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ORIGINAL ARTICLE

Synergistic Protective Effect of Allium Cepa Linn and Amlodipine Against Isoproterenol Induced Myocardial Infarction in Rats

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ABSTRACT

Objectives: Allium cepa L. is an important dietary vegetable that has been used as a herbal medicine for centuries because of its potent antioxidant properties. Recently, amlodipine has been widely recognized for its potent antioxidant properties, attributed to its unique interactions with the membrane lipid bilayer. This mechanism may contribute to its cytoprotective action against cardiovascular disease. This study aimed to examine the synergistic protective effects of Allium cepa L. and amlodipine against isoproterenol-induced myocardial infarction in rats.

Methods: Male Wistar albino rats were divided into six groups (n=6). Daily pre-treatment with Allium cepa L., amlodipine, and their combination for respectively period of 30 days. At the end of this period, rats were administered isoproterenol (85 mg/kg, s. c.) for two consecutive days to induce myocardial injury. After induction, the rats were anaesthetized with ketamine (22 mg/kg, i. m.) and sacrificed, and biochemical assays of heart tissues were performed.

Findings: The results revealed the cardioprotective effect observed in the combination group showed significantly reduced isoproterenol-induced elevations in diagnostic marker enzyme levels and almost restored normal levels. This combination demonstrated a synergistic antioxidant effect by inhibiting the induction of lipid peroxidation and increasing superoxide dismutase activity. Histopathological observations supported these biochemical results.

Novelty: Allium cepa L. and amlodipine can be used in combination as cardioprotective agents against myocardial infarction.

Keywords: Allium cepa L; Amlodipine; Isoproterenol; Myocardial Infarction

INTRODUCTION

Ischaemic heart disease (IHD) is a major non-communicable disease that has become an important problem worldwide. Acute myocardial infarction (AMI) is the most significant cause of ischaemic heart disease and is caused by an imbalance between coronary blood supply and myocardial demand. Reactive oxygen species (ROS) play a significant role in the pathogenesis of myocardial damage, which is a key contributing factor in the development of various cardiovascular diseases. This condition is often linked to an imbalance between ROS levels and antioxidant defence mechanisms[1]. Isoprenaline (ISO) is a potent non-selective beta-adrenergic agonist that exhibits low affinity for alpha-adrenergic receptors. It is known to produce infarct-like necrosis of the myocardium at high doses[2]. The isoprenaline-induced MI model is widely utilized to assess the cardioprotective effects of different medications[3]. Administering isoprenaline in large amounts leads to myocardial lesions similar to those observed in human myocardial infarction. While contemporary medications are efficient in preventing heart ailments, their application is restricted due to a range of adverse consequences[4].

An increasing number of plant-based dietary elements and food antioxidants have been acknowledged for their role in promoting cardiovascular health[5]. Research has indicated that Allium cepa Linn, which belongs to the Liliaceae family, possesses qualities that may help guard against a range of chronic ailments[6]. This is likely due to the presence of a significant quantity of the flavonoid quercetin in Allium cepa L., which has been shown to offer protection against cardiovascular diseases. Organosulphur compounds present in Allium cepa L. have been found

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to offer several health benefits, including a decrease in cholesterol and blood pressure levels. Additionally, these compounds have been shown to help in the prevention of heart disease, inhibit the occurrence of strokes, and stimulate the immune system[7]. The Allium family, comprising garlic and onions, has been found to possess the ability to mitigate the harmful consequences of cardiovascular risk factors. It is believed that the beneficial effects of these vegetables are related to their antioxidant properties. Fewer publications have reported the beneficial cardiac effects of onions compared with those of garlic. Amlodipine, a calcium channel antagonist, has been used as an effective antihypertensive agent. Calcium-channel antagonists exert beneficial effects on the myocardium by inhibiting the slow Ca^{2+} inward current through L-type Ca^{2+} channels into cardiac cells. In light of these factors, the current study was conducted to assess the synergistic cardioprotective activity of onion extract and amlodipine in an ischaemic myocardial infarct model in Wistar albino rats.

METHODOLOGY

Collection of Material

Fresh bulbs of Allium cepa L. were purchased from a local market in Bangalore. Samples were randomly selected from the shelf based on their freshness.

Preparation of Allium cepa L Extract

Allium cepa L. bulbs were thoroughly rinsed, dried, and blended into a paste. The extract was obtained from fresh bulbs that were squeezed and filtered. The filtrate extract was prepared daily by using the same procedure.

Preliminary phytochemical screening

Preliminary phytochemical tests were performed to determine the different chemical constituents present in Allium cepa L. extract.

Tests for alkaloids

The extract was evaporated and to the residue, dilute HCl was added and properly mixed and filtered. The filtrate was tested for the presence of alkaloids.

