocardial infarct model in Wistar albino rats, and if so, whether the beneficial effect of onion is due to its antioxidant property.

MATERIALS AND METHODS

All experiments were performed after prior permission from Institutional Animal Ethics Committee (IAEC), Government Medical College, Bhavnagar, Gujarat, India (IAEC No.35/2014, dated: 08/02/2014). Care of experimental animals was taken as per the guidelines given by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forests (Animal Welfare Division), Government of India, New Delhi, India.

Experimental animals

Wistar albino rats (250 ± 50 g) of both sexes were procured from central animal house of the institution. They were housed in standard transparent polypropylene cages with wheat husk bedding, renewed every 24 h. They were kept under controlled room temperature and humidity (18 to 29 °C; 30 to 70%) in a 12 h light-dark cycle. Animals were acclimatized for one week to laboratory conditions before starting the experiment. The rats were given standard laboratory diet and water *ad libitum*.

Chemicals and reagents

Plant authenticated by Dr. S. K. Mehta, Dept. of Botany, Sir. P. P. Institute of sciences, MK Bhavnagar University, Bhavnagar. After plant authentication, aqueous extract of *A. cepa* was provided by Leopard Investments Ltd. Vapi, Gujarat. ISO, Gallic acid, quercetin, urethane, and 2, 2'-diphenyl-1-picrylhydrazyl hydrate (DPPH) were obtained from the Sigma chemical company, St. Louis, USA. Carvedilol was purchased from Selleck chemicals, Germany. ISO solution was freshly prepared in normal saline and given subcutaneously at the time of injection; Carvedilol and *A. cepa* was dissolved in distilled water and given orally.

Determination of total phenols and flavonoids

Total phenolic content was determined according to Folin-Ciocalteu method (9). Flavonoid content in the extract was determined by a colorimetric method (10).

2, 2'-diphenyl-1-picrylhydrazyl hydrate radical scavenging assay

In this assay, free radical scavenging activity of *A. cepa* was determined by measuring the bleaching of purple-colored methanol solution of DPPH (11).

Experimental groups

Total 72 Wistar albino rats were allocated randomly into 6 groups using Rando software (12 animals per group). MI was induced in rats by giving ISO (85 mg/kg) subcutaneously (s.c.) for two subsequent days, on day 28 and 29 at the interval of 24 h. Distribution of study groups was as follow: Group 1 (normal control): rats were given distilled water orally for 30 days and normal saline s.c. on the day 28 and 29. Group 2 (ISO control): rats were given distilled water orally for 30 days and ISO (85 mg/kg) s.c. on the day 28 and 29. Group 3 (active control): rats were given carvedilol (1 mg/kg) orally for 30 days and ISO (85 mg/kg) s.c. on the day 28 and 29. Group 4 (*A. cepa* low dose): rats were given *A. cepa* extract 400 mg/kg orally for 30 days and ISO (85 mg/kg) s.c. on the day 28 and 29. Group 5 (*A. cepa* high dose): rats were given *A. cepa* extract 800 mg/kg orally for 30 days and ISO (85 mg/kg) on the day 28 and 29 s.c.

Group 6 (extract control): rats were given *A. cepa* extract 800 mg/kg orally for 30 days.

After administration of ISO, the following parameters were recorded to evaluate the cardioprotective effect.

Electrocardiography recording

At the end of 30 days, 48 h after the first dose of ISO, all the animals were anaesthetized with intraperitoneal injection of urethane (125 mg/100 g, i.p) and was given stabilization period of 30 min before recording of electrocardiography (ECG). ECG was recorded with the help of student's physiograph and EKG coupler (Inco Ambala Co, India; paper speed -50 mm/s, sensitivity -200 μV/cm, gain-maximum). The electrodes constructed from 26 gauge hypodermic needle were attached to both front and hind paw. One precordial lead was used and was placed in a position corresponding to V_4 in human. Heart rate, RR interval, QT interval, corrected QT interval and QRS interval were calculated and compared between the groups.

Biochemical estimations

After recording of ECG, blood was collected through retro-orbital plexus using capillary tube. The serum was separated by centrifugation and used for the estimation of lactate dehydrogenase (LDH, measured by UV kinetic method), creatinine kinase-MB (CK-MB, measured by Immuno inhibition method), troponin I (measured by Immuno-fluorescence method), aspartate aminotransferase (AST, measured by UV kinetic method), alanine aminotransferase (ALT, measured by UV kinetic method) and uric acid (measured by Uricase pap method). After blood collection, rats were sacrificed and heart was dissected out with mid abdominal incision. It was blotted in filter paper, weighed and further processed for histopathology or antioxidant analyses. From all groups, 6 hearts were used for histopathological analysis and 6 hearts were used for antioxidant estimation.

