ABSTRACT

The present study was carried out to explore the cardioprotective potency of pineapple (*ananas comosus*) with losartan in isoprenaline (ISO) induced acute and chronic myocardial damage in rats. The purpose of the study research was to uncover the potency of anti-inflammatory activity of pineapple on necrotic myocardium, in combination with angiotensin blocker losartan (LTN). In acute model, Sprague Dawley rats were pre-treated with losartan (LTN) (30 mg/kg, ten days, *po*), low, intermediate and high doses of pineapple (100, 250 and 500 mg/kg, 30 days, *po*) and subsequently subjected to ISO administration (150 mg/kg, *s.c*) for two consecutive days. The influence of prophylactic treatment was analysed by quantification of biomarkers, antioxidants and histopathological observations. LDH and CK-MB activities were studied in serum and heart tissue homogenate (HTH) where as SOD, catalase and TBARS were estimated in HTH.
There was significant elevation in LDH and CK-MB activities in HTH of all treated groups as compared to ISO control. SOD and catalase activities were also significantly increased in treated groups. In chronic model, ISO (3 mg/kg/day, s.c) was administered to Sprague Dawley rats treated with low, intermediate and high doses of pineapple (100, 250 and 500 mg/kg, p.o. thirty days) and LTN (30 mg/kg, p.o. ten days). Through retro orbital route blood is withdrawn for LDH and CK-MB estimations. Antioxidants were estimated by biochemical method and histopathological studies were carried out to support biochemical studies. Prior treatment of animals with Pine apple and LTN resulted in elevation of biomarker activities in heart tissue homogenate and decline in serum. The SOD and Catalase activities were significantly elevated with depletion of TBARS and histological scores in groups subjected to prophylactic treatment. Among various treatments, high dose of Pine apple and LTN was found to be most effective. From the present results it may be concluded that high dose of Pine apple (500 mg/kg) possess cardioprotective efficacy when given prophylactically in experimental animals. However, further studies should be carried out to explore the ultrastructural changes and underlying mechanisms of protection when these substances are used together.

**KEYWORDS:** Isoprenaline, Losartan, Pine Paple, Myocardial Damage, LDH, CK-MB, SOD, Catalase, TBARS.

**INTRODUCTION**

The treatment of diseases with medicines of plant origin is an integral part of many cultures throughout the world.\(^1,2\) Natural drugs are gaining greater acceptance from the public and the medical profession due to greater advances in understanding the mechanism of action by which herbs can positively influence health and quality.\(^3\) When herbs were combined with pharmaceutical agents, posing the possibility of potential interaction between the two groups of substances.\(^4\) Although modern drugs are effective in preventing the cardiovascular disorders, their use is often limited because of their side effects and adverse reactions.\(^5\) Hence it is imperative to promote credible research on the safety and efficacy of combined herb-drug treatment for variety of ailments including cardiovascular diseases.\(^6\) Ananças comosus belongs to the family Bromeliaceae, commonly called as pineapple.\(^7\) Pineapple mainly contains enzyme complex called protease (bromelain), which contains peroxidase, acid phosphate, several protease inhibitors and organically bound calcium which is found to have cardioprotective activity.\(^8\) It is mainly reported in treatment of Rheumatoid
arthritis,[9-12] Cancer[13-16] and Gastro intestinal disturbances.[17,18] And found to have actions like immunogenicity, platelet aggregation, wound healing,[19-25], antioxidant, antimicrobial and antidiarrheal. Its anti-inflammatory activity is helpful in the treatment of angioedema.[26] Bromelain on long term administration was found to have anti hypertensive effect.[27] Bromelain have the protective action on the myocardial ischemia-reperfusion by the up regulation of survival kinases known to attenuate the process of apoptosis particularly the serine or threonine kinase Akt is well established to play an important role in endothelial and cardiomyocyte cell biology that activates an antiapoptotic or prosurvival signaling cascade.[28]

Losartan is a competitive and selective Angiotensin II receptor antagonist, that is, it blocks the action of Angiotensin II on ATI Receptor[29] and lowers the blood pressure.[30] It isolated perfused rat heart, losartan reduced the duration of ventricular fibrillation induced by reperfusion.[31] However at high doses losartan has side effects[32] on all over body systems like allergic reactions, irregular heartbeat, fever, chills, or persistent sore throat; mental or mood changes; muscle pain or cramps; one-sided weakness; persistent headache; severe or persistent stomach pain (with or without nausea or vomiting); shortness of breath, severe nausea or vomiting; symptoms of liver problems (eg, dark urine, loss of appetite, pale stools, stomach pain, yellowing of the eyes or skin); symptoms of low blood pressure (eg, fainting, severe dizziness, light-headedness); tremor; unusual bruising or bleeding; unusual sweating; unusual tiredness or weakness; vision changes. So, necessity to be used along with other safe cardioprotective regimen. As pineapple has the great benefits of providing protection to myocardium and losartan is potent cardioprotective, it is of interest to determine the role of combined therapy of fresh pineapple juice at different doses and losartan during isoprenaline induced acute and chronic cardiac dysfunction in rats.