- **Dragendroff's test:** Few drops of Dragendroff's reagents were added to 2-3 mL of filtrate, orange brown-coloured precipitates confirms the presence of alkaloids.
- **Mayer's test:** Few drops of Mayer's reagent was added to 2-3ml of filtrate, the formation of the precipitates indicates the presence of alkaloids.
- **Hager's test:** The extract was treated with few drops of Hager's reagent (saturated picric acid solution). The formation of yellow precipitate shows a positive result for the presence of alkaloids.
- **Wagner's test:** 2 mL of Wagner's reagent (iodine in potassium iodide) was added to 1 mL of extract. The presence of alkaloids was confirmed by the formation of a reddish-brown precipitate.

Tests for carbohydrates

- **Benedict's test**: Equal volumes of Benedict's reagent and the extract were mixed in a test tube and heated for 5 min in a boiling water bath. Yellow, green, and red solutions indicate the presence of reducing sugars.
- **Molish's test**: 2 mL of Molisch's solution with crude plant extract and 2 mL of concentrated H_2SO_4 was mixed and poured along the side of the test tube. The appearance of a violet ring in the interphase of the test tube indicated the presence of carbohydrates.
- **Barfoed's test**: Equal volumes of Barfoed's reagent (copper acetate in water and glacial acetic acid) and the extract were mixed and heated for 1-2 minutes in a boiling water bath and cooled. The red precipitate indicates the presence of sugars.
- **Felhing's test:** One millilitre of Fehling's A (copper sulphate in water) and Fehling's B solution (sodium tartrate) were mixed in a test tube and boiled for 1 min. An equal volume of the test solution was added and heated in a boiling water bath for 5-10 min. The brick-red precipitate confirms the presence of carbohydrates.

Tests for anthraquinone glycosides

- **Borntrager's test:** Dilute H_2SO_4 was added to 3 ml of Allium cepa L. extract, boiled, and filtered. Equal volumes of benzene and chloroform were added to the cooled filtrate, shaken well, and the organic layer was collected. A few drops of strong ammonia solution were shaken slightly, and the test tube was kept aside for a few minutes. The lower ammoniacal layer turned pink or red, indicating the presence of anthraquinone glycosides.

Tests for saponins glycosides

- **Foam test:** The stock extract solution (1 mL) was diluted with 20 mL of distilled water in a test tube and vigorously shaken. The presence of a foam layer on top of the test tube indicated the presence of saponins.

Cardiac glycosides

- **Legal test:** 1 mL of pyridine was added to 1 mL of sodium nitroprusside, and changing the pink colour to red indicated the presence of glycosides.
- **Keller-Killiani test:** The extract was mixed with a few drops of glacial acetic acid and a ferric chloride solution, concentrated sulphuric acid was added, and the formation of two layers was observed. A lower reddish-brown layer and upper acetic acid layer which turn bluish green, would indicate a positive test for

glycosides.

Tests for Flavonoids

- **Shinoda test:** To 3 mL of the extract, 5 mL of 95% ethanol and a few drops of concentrated HCl and magnesium turnings (0.5 g) were added. The pink colour indicated the presence of flavonoids.
- **Alkaline reagent test:** The extract was treated with sodium hydroxide solution and showed an increase in the intensity of the yellow colour which would become colourless upon addition of a few drops of dilute hydrochloric acid, indicating the presence of flavonoids.

Tests for tannins

- **Lead acetate solution:** The extract was treated with a few drops of lead acetate (10%) solution, resulting in the formation of a yellow precipitate, indicating the presence of tannins.
- **Ferric chloride solution:** 5% $FeCl_3$ solution was added to 2-3 mL of aqueous or alcoholic extract, and the formation of a deep blue-black colour indicates the presence of tannins.

Tests for steroids and terpenoids

- **Libermann-Burchard reaction:** A few drops of acetic anhydride were added to the crude extract, which was boiled and cooled. Concentrated sulphuric acid was then added to the sides of the test tube. Formation of a brown ring at the junction of the two layers was observed. The green colouration of the upper layer and the formation of a deep red colour in the lower layer indicate a positive test for steroids and triterpenoids, respectively.
- **Salkowski test:** 2mL chloroform and 2mL concentrated H_2SO_4 were then added to the extract (2 mL) and mixed thoroughly. The chloroform layer appears red and the acid layer shows greenish-yellow fluorescence which indicates the presence of steroids and terpenoids.

Evaluation of in vitro antioxidant activity

DPPH radical scavenging activity: (1, 1-diphenyl-2-picrylhydrazyl[8]

Antioxidant activity of Allium cepa L. extract was measured using the 2,2-diphenyl-2-picrylhydrazyl (DPPH) method. One millilitre of DPPH (0.01mM) was added to 3 mL of METP at various concentrations. The reaction mixture was then incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm against a blank. The free radical-scavenging activity of the plant extract was determined by comparison with that of the methanol control. The % DPPH scavenging activity is calculated using formula:

$$\%\ DPPH\ Scavenging\ Activity = \frac{A(Control - A(Test)}{A(Control)} X\ 100$$

Reducing power activity[9]

Various concentrations of onion extract in 1.0 mL of deionized water were mixed with phosphate buffer (2.5 mL, 0.2M, pH 6.6) and potassium Ferricyanide (2.5 mL, 1%) and incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared ferric chloride solution (0.5 mL, 0.1%). Absorbance was measured at 700 nm. Phosphate buffer (pH 6.6) was used as blank solution. Ascorbic acid at various concentrations was used as a standard. The % increase in reducing power is calculated using the formula:

$$\%\ Increase\ in\ Reducing\ Power = \frac{A(Test) - A(Blank)}{A(Blank)}\ X\ 100$$

where A (Test) is the absorbance of the test solution, and A (Blank) is the absorbance of the blank. The antioxidant activity of the extract was compared with that of the standard ascorbic acid.