Antioxidant estimation in heart tissue homogenate preparation

Hearts were washed in ice cold saline followed by in 0.25 M sucrose solution and were finely sliced. Homogenate was prepared in chilled tris-HCl buffer solution (10% w/v, 0.1 M, pH 7.4). The homogenate was then centrifuged at 5000 rpm at 4 °C using compact high speed refrigerated centrifuge (Kubota 6500, Japan). The clear supernatant obtained was used for the estimation of superoxide dismutase (SOD) by using the method of Misra and Fridovich (12), catalase by the method of Hugo e. Aebi (13) and marker of lipid peroxidation malondialdehyde (MDA) by the method of Slater and Sawyer (14). Heart weight to body weight ratio was calculated in each group. Body weight was the weight of animal on the day of sacrifice. Heart weight was measured after washing it in ice cold saline after removal from the body, squeezing out the blood and blotted on the filter paper.

Histopathological analysis

Six hearts from each group were preserved in 10% formalin, processed and embedded in paraffin wax. 5-6 μm thick sections were cut and stained with hematoxylineand eosin (H&E) stain and observed with light microscope to evaluate myocardial injury. All slides were analyzed by the trained pathologist from our institute. Histological observations for oedema, infiltration and necrosis were categorized in to 5 different grades ranging from grade 0 to 4. Grade 0 indicates no change and +1, +2, +3, +4 indicates slight, mild, moderate and severe histopathological changes, respectively.

Statistical analysis

All the values were presented as Mean ± SEM. Parametric data were analyzed by one way ANOVA followed by Tukey-Kramer multiple comparison test and non-parametric data were analyzed by Kruskal-Wallis followed by Dunn's multiple comparison test using GraphPad InStat (version 3.00, GraphPad Software, California USA). A P value < 0.05 was considered as statistically significant.

RESULTS

During *in vitro* evaluation of antioxidant property of aqueous extract of *A. cepa*, we

found that extract had total phenolic content 838.87 mg/g gallic acid equivalent (GAE), total flavonoid content 0.48 mg/g quercetin equivalents (QUE) and IC_{50} value for DPPH scavenging activity 237 ± 4.33 µg/mL (Table 1).

As shown in Table 2, rats in ISO group showed significant increase in cardiac injury markers like troponin-I, LDH, CK-MB, *serum AST,* and ALT as compared to normal control group ($P < 0.05$). In test groups, administration of *A. cepa* extract in dose 400 mg/kg could significantly restore the elevated parameters which altered due to ISO administration ($P < 0.05$), whereas, *A. cepa* extract in dose 800 mg/kg and carvedilol in active control group could not significantly restore the cardiac injury markers ($P > 0.05$) (Table 2).

Subcutaneous ISO administration caused marked increase in HR and decrease in RR interval, altered QRS and QT intervals compared to normal control group ($P < 0.05$; Table 3). HR and RR interval was significantly restored in *A. cepa* in dose 400 mg/kg while carvedilol group and *A. cepa* in dose 800 mg/kg only able to rest HR.

The rats in ISO control group showed marked increase in heart weight and heart weight/body weight ratio (HW/BW) but ratio was decreased in active control group, *A. cepa* in dose 400 mg/kg and *A. cepa* in dose 800 mg/kg (Table 4).

Table 1. Total phenolic, flavonoid content in *Allium cepa* Linn. and its DPPH scavenging activity.

Parameters	Aqueous extract of *A. cepa*
Total phenolic content (GAE)	838.87 ± 385.31 mg/g
Total flavonoid content (QUE)	0.48 ± 0.15 mg/g
DPPH scavenging activity (IC_{50})	237 ± 4.33µg/ml

Values are expressed as Mean ± SEM. (GAE) Gallic Acid Equivalents, (QUE) Quercetin Equivalents, (DPPH) 2, 2'-Diphenyl-1-picrylhydrazyl hydrate, (IC_{50}) inhibitory concentration 50%.

Table 2. Comparison of cardiac injury biomarkers between the experimental groups (n = 12 in each group).