OBJECTIVES

Objective of study: The objective of the present study will be to evaluate the potency of combined therapy of losartan and Ananas comosus during isoprenaline mediated cardiac dysfucntion in rats.

SPECIFIC OBJECTIVES

1) To collect and authenticate the cloves of Ananas comosus.
2) To prepare and carryout phytochemical evaluation of Ananas comosus homogenate.
3) To standardized acute and chronic models of isoprenaline for induction of myocardial damage.

4) To select the dose of losartan.

5) To study the biochemical and antioxidant profile in serum and heart tissue homogenate upon chronic administration of Ananas comosus homogenate with or without losartan.

6) To explore the role of losartan in presence/absence of Ananas comosus during isoprenaline induced acute myocardial derangement in rats.

7) To study the benefits of adding Ananas comosus to cardioprotective therapy of losartan in isoprenaline mediated chronic myocardial damage in rats.

**METHODOLOGY**

**Experimental Animals**

Sprague Dawley rats of either sex weighing 150-200 gm and Male Swiss albino mice weighing between 25-30 gm were housed at 25° ± 5°C in a well-ventilated animal house under 12:12 h light dark cycle. Institutional Animal Ethics Committee approved the experimental protocol (KCP/IAEC-74/2011-12). The animals were maintained under standard conditions in an animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

**Preparation of Ananas comosus homogenate and Dose selection**

Pineapple fruits will be purchased from the local fruit market. The fruit will be peeled, sliced and ground into a paste and suspended in distilled water. Three different concentrations of the pineapple will be selected based on OECD guidelines\(^\text{[121]}\) and will be administered within 30 min of preparation. The dose selections of pineapple for isoproterenol model were done based on acute toxicity studies, carried out according to OPPTS (Office of Prevention, Pesticide and Toxic Substance) following the limit test procedure.\(^\text{[122]}\) The animals were fasted over night prior to the studies. Mice were divided into three groups of three each. Test dose of 2 g/kg body weight, 3 g/kg body weight and 5 g/kg body weight were given orally to all group of mice. Mice were observed for 72 hours for mortality. 1/10\(^\text{th}\), 1/25\(^\text{th}\) and 1/50\(^\text{th}\) of the maximum safe dose corresponding to 500 and 250 mg/kg and 100mg/kg body weight were selected as high, intermediate and low doses respectively.

**Apparatus and chemical used**

Analytical balance (Schimadzu, Japan), Autoanalyser (Qualigens, Mumbai), Centrifuge, Colorimeter, Students Physiograph (INCO, India), Spectrophotometer, CK-MB (Crest
Biosystems, Coral Clinical Systems, Goa, India), LDH kits (Crest Biosystems, Coral Clinical Systems, Goa, India), DL- Isoproterenol hydrochloride (Sigma Aldrich, U.S.A), losartan (Sun Pharmaceuticals Industries), Copper sulphate, Disodium hydrogen orthophosphate (Merck Specialities Private Limited, Mumbai, India), EDTA (Nice Chemicals Pvt Ltd, Cochin, India), Ethanol (Hong yang chemical corp. China), Heparin (Gland Pharmaceutical Ltd, Hyderabad, India), Hydrogen peroxide, Hydroxylamine HCL, Ketamine (Prem Pharmaceutical Ltd, Indore, India), Malondialdehyde (MDA), N- Butanol, Nitro blue tetrazolium (NBT) (Loba Chemicals, Mumbai, India), Phenol reagent, Phosphoric acid, Potassium dihydrogen orthophosphate, Sodium carbonate (Merck Specialities Private Limited, Mumbai, India), Sodium chloride (Merck Specialities Private Limited, Mumbai, India), Sodium hydroxide (Merck Specialities Private Limited, Mumbai, India), Standard bovine albumin, Sucrose (S D Fine Chemicals, Mumbai, India), Thiobarbituric acid, Xylazine (Indian Immunological, Guntur, India).

**Phytochemical estimations of the Almond**

Bitter almond was subjected to qualitative analysis to investigate the presence of various phytochemical constituents such as alkaloids, carbohydrates, glycosides, phytosterols, proteins, amino acids, saponins, tannins and flavonoids.