In Vivo study experimental Animals

All experiments were carried out with Male Wistar albino rats weighing 200-250g, obtained from the Experimental Animal Care Centre, Krupanidhi College of Pharmacy (Rajiv Gandhi University of Health Science). Animals were housed in polypropylene cages (three rats per cage) lined with husk, renewed every 24 h, under a 12 h light/dark cycle at around 24 °C with 50% humidity. The rats had free access to tap water and a standard pellet diet (Purina Chow). The study protocol was approved by the Institutional Animal Ethics Committee (2016/PCOL/05/KCP/IAEC). The animals were maintained under standard conditions in an animal house according to the guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA).

Experimental Model and experimental groups

Male Wistar Albino rats were weighed and divided into the following groups consisting of six animals each group[10–12].

- **Group 1**: Negative group: Normal control rats (rats fed a normal diet).
- **Group 2**: Positive group: isoproterenol (85 mg/kg, s. c.) control rats.
- **Group 3**: Rats were pre-treated with Allium cepa L. extract (7.5 ml/kg /day, p. o.) and then injected with isoproterenol (85 mg/kg, s. c.).

- **Group 4**: Rats were pre-treated with Allium cepa L. extract (10 ml/kg/day, p. o.) and then injected with isoproterenol (85 mg/kg, s. c.).
- **Group 5**: Rats pre-treated with amlodipine (5 mg/kg/day, p. o.) and then injected with isoproterenol (85 mg/kg, s. c.).
- **Group 6**: Rats were pre-treated with a combination of Allium cepa L. extract (10 ml/kg/day, p. o.) and amlodipine (5 mg/kg/day, p. o.), and then injected with isoproterenol (85 mg/kg, s. c.). The experiment continued for 30 days. Animals were pre-treated with Allium cepa L. extract for 30 days, and at the end of the experimental period on the 29th and 30th day, rats received isoproterenol (85 mg/kg, s. c.), and injected once every 24 h.

Body weight and heart weight

The body weight of the rats was monitored at regular intervals during the experimental period. At the end of the experiment, the animals were sacrificed, their hearts were removed and weighed, and histological examinations were performed. The relative heart weight to body weight ratio was calculated to assess the degree of myocardial weight gain[13].

Biochemical Parameters in Serum

At the end stage of the study, animals were anaesthetized with Ketamine (22-24 mg/kg- i.m), the blood was collected by retro-orbital route without using anticoagulant and serum was separated for the estimation of various cardiac marker enzymes such as SGOT, SGPT, LDH and CK-MB in serum were estimated spectrophotometrically, using Erba Diagnostic kits. All marker enzymes were expressed as U/L.

Estimation of Serum Glutamic Oxaloacetic Transaminase (SGOT or AST[14,15]

SGOT was calculated using the formula - SGOT (AST) activity [IU/L] = ΔA/min X Factor (3376)

Estimation of Serum Glutamate Pyruvate Transaminase (SGPT

SGPT was calculated using the formula - SGPT (ALT) activity [IU/L] = ΔA/min X Factor (3376)

Estimation of serum lactate Dehydrogenase (LDH[16,17]

LDH was calculated using the formula (U/L) = ΔA /min X concentration of calibrator (IU/L)

Estimation of serum creatine kinase isoenzyme (CK-MB[18–20]

LDH was calculated using the formula (U/L) = f x ΔA/min, where, f = factor - 4127 (at 340 nm)

Measurement of Myocardial Infarct Size Procedure

A direct triphenyl tetrazolium chloride (TTC) assay according to the method was used to determine the myocardial infarct size. The heart was transversely cut across the left ventricle, and sections of 2–3 mm thickness were incubated in 1% TTC solution prepared in phosphate buffer (pH 7.4) for 30 min at 37 °C, followed by fixation with 10% formalin. The non-ischaemic myocardium and viable ischaemic myocardium were stained red, whereas the infarcted myocardium appeared pale grey or white. The slices were photographed using a digital camera, and the percentage of infarctions was analyzed using Adobe Photoshop software.

In vivo antioxidant activity

After collecting blood samples, the animals were sacrificed by cervical dislocation. The heart was excised, rinsed in ice-cold normal saline solution followed by cold 0.15 M Tris-Hcl (pH 7.4), blotted dried, and weighed. A 10% w/v homogenate was prepared in 0.15 M Tris-Hcl buffer and was used for the estimation of lipid peroxidation and superoxide dismutase.