Groups	Troponin-I (ng/mL)	LDH (IU/L)	CK-MB (IU/L)	AST (IU/L)	ALT (IU/L)	Serum uric acid (mg/dL)
Group 1 (Normal Control)	0.42 ± 0.15	2086 ± 414.28	920.75 ± 64.78	342.19 ± 56.07	82.41 ± 31.92	3.1 ± 0.47
Group 2 (ISO Control)	2.02 ± 0.34$^{\#\#}$	2266.16 ± 276.89	2216.16 ± 278.02$^{\#\#}$	594.66 ± 63.77$^{\#}$	135.41 ± 36.17$^{\#}$	1.79 ± 0.223$^{\#\#}$
Group 3 (Active Control)	1.09 ± 0.31	1198.33 ± 108.24	1229.16 ± 118.49	383.66 ± 64.19	95 ± 29.6*	2.87 ± 0.48
Group 4 (*A. cepa*400 mg/kg)	0.57 ± 0.14**	1668.66 ± 195.05	1091.25 ± 81.43**	437.58 ± 73.11	78.66 ± 25.03*	2.38 ± 0.16
Group 5 (*A. cepa*800 mg/kg)	0.88 ± 0.21	2109.08 ± 310.74	1342 ± 128.79	458.66 ± 58.4	118.33 ± 37.89	2.25 ± 0.3
Group 6 (EC 800 mg/kg)	0.37 ± 0.1	1487.25 ± 110.7	1018.58 ± 73.04	368 ± 21.004	65.75 ± 25.14	3.59 ± 0.33
P value	0.0001	0.0347	0.0003	0.0440	0.0001	0.0003

Data are expressed as Mean ± SEM (n = 12 for each group).$^{\#}P < 0.05$ as compared to normal control, $^{*}P < 0.05$ as compared to ISO control analyzed by one way ANOVA followed by Tukey Kramer's multiple comparison test. $^{\#\#}P < 0.05$ as compared to normal control, $^{**}P < 0.05$ as compared to ISO control analyzed by Kruskal Wallis followed by Dunn's multiple comparison test.

Table 3. Comparison of ECG parameters experimental groups (n = 12 in each group).

Groups	HR (beats/min)	QRS (s)	QT (s)	R-R interval (s)	QTc (s)
Group1 (Normal Control)	325.083 ± 10.74	0.05 ± 0.0046	0.15 ± 0.012	0.38 ± 0.006	0.41 ± 0.036
Group 2 (ISO Control)	477.83 ± 21.48$^{\#\#}$	0.078 ±0.0067$^{\#\#}$	0.22 ± 0.016$^{\#\#}$	0.25 ± 0.011$^{\#\#}$	0.58 ± 0.051
Group 3 (Active Control)	366 ± 10.12**	0.06 ± 0.0049	0.17 ± 0.013	0.33 ± 0.012	0.51 ± 0.041
Group 4 (*A. cepa* 400 mg/kg)	349.83 ± 5.027**	0.051 ± 0.0029	0.19 ± 0.016	0.34 ± 0.0045**	0.54 ± 0.045
Group 5 (*A. cepa* 800mg/kg)	356.83 ± 11.18**	0.055 ± 0.0035	0.16 ± 0.013	0.33 ± 0.01	0.49 ± 0.035
Group 6 (EC 800 mg/kg)	316.91 ± 18.08	0.051 ± 0.0038	0.14 ± 0.009	0.40 ± 0.035	0.59 ± 0.053

Data are expressed as Mean ± SEM (n = 12 for each group). (HR) Heart rate, (QTc) Corrected QT interval. $^{\#\#}P < 0.05$ as compared to normal control, $^{**}P < 0.05$ as compared to ISO control analyzed by Kruskal Wallis followed by Dunn's multiple comparison test.

Table 4. Comparison of body weight, heart weight, heart weight/body weight ratio in experimental groups (n= 12 in each group).

Groups	Heart weight (g)	Body weight (g)	HW/BW ($\times 10^{-3}$)
Group 1 (Normal Control)	0.86 ± 0.016	244 ± 6.90	3.55 ± 0.037
Group 2 (ISO Control)	0.96 ± 0.024$^{\#}$	235 ± 7.53	4.12 ± 0.075$^{\#}$
Group 3 (Active Control)	0.93 ± 0.018	255 ± 9.73	3.69 ± 0.091*
Group 4 (*A. cepa* 400 mg/kg)	0.87 ± 0.019*	264 ± 7.73	3.32 ± 0.054*
Group 5 (*A. cepa* 800 mg/kg)	0.89 ± 0.015*	238.33 ± 5.48	3.74 ± 0.062*
Group 6 (EC 800 mg/kg)	0.87 ± 0.015	272.5 ± 6.29	3.21 ± 0.030

Data are expressed as Mean ± SEM (n = 12 for each group). $^{\#}P < 0.05$ as compared to normal control, $^{*}P < 0.05$ as compared to ISO control analyzed by one way ANOVA followed by Tukey Kramer's multiple comparison test.