Estimation of Superoxide Dismutase (SOD[21]

To 100 μl of 10% heart tissue homogenate, a mixture containing 1 ml sodium carbonate, 0.4 ml NBT and 0.2 ml EDTA was added and a zero-minute reading was taken at 560 nm. The reaction was initiated by adding 0.4 ml of 1 mM hydroxylamine HCL. The reaction mixture was then incubated at 25 °C for 5 min. The reduction in NBT was measured at 560 nm. A parallel control without the tissue homogenate was prepared in the same manner. One enzymatic unit of SOD is equal to the amount in the form of protein in 100μl of 10% tissue homogenate required to inhibit the reduction of 24mM NBT by 50% and is expressed as units/mg of protein. Enzyme concentration was calculated using the following formula:

Unit/mg = 10000/ μgm of enzyme resulting in $\frac{1}{2}$ max inhibition

Estimation of Lipid Peroxidation (LPO[21–23]

One millilitre of 25% trichloroacetic acid (TCA) and 1 mL of thiobarbituric acid (TBA) were added to 1 ml of tissue homogenate. The solution and blank were heated in a boiling water bath for 20 min, cooled, and centrifuged at 4000 g for 20 min. The absorbance of the supernatant was measured at 535 nm against a blank using a UV-VIS spectrophotometer. MDA concentration was calculated using a molar extinction coefficient of 1.56x105 L mol^{-1} cm^{-1} and expressed in nmol g-1 of tissue[24].

Histopathological examination[25]

At the end of the study, all rats were sacrificed by anesthesia followed by cervical dislocation, and the hearts

were collected from each group and washed in ice-cold saline. Heart tissues were immediately fixed in 10% buffered neutral formalin solution and processed for histopathological analysis.

Statistical analysis

Statistical significance was assessed using one-way analysis of variance (ANOVA), followed by Dunnett's test. The value is expressed as the mean ± SEM, where $P<0.05$ was considered significant.

RESULTS

Preliminary Phytochemical Investigation

Phytochemical screening was performed to determine the chemical composition of the drug. Allium cepa L. extract showed positive results for alkaloids, carbohydrates, saponin glycosides, flavonoids, cardiac glycosides, steroids, and terpenoids.

In vitro Antioxidant Activity

DPPH free Radical Scavenging Assays

Free radical scavenging activity was observed at different concentrations. The radical scavenging activity of Allium cepa L. extract increased with increasing concentrations, with 13.5%, 31.81%, 44.30%, 55.55, 67.17%, and 74.74% scavenging activity at 5, 10, 15, 20, 25, and 30 mg/ml, respectively. The IC_{50} values were found to be 18.39 mg/ml. The radical scavenging activity of standard ascorbic acid increased with increasing concentration, with 24.74%, 39.9%, 48.48%, 58.48%, 72.9%, and 90.45% scavenging activity at 5, 10, 15, 20, 25, and 30 μg/ml, respectively. The IC_{50} value was found to be 15.17 μg/ml. This result indicates that Allium cepa L. extract exhibited the ability to quench DPPH radicals, indicating that Allium cepa L. extract is a good antioxidant with radical-scavenging activity. However, the antioxidant activity of Allium cepa L. extract was lower than that of the standard ascorbic acid.

Reducing Power Activity

At 5, 10, 15, 20, 25 and 30 mg/ml, % reducing power of Allium cepa L. extract were found to be 4.5%, 11.38%, 39.28%, 73.26%, 96.1%, 116.31% respectively. The IC50 values were found to be 15.83 mg/ml. The reducing power of the Allium cepa L. extract might be due to its hydrogen-donating ability. For the standard solutions of ascorbic acid at different concentrations, such as 5, 10, 15, 20, 25, and 30 μg/ml, the estimated % reducing power of ascorbic acid was 12.8%, 48.26%, 75.69%, 112.5%, 169.9%, and 181.9%, respectively. The IC_{50} value was found to be 10.45 μg/ml. It is possible that the Allium cepa L. extract contains high amounts of reduction, which could react with radicals to stabilize and terminate the radical chain reaction. However, the antioxidant activity of Allium cepa L. extract was lower than that of the standard ascorbic acid.

In vivo study

Effect of Allium cepa L., amlodipine and their combination on heart weight to body weight ratio and % mortality in isoproterenol infarcted rats

The effects of Allium cepa L. extract, amlodipine, their combination, and isoproterenol treatment on heart weight, body weight, and mortality are shown inTable 1. No significant difference in body weight was observed at baseline or at the end of the experiment. The isoproterenol-treated animals showed a slight reduction in body weight. In the present study, the heart weight of rats treated with isoproterenol (85 mg/kg administered at 24 h interval for 2 days) was significantly higher than that of the normal control rats. Pre-treatment with Allium cepa L. extract, amlodipine, and their combination significantly reduced the increase in heart weight/body weight ratio in isoproterenol-treated rats. Twelve hours after the last isoproterenol injection, the total mortality rate of the isoproterenol group was 16%, with no mortality observed in the control, amlodipine, or combination treatment groups.

Effect of Allium cepa L., amlodipine, and their combination on isoproterenol-induced changes in serum biochemical parameters

Isoproterenol treatment (85 mg/kg administered at 24 h intervals for 2 days) caused a significant increase in the serum activities of myocardial injury marker enzymes (lactate dehydrogenase, creatine kinase, alanine aminotransferase, and aspartate aminotransferase) when compared to control rats. Prior treatment with Allium cepa L., amlodipine and their combination of these agents showed a significant and restored the serum diagnostic marker enzymes to near-normal levels following isoproterenol treatment (Table 1).

Measurement of myocardial infarct size

Detection of myocardial infarct size by the direct staining method using 2,3,5 triphenyltetrazolium chloride (TTC) dye, which forms a red formazan precipitate with dehydrogenase of the viable myocardial tissue, shows normal viable tissue with red color/dark spots. The size of the myocardial infarction stained with TTC is shown in Figure 1 and Table 2. ISO administration resulted in a large unstained area with more necrotic patches. However, the heart slices of the combination group treated with Allium cepa L. and amlodipine rats exhibited a major portion stained positively, showing tissue viability with less necrotic tissues compared to the individual treated group. The isoproterenol group showed increased infarction size (57.39%), which was significantly reduced to 17.81% with combined pre-treatment with Allium cepa L. and amlodipine.

Table 1: Effect of Allium cepa L. extract, amlodipine and their combination on heart weight to body weight ratio on Isoproterenol treated rats and LDH, CK-MB, AST, ALT in serum isoproterenol (85 mg / kg / day, s.c) treated, myocardial infracted rats

Sr. No.	Animal Group	Body Weight (gm)		HeartWeight (gm)	Heart weight/ Body Weight (%)
		Initial	Final		
1.	Negative Control	247.2±0.945	255.5±1.118	0.84±0.014	0.26±0.0144
2.	Isoproterenol	248.0±1.148	244.7±3.116	1.10±0.006***	0.41±0.008***
3.	Low Dose + ISO	248.3±0.988	251.8±0.909	1.02±0.004***	0.38±0.014*
4.	High Dose + ISO	248.7±1.358	251.0±1.770	0.96±0.011***	0.36±0.011**
5.	Amlodipine + ISO	243.7±1.282	256.8±1.078	0.93±0.015***	0.35±0.010**
6.	Combination + ISO	248.0±1.125	254.5±0.718	0.88±0.007***	0.30±0.006***
Sr. No.	**Animal Group**	**CK-MB**	**LDH**	**AST**	**ALT**
1.	Negative Control	47.8±2.301	123.1±2.227	26.2±1.925	21.1±1.118
2.	Isoproterenol	162.7±8.578***	278.0±4.787***	73.2±3.130***	68.3±3.359***
3.	Low Dose + ISO	132.0±2.611***	175.4±3.202***	62.5±3.208*	54.5±3.459**
4.	High Dose + ISO	114.6±3.600***	152.0±2.452***	57.1±2.520***	48.1±2.154***
5.	Amlodipine + ISO	99.4±2.275***	149.3±2.621***	52.6±0.926***	43.3±2.154***
6.	Combination + ISO	66.1±3.186***	132.9±1.536***	32.9±1.380***	28.5±1.729***

All the values were expressed as Mean± SEM (N= 6). *P<0.05, **P<0.01, ***P<0.001; ANOVA was applied followed by Dunnett's test. CPK-MB, LDH, AST, and ALT concentrations are expressed in U/l. Comparisons were made between the Negative control and ISO groups; and, ISO induced group with Low dose, High dose, Amlodipine and Combination treated group

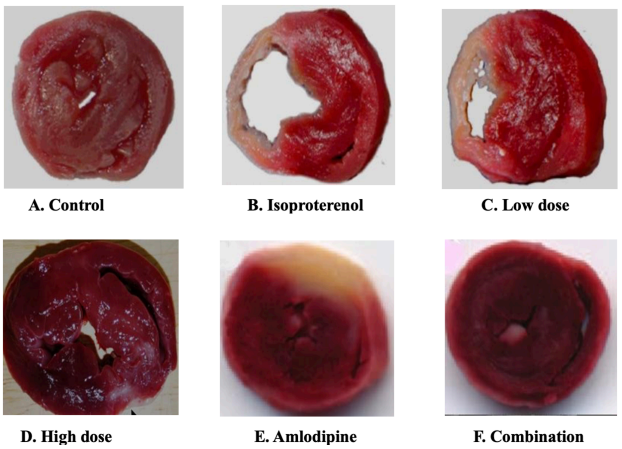

Fig. 1: Comparison of infarct area in transversely sectioned hearts after the onset of myocardial infarction

Table 2: Effect of Allium cepa L., amlodipine and their Combination pretreatment on % infarct size

Sl. No.	Animal Group	% Infarct size
1.	Negative Control	0
2.	Isoproterenol	57.39±0.808***
3.	Low Dose + ISO	41.333±0.793***
4.	High Dose + ISO	30.61±0.645***
5.	Amlodipine + ISO	26.39±0.832***
6.	Combination + ISO	17.81±0.745***

All the values were expressed as Mean± SEM (N= 6). *P<0.05, **P<0.01, ***P<0.0 ANOVA was applied, followed by Dunnett's' test. Comparisons were made between the Negative control and ISO groups; and, ISO induced group with Low dose, High dose, Amlodipine and Combination treated groups