Table 5. Comparison of biomarkers of oxidative stress (in heart tissue homogenate) between the groups (n = 6 in each group).

Groups	SOD (Unit/g of tissue)	Catalase (µml of H_2O_2 consumed/min/g of tissue)	TBARS (nml MDA/ g of tissue
Group 1 (Normal Control)	13.24 ± 1.50	2.98 ± 0.24	2.22 ± 0.18
Group 2 (ISO Control)	7.74 ± 0.73$^{\#}$	0.63 ± 0.04$^{\#}$	4.06 ± 0.22$^{\#\#}$
Group 3 (Active Control)	12.08 ± 0.64*	1.78 ± 0.34*	2.71 ± 0.31
Group 4 (*A. cepa* 400 mg/kg)	11.75 ± 0.58*	1.78 ± 0.37*	2.48 ± 0.28**
Group 5 (*A. cepa* 800 mg/kg)	11.26 ± 0.75*	1.86 ± 0.31	2.63 ± 0.58
Group 6 (EC 800 mg/kg)	12.25 ± 0.62	2.06 ± 0.29	2.47 ± 0.30

Data are expressed as Mean ± SEM (n = 6 for each group). $^{\#}P < 0.05$ as compared to normal, $^{*}P < 0.05$ as compared to ISO control analyzed by one way ANOVA followed by Tukey Kramer's multiple comparison test. $^{\#\#}P < 0.05$ as compared to normal control, $^{**}P < 0.05$ as compared to ISO control analyzed by Kruskal Wallis followed by Dunn's multiple comparison test.

SOD and catalase level were decreased and thiobarbituric acid reactive substance (TBARS) level was increased significantly in ISO control group as compared to normal control group ($P < 0.05$). In *A. cepa* treated groups, the levels of antioxidant enzymes was restored in dose 400 mg/kg while *A. cepa* in dose 800 mg/kg only restored SOD level ($P < 0.05$, Table 5). Average grading of hisopathological findings shown in (Table 6, Fig. 1) indicate histopathological texture of myocardium in study groups. Administration of ISO significantly caused necrosis, oedema and neutrophilic infiltration in myocardium as compared to normal control group. Myocardium of animals treated with *A. cepa* extract and carvedilol showed improvement as compared to ISO control group. However, it could not achieve the statistical significance. The extract control group was comparable to normal control group in relation to all observed parameters.