In vivo antioxidant study

Lipid peroxidation

In the present study, we observed a significant elevation in lipid peroxidation to an extent of, (12.35±0.332 nmol/mL) in the isoproterenol-induced group as compared to normal control rats which exhibited (1.192±0.018 nmol/mL) in heart tissue. The results clearly depict the state of myocardial injury. Pre-treatment with Allium cepa L. extract, amlodipine, and their combination significantly inhibited isoproterenol-induced changes in lipid peroxidation. The combination of both agents exerted a synergistic antioxidant effect by blocking the induction of lipid peroxidation (2.71±0.248 nmol/ml) (Table 3).

Estimation of Superoxide Dismutase

In the present study, rats injected with isoproterenol showed significantly reduced superoxide dismutase levels relative to the control (1.335±0.026 vs. 4.632±0.0563). Pre-treatment with Allium cepa L. extract, amlodipine, and their combination significantly inhibited isoproterenol-induced changes in cardiac antioxidant parameters. The combination of Allium cepa L. extract and amlodipine significantly increased the level of superoxide dismutase (4.183±0.0549) (Table 3).

Histopathological Examination

Histopathological observations of the normal control group showed clear integrity of the myocardial membrane and endocardium. Isoproterenol (85 mg/kg administered at 24 h intervals for 2 days) showed massive vascular haemorrhage, myocardial necrosis, endocardium oedema, and mononu-

Table 3: Effect of Allium cepa L., amlodipine and their combination on the cardiac content Malondialdehyde and on superoxide dismutase activity in isoproterenol induced myocardial infarction in rats

Sr. No.	Animal Group	MDA (nmoles/ml)	SOD Units/ml
1.	Negative Control	1.192±0.018	4.632±0.0563
2.	Isoproterenol	12.35±0.332***	1.335±0.026***
3.	Low Dose + ISO	7.46±0.154***	2.313±0.07***
4.	High Dose + ISO	6.44±0.114***	3.150±0.063***
5.	Amlodipine + ISO	5.61±0.110***	3.175±0.1004***
6.	Combination + ISO	2.71±0.248***	4.183±0.0549***

All the values were expressed as Mean± SEM (N= 6). *P<0.05, **P<0.01, ***P<0.001; ANOVA, followed by Dunnett's' test. Comparisons were made between the Negative control and ISO groups; and, ISO induced group with Low dose, High dose, Amlodipine and Combination treated groups

clear cell infiltration. Rats pre-treated with Allium cepa L. extract, amlodipine, and a combination of both agents prior to ischaemia (isoproterenol injection) exhibited a gradual repair of the myocardium. Maximal improvement was observed when the rats were administered a combination of both agents (Figure 2).

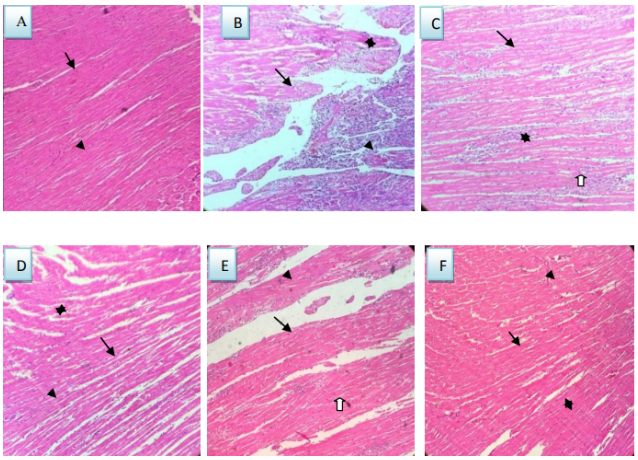

Fig. 2: Light photomicrographs in the wall of rat heartstained by H&E and magnified X10

A represent normal control heart with normal endocardium (arrow) and myocardium (arrow-head); **B** represent ischemic heart (induced by isoproterenol) showed severe endocardiac edema (arrow-head), mononuclear inflammatory cells infiltration (arrow) and massive myocardium coagulative necrosis (star); **C** represent heart tissue of rat received low dose Allium cepa L. extract prior to isoproterenol injection showed focal myocardium coagulative necrosis (star), focal areas of cellular infiltration (thick arrow), some congested vessels (arrow); **D** represent heart tissue of rat received high dose of Allium cepa L. extract prior to isoproterenol injection showed focal areas of coagulative necrosis (star), minimal cellular infiltration (thick arrow) and apparently healthy myocardium (arrow-head); **E** represent heart tissue of rat received amlodipine prior to isoproterenol injection showed focal areas of coagulative necrosis (arrow-head), sporadic vascular congestion (arrow) and minimal cellular infiltration (thick arrow); **F** Showed heart tissue of rat that received combination of Allium cepa L. extract and amlodipine prior to isoproterenol injection showed maximum reduction of vascular congestion (arrow), endocardiac edema (arrow-head) and most of myocardium was histological normal (star).

DISCUSSION

There is an urgent need for the clinical development of safe and non-toxic cytoprotective agents to adequately manage cardiovascular diseases, particularly myocardial infarction. MI occurs due to an imbalance between myocardial blood supply and demand, resulting in the development of ischaemia, followed by necrosis[25]. Catecholamine-induced MI serves as a standardized model to study the beneficial effects of many drugs on cardiac function. Isoproterenol, a synthetic catecholamine and β-adrenergic agonist, increases myocardial oxygen demand by its positive inotropic and chronotropic actions, leading to ischaemic necrosis[26]. The present study was initiated to investigate whether Allium cepa L. extract could offer cardioprotection against isoproterenol-induced myocardial infarction, and if so, whether Allium cepa L. extract in conjunction with amlodipine could result in a synergistic cardioprotective effect against isoproterenol-induced myocardial injury. In the present study, the cardioprotective effects of Allium cepa L. extract, amlodipine, and their combination were evaluated by in vitro and in vivo studies.

The presence of alkaloids, carbohydrates, saponin glycosides, flavonoids, cardiac glycosides, steroids, and terpenoids in Allium cepa L. extract suggests its rich chemical composition. These compounds are known for their diverse pharmacological activities, including their antioxidant and cardioprotective effects. The in vitro antioxidant activity of Allium cepa L. extract was determined by DPPH and Reducing power method using spectrophotometer. The antioxidant properties of Allium cepa L. were found to be concentration-dependent. Hence, it was found to possess free radical scavenging activity. Allium cepa L. extract showed a concentration-dependent increase in reducing properties. The IC50 value of ascorbic acid was found to be 10.45 μg/ml and IC50 value of Allium cepa L. extract was found to be 15.83 mg/ml. The results showed that Allium cepa L. is rich in flavonoids, which are known to have higher antioxidant activity.

An increase in relative heart weight is an index of cardiac hypertrophy. Heart hypertrophy is an adaptive response to any intrinsic or extrinsic stimuli, or during the remodelling that occurs during the evolution of ischaemic heart disease. Hypertrophy is a compensatory response to necrosis of the heart muscle caused by severe stress of the heart induced by the administration of isoproterenol. This may be due to an increase in overall protein biosynthesis during the development of hypertrophy accompanied by oedema or overexpression of genes encoding proteins involved in the contractile unit. The elevation of cytoplasmic calcium during MI could also be responsible for the activation of intracellular signals governing the hypertrophic response of cardiac cells. An augmentation in heart weight might be ascribed to an upsurge in water content, or the presence of oedematous intramuscular spaces[27] and increased protein content. In the present study, we observed a significant increase in heart weight and heart weight-to-body weight ratio (i.e. relative heart weight) in isoproterenol-induced rats. The relative heart weight (0.41±0.008%) in the positive control (isoproterenol-induced) group rats was significantly higher than that in the control rats (0.26±0.0144%). Pre-co-treatment with Allium cepa L. extract, amlodipine, and their combination significantly reduced the increase in heart weight observed in isoproterenol-induced rats, whereas more improvement was observed in the combination groups, which may be due to calcium channel antagonist, thus reducing calcium overload during stress conditions[28]. These results are consistent with a previous report[29], which observed extensive oedematous intramuscular space, accumulation of mucopolysaccharides, and cellular infiltration 4 h after induction of myocardial infarction. It has been proposed that a 1% increase in myocardial water content could result in a 10% reduction in myocardial function[30]. Myocardial cells are characterized by the presence of various enzymes and macromolecules. When these cells experience metabolic damage, they release extracellular fluid that serves as a diagnostic marker of myocardial injury[31]. The release of these enzymes indicates a change in the integrity and permeability of the plasma membrane owing to adrenergic stimulation. Biomarkers, including AST, ALT, CK, and LDH, are indicators of myocardial injury. In this study, the administration of ISO resulted in an enhanced level of diagnostic marker enzymes AST, ALT, LDH, and CK due to the leakage of these enzymes from the tissue to the blood serum as a consequence of damaged or ruptured cardiomyocytes and other cells. This occurred because of insufficient oxygen supply and oxidative damage to the myocardium, which weakened the cell membrane, making it more fragile, porous, or susceptible to rupture. Elevated levels of these enzymes signify the extent of cell necrosis and ISO-induced peroxidative damage to the cardiac muscle cells. The serum concentrations of CK and LDH are used as both early and late diagnostic indicators of MI. CK levels increase within 2–8 h of MI onset, and LDH starts to rise within 12–24 h of MI occurrence, reaching its peak at 2-3 days. Isoproterenol-induced myocardial infarction is associated with the release of cardiac enzymes from cells, which is influenced by alterations in plasma membrane integrity and/or permeability in response to β-adrenergic stimulation. The potential cause of this could be the harm caused to the sarcolemma by the β-agonist, resulting in increased permeability. This hypothesis is supported by the results of our study, as we observed a significant increase in serum levels of cardiotoxicity enzymatic indices (CK-MB, LDH, AST, and ALT) after isoproterenol treatment. Pre-treatment with Allium cepa L. extract, amlodipine, and the combination of both drugs prevented the increase in serum CK-MB, LDH, AST, and ALT induced by isoproterenol. Our biochemical findings were supported by the improvement in histological architecture of heart tissues in all treated groups, with maximal improvement in the combination group, suggesting that these drugs may have a potential protective effect against isoproterenol-induced cardiac damage. Hence, it has been reported that oral administration of Allium cepa L. extract prevents the prognosis of atherosclerosis (hardening of blood vessels) and reduces serum cholesterol levels[32]. The protective effect of Allium cepa L. extract could be attributed to its antioxidant properties, which reduce myocardial damage and consequently decrease the release of cardiac enzymes[33]. Additionally, pre-treatment of isoproterenol-induced rats with amlodipine and/or nicorandil produced a synergistic effect on myocardial marker enzymes and mitochondrial enzymes[34]. The extent of myocardial infarction was determined by direct staining with triphenyl tetrazolium chloride (TTC) dye. In the present study, we observed an increased infarct size which was indicated by a clear bright/grey spot in the heart slices of ISO-induced rats. The utilization of NADH by the free radicals produced by ISO and leakage of dehydrogenase enzymes from damaged myocardial cells are possible mechanisms that might prevent the conversion of TTC into TPF. This may be the reason for the colourless bright spots in ISO-induced rat hearts and reflects the presence of non-viable cells. This finding was also supported by the increased levels of LDH in the serum of ISO-induced rats, where LDH may be released from the myocardium. The increased infarction area in ISO administered group (57.39%), which was significantly reduced to (17.81%) with the combined pre-treatment with Allium cepa L. extract, amlodipine, and their combination, further supported the better protection from cardiac damage by the combination of Allium cepa L. and amlodipine. Our data also showed a significant increase in myocardial lipid peroxide levels following the subcutaneous injection of isoproterenol. It has been proposed that isoproterenol acts as a cardiotoxic agent, and its oxidative metabolism leads to excessive production of free radicals, which destroy myocardial cells[35].