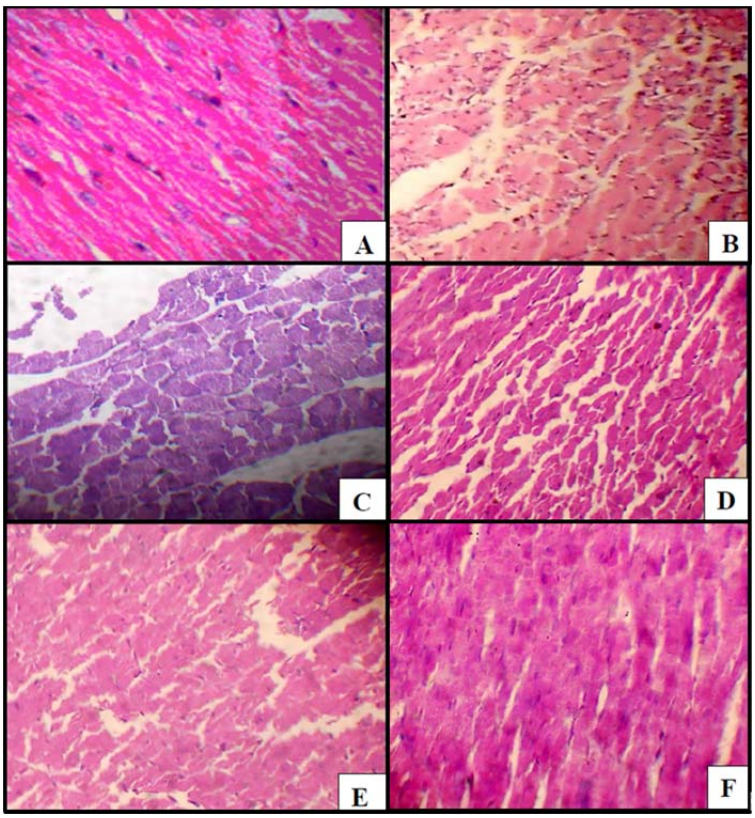

Fig. 1. Histopathological images of heart sections after Hematoxylin and Eosin staining (×40), (A) Normal control, (B) ISO control, (C) Active control, (D) *A. cepa* 400 mg/kg, (E) *A. cepa* 800 mg/kg, (F) Extract control 800 mg/kg.

Table 6. Comparison of histopathological score in experimental groups (n = 6 in each group).

Groups	Oedema	Infiltration	Necrosis
Group 1 (Normal Control)	0	0	0
Group 2 (ISO Control)	2.08 ± 0.27$^{\#\#}$	2.41 ± 0.23$^{\#\#}$	2.5 ± 0.25$^{\#\#}$
Group 3 (Active Control)	0.66± 0.10	0.5 ± 0.18	0.91 ± 0.15
Group 4 (*A. cepa* 400 mg/kg)	0.58 ± 0.15	0.5 ± 0.12	0.41 ± 0.08
Group 5 (*A. cepa* 800 mg/kg)	0.75± 0.11	0.91 ± 0.15	0.83 ± 0.16
Group 6 (EC 800 mg/kg)	0	0	0

Data are expressed as Mean ± SEM (n = 6 for each group). $^{\#\#}P < 0.05$ as compared to Normal control by Kruskal Wallis followed by Dunn's multiple comparison test in intra group comparison.

DISCUSSION

In vitro evaluation showed a good antioxidant property of aqueous extract of *A. cepa* Linn. bulb. So, we used ISO-induced I model in Wistar albino rats for evaluation of cardio protective effect of *A. cepa* Linn. Bulb extract.

In our study flavonoid have major role to produce cardioprotective effect of *A. cepa*. ISO is a synthetic catecholamine and non-selective β agonist. It is proven that catecholamine, if given in high doses, produces ischemic necrosis of heart muscle (15). Exact mechanism of ISO induced MI is not proven yet. Intracellular calcium overload due to massive influx (16), hypoxemia due to increased cardiac work and O_2 demand, free oxygen radical generation by auto oxidation of catecholamines, alteration of myocardial cell membrane permeability due to lipid peroxidation, mitochondrial oxidative phosphorylation interruption by free fatty acid and changes in electrolyte contents are various proposed mechanisms. Among them, oxidative stress and free radical mediated injury appears to be the most important mechanism for ISO induced MI (17). In our study, it was observed that 85 mg/kg dose of ISO significantly altered various biochemical parameters. Therefore, the cardioprotective activity of *A. cepa* was evaluated against this dose.

After injection of ISO, enormous amount of reactive oxygen species are generated due to auto-oxidation which reacts with polyunsaturated fatty acids present within cell membrane initiating chain of lipid peroxidation. Myocardium contains an abundant concentration of diagnostic marker enzymes and once metabolically damaged, it releases its contents into the extracellular fluid (18). ISO raised concentration of Troponin I, CKMB, AST, and ALT in serum which confirms myocardial necrosis of the heart. Restoration of these enzymes (Troponin I, CKMB, and ALT) indicates cardioprotective effect of low dose *A. cepa* (Table 2). LDH level was not found to elevated in our study might be the reason that normally in MI LDH rise after 24 h and reach up to peak level after 3 to 6 days, but in our study we sacrificed animal within 48 h of MI (19). In our study serum uric acid level was not significantly increased. Further research needs to be done in this area and also raised serum uric acid level should not considered as less cardioprotective effect of *A. cepa* since all other parameters clearly demonstrate a protective effect.

Lipid peroxidation is an important pathogenic event in MI and the accumulated lipid peroxides reflect the various stages of the disease and its complications (20).