Rats treated with Allium cepa L. extract, amlodipine, and their combination showed a significant decrease in lipid peroxidation within the myocardium, as compared to that of isoproterenol-infarcted rats. Interestingly, the combination of Allium cepa L. extract and amlodipine resulted in a nearly complete reversal of isoproterenol-induced lipid peroxidation to normal control values, indicating a synergistic antioxidant effect of these drugs within infarcted heart tissue. Our results are supported by previous reports that Allium cepa L. has potent antioxidant properties. On the other hand, it has been reported that lipophilic calcium antagonists, such as amlodipine, inhibit lipid peroxidation in cellular membranes by modulating the physicochemical properties of the membrane lipid bilayer, independent of calcium channel inhibition. This observation aligns with our results as well as with the data of previous studies, which reported that amlodipine pre-treatment reversed the increase in myocardial lysosomal enzyme activities within isoproterenol-infarcted rat myocardium with a consequent reduction in the extent of myocardial damage, which is due to the inhibitory effect of amlodipine on lipid peroxidation [36]. In the current study, isoproterenol induction caused oxidative stress in the heart tissues, as evidenced by the significant inhibition of antiperoxidative enzyme activities (superoxide dismutase) within the myocardium. This is consistent with the findings of several previous studies. The decrease in the function of these enzymes in rats treated with isoproterenol might be attributed to the heightened production of reactive oxidising agents, including superoxide and hydrogen peroxide, which have exceeded the collective ability of free radical scavenging enzymes to quench these radicals, resulting in myocyte lesions and a reduction of scavengers. Allium cepa L., amlodipine, and their combination pre-treated isoproterenol-induced rats showed significant elevations in the activities of antioxidant enzymes in heart tissue as compared with infarcted rats, which indicates that both drugs are capable of scavenging free radicals produced by isoproterenol and also explains the apparent reduction of myocardial damage. Amlodipine, on the other hand can reduce Oxidative stress as it contains an aromatic ring which can scavenge free radicals. Furthermore, the dihydropyridine ring in this drug can donate a proton, which stabilize free electrons [36]. This hypothesis is supported by the results of, which showed that pre-treatment with Allium cepa L. in combination with amlodipine significantly increased SOD activity when compared to myocardial infarcted rats. Histopathological assessments aligned with the outcomes of this study, indicating that the control group presented with an intact myocardial membrane without any apparent pathological changes. Architectural changes found in isoproterenol treated cardiomyocytes. Rats pre-treated with Allium cepa L. extract, amlodipine, and a combination of both drugs prior to ischaemia (isoproterenol injection) exhibited a gradual repair of the myocardium. Maximal improvement was observed when the rats were administered a combination of both agents.

CONCLUSION

This study demonstrated that oral pre-treatment with Allium cepa L. extract, amlodipine, and their combination significantly potentiated cardioprotective effects against ISO-induced myocardial infarction, making them potential candidates for the management of myocardial infarction. Further studies, including clinical trials, are warranted to validate these findings and explore their therapeutic potential in human subject.

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CONFLICT OF INTEREST

There are no conflicts of interest

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