SOD catalyzes the dismutation of the superoxide anion ($O2^-$) into hydrogen peroxide and molecular oxygen (21). It's over expression protects against apoptosis and promote cell differentiation (22). Estimation of SOD gives information regarding the cell oxidative stress. Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen (23) and facilitate the removal of hydrogen peroxide. Catalase assay is done to estimate the oxidative state of the cell. TBARS assay is used for screening and monitoring lipid peroxidation, a major indicator of oxidative stress (24-26). The assay provides important information about free radical activity in disease states. MDA is one of the lipid peroxidation products which are measured in this assay.

Levels of antioxidant enzymes like SOD and catalase were decreased and level of TBARS (marker of lipid peroxidation) was increased in ISO treated group which indicates oxidative stress, while SOD and catalase were increased and TBARS was decreased suggests cardioprotective effect of low dose of *A. cepa*.

Administration of aqueous extract of *A. cepa* in a dose of 800 mg/kg for 30 days restored the altered parameters (HR, R-R interval, SOD level, HW/BW ratio). While the other parameters like (Troponin-I, CK-MB, ALT, ECG parameters, biomarkers of oxidative stress) altered as compared to ISO control group but not reach up to statistical significant level suggesting the lack of cardio protective effect of *A. cepa* in a dose of 800 mg/kg.

In our study, administration of aqueous extract of *A. cepa* in a dose of 400 mg/kg for 30 days restored the altered parameters (Troponin-I, CK-MB, ALT, ECG parameters,

biomarkers of oxidative stress, HW/BW ratio) which suggests cardio protective effect of aqueous extract of *A. cepa* Linn. bulb at given dose.

Onions are rich in different types of phenolics, mainly flavonols and anthocyanins. This is significant because these classes of phenolics are antioxidants and hence may impart important functional properties to onions. Flavonoids, known for their high antioxidant activity, are the main polyphenols present in onion bulbs. Quercetin is the major flavonoid of interest in *A. cepa*. Mechanisms of action include free radical scavenging, chelation of transition metal ions, and inhibition of oxidases such as lipoxygenase (27). Flavonoids act as antioxidants by a variety of pathway including inhibition of enzymes responsible for superoxide anion production, trapping of reactive oxygen species, chelation of transition metals involved in process forming radicals and by reducing alcoxyl and peroxyl radicals prevent the peroxidation process. Carvedilol dose 1 mg/ kg did not show cardioprotective effect compared to ISO control group. Suboptimal dose of carvedilol may be the reason in this study. We have referred many works in which they analyzed the phenolic, flavonoid and various properties of various plant (28,29).

In our study histopathological parameters did not reach up to significance level which may be possibly due to shorter duration of action. Further study for longer duration can be planned to evaluate histopathological parameters.

One study reporting the cardioprotective and antioxidant activity of onion *(A. cepa)* leaves extract in doxorubicin-induced cardiotoxicity in rats support the cardioprotective effect of *A. cepa* (30). Further studies with different doses of *A. cepa* required for further evaluation of cardioprotective effect of *A. cepa*.

CONCLUSION

The aqueous extract of *A. cepa* at 400 mg/kg was found to be cardioprotective against myocardial injury while *A. cepa* at 800 mg/kg did not show significant cardioprotectiv activity. So, we presume that *A. cepa* might be effective within certain dose range only.

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REFERENCES

1. Abubaker S, Shanmukha I, Jyoti TM, Gupt K. Cardioprotective effect of *Spathodea campanulata* bark on isoproterenol induced myocardial infarction in rats. Asian Pac J Trop Dis. 2012;2:S1-S5.
2. Boudina S, Laclau MN, Tariosse L, Daret D, Gouverneur G, Bonoron-Adèle S, *et al.* Alternation of mitochondrial function in a model of chronic ischemia *in- vivo* in rat heart. Am J Physiol Heart Circ Physiol. 2002;282(3):H821-H831.
3. Wexler BC. Myocardial infarction in young *vs* old male rats; pathophysiologic changes. Am Heart J. 1978;96(1):70-80.
4. Grimm D, Elsner D, Schunkert H, Pfeifer M, Griese D, Bruckschlegel G, *et al.* Development of heart failure following isoproterenol administration in the rat: role of rennin angiotensin system. Cardiovasc Res. 1998;37(1):91-100.
5. Rajadurai M, Prince PS. Comparative effects of *Aegle marmelos* extract and alpha tocopherol on serum lipids, lipid peroxides and cardiac enzyme levels in rats with isoproterenol-induced myocardial infarction. Singapore Med J. 2005;46(2):78-81.
6. Upaganlawar A, Gandhi H, Balaraman R. Isoproterenol induced myocardial infarction: protective role of natural products. J Pharmacol Toxicol. 2011;6:1-17.
7. Fakim AG. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspects Med.2006.;27(1):1-93.
8. Augusti K. Therapeutic values of onion (*Allium cepa L.*) and garlic (*Allium sativum L.*). Indian J Exp Biol. 1996;34(7):634–640.
9. Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx M, *et al*. Phenolic compounds and antioxidant activities of buckwheat (Fagopyrum esculentum Moench) hulls and flour. J Ethnopharmacol. 2000;72(1-2):35-42.
10. Hatano T, Kagawa H, Yasuhara T, Okuda T. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem Pharm Bull (Tokyo).1988;36(6):2090-2097.
11. Amin I, Zamaliha MM, Chin WF. Total antioxidant activity and phenolic content in selected vegetables. Food Chem. 2004;87(4):581-586.

12. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem.1972;247(10):3170-3175.
13. Aebi H. Catalase *in-vivo*. Methods Enzymol.1984;105:121-126.
14. Slater TF, Sawyer BC. The stimulatory effects of carbon tetrachloride and other halogenoalkanes or peroxidative reactions in rat liver fractions *in vitro*. General features of the systems used. Biochem J.1971;123(5):805-814.
15. Vivek B, Wilson E, Nithya SV, Velmurugan C, Kannan M. Cardioprotective activity of *shilajit* in isoproterenol - induced myocardial infarction in rats: A biochemical and histopathological evaluation. Int J Res Phytochem Pharmacol. 2011;1(1):28-32.
16. Bloom S, Davis DL. Calcium as a mediator of isoproterenol induced myocardial necrosis. Am J Pathol. 1972;69(3):459-470.
17. Rahmathulla SB, Lakshmi Devi K. Origination and development of isoproterenol-induced myocardial infarction in male wistar rats. Int Res J Pharm.2013;4(5):26-35.
18. SabeenazFarvin KH, Anandan R, Kumar SH, Shiny KS, Sankar TV, Thankappan TK. Effect of squaleneon tissue defense system in isoproterenol-induced myocardial infarction in rats. Pharmacol Res.2004;50(3):231-236.
19. Mohan H. Text book of Pathology, Anshan Publishers, 2005, 5th edition, 324-325
20. Radhika S, Smila K H, Muthezhilan R. Cardioprotective activity of Hybanthus enneaspermus (Linn.) on isoproterenol induced rats. Ind J Fund Appl Life Sci. 2011;1(3):90-97.
21. Fukai T, Ushio-Fukai M. Superoxide dismutases: role in redox signaling, vascular function, and diseases. Antioxid Redox Signal. 2011;15(6):1583–1606**.**
22. Laukkanen MO. Extracellular superoxide dismutase: growth promoter or tumor suppressor? Oxid Med Cell Longev. 2016;2016:3612589.
23. Chelikani P, Fita I, Loewen PC. Diversity of structures and properties among catalases. Cell Mol Lif Sci. 2004;61(2):192–208.
24. Armstrong D, Browne R. The analysis of free radicals, lipid peroxidases, antioxidant enzymes and compounds related to oxidative stress as applied to the clinical chemistry laboratory. Adv Exp Med Biol. 1994;366:43-58.
25. Lef'evre G. Evaluation of lipid peroxidation by measuring thiobarbituric acid reactive substances. Ann Biol Clin (Paris). 1998;56(3):305-319.
26. Yagi K. Simple procedure for specific assay of lipid hydroperoxides in serum or plasma. Methods Mol Biol. 1998;108:107-110.
27. Udayan PS, Venkatesh S. Some common medicinal plant used by the Nayaka community, savandurga forest of Magaditaluk, Bangalore District, Karnataka, India. J Nat Remedies. 2005,5(1):35-40.
28. Cheng A, Chen X, Jin Q, Wang W., Shi J., *Liu* Y. Comparison of phenolic content and antioxidant capacity of red and yellow onions. Czech J Food Sci.2013;31(5):501–508.
29. Srivastava MP, Tiwari R, Sharma N. Assessment of phenol and flavonoid content in the plant materials. J New Biol Rep. 2013;2(2):163-166.
30. NehaNausheen Q, Ayaz Ali S, Subur K. Cardioprotective and antioxidant activity of onion *(Allium cepa)* leaves extract in doxorubicin induced cardiotoxicity in rats. Annals Exp Biol. 2014;2(2):37-42.