**Test for Alkaloids**

1. **Hager’s Test:** Extract was treated with Hager’s reagent (saturated picric acid solution). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

2. **Mayer’s Test:** Extract was treated with Mayer’s reagent (potassium mercuric iodide solution). Formation of a cream coloured precipitate indicates the presence of alkaloids.

3. **Dragendorff’s Test:** Extract was treated with dragendorff’s reagent (potassium bismuth iodide solution). Formation of orange brown precipitate indicates the presence of alkaloids.

4. **Wagner’s Test:** Extract was treated with Wagner’s reagent (iodine potassium solution). Formation of reddish brown precipitate indicates the presence of alkaloids.

**Test for carbohydrates**

1. **Molisch Test:** Extract was treated with molisch reagent (α-naphthol in 95% ethanol) and few drops of sulphuric acid were added through the side of the test tube. Appearance of violet ring at the junction indicates the presence of carbohydrates.
2. Fehling’s Test: A small portion of the extract was treated with felhing’s reagent A (Copper Sulphate in water) and felhing’s reagent B (sodium potassium tartarate) and heated in a water bath. Formation of red colour precipitate indicated the presence of reducing sugars.

3. Barfoed’s Test: Extract was treated with barfoed’s reagent (copper acetate in water and glacial acetic acid), and heated in a water bath. Red coloured precipitate indicates the presence of sugars.

4. Benedict’s Test: Extract was treated with benedict’s reagent (copper sulphate + sodium citrate + sodium carbonate in water), and heated for 10 minutes. Red coloured precipitate indicates the presence of sugars.

**Test for Proteins and amino acids**

1. Millon’s Test: Extract was treated with millons’s reagent (mercuric nitrate in nitric Acid). Red colour indicates the presence of proteins.

2. Biuret Test: Extract was treated with sodium hydroxide. Copper sulphate solution was added drop wise. Violet colour is produced which indicates the presence of proteins.

3. Ninhydrin Test: Extract was treated with ninhydrin reagent and ammonia and heated. Violet colour indicates the presence of proteins.

**Test for Steroids, Triterpenoids and Cardiac Glycosides**

1. Salkowski Test: To the solution of extract in chloroform, a few drops of sulphuric acid were added and the moisture was shaken and allowed to stand for some time. Red colour is produced in the chloroform layer, which indicates the presence of steroids.

2. Liebermann-Burchard Test: Small portion of extract was dissolved in chloroform. To this 1ml acetic anhydride and then 2ml concentrated sulphuric acid was added through the side of the test tube. A reddish violet colour at the junction of two liquids indicates the presence of steroids, triterpenoids and cardiac glycosides.

3. Baljet Test: To the solution of extract in water a few drops of sodium picrate reagent was added. Formation of yellow colour indicates the presence of cardiac glycosides.

4. Keller Killani’s test: A portion of extract was treated with 1ml of glacial acetic acid and a few drops of ferric chloride were added to it. To this mixture 2ml concentrated sulphuric acid, carefully through the sides of the test tube. A reddish brown colour is formed at the junction of two layers and bluish green colour in the upper layer which indicates the presence of deoxy sugars in the carbohydrates.
Test for Tannins
1. **Ferric Chloride Test:** Extract was treated with ferric chloride solution. Formation of blue colour indicates the presence of tannins.
2. **Lead Acetate Test:** Extract was treated with lead acetate solution; yellow precipitate indicates the formation of tannins.

Test for Saponins
1. **Froth test:** Diluted 1ml of the extract with distilled water to 20ml and shaken in a graduated cylinder for 15min. One-centimeter layer of foam indicates the presence of saponins.
2. **Haemolysis test:** 2ml of 1% Nacl was mixed with 2ml of extract, to this 5 drops of blood was added. Haemolysis indicates the presence of saponin.

Test for Flavonoids
1. **Ferric Chloride Test:** Extract was treated with few drops of ferric chloride solution. Formation of blackish blue colour indicates the presence of flavonoids.
2. **Lead Acetate Test:** Extract was treated with lead acetate solution; yellow precipitate indicates the formation of flavonoids.

Experimental Models
The cardioprotective role of combined therapy of losartan with *Ananas comosus* will be determined in isoprenaline induced ischemia-reperfusion rats. The Sprague dawley rats of either sex will be divided into following groups consisting of six animals each:
1. Group-I- animals kept as control without pretreatment.
2. Group II- ISO control.
5. Group-V- *Ananas comosus* (30 days oral treatment).
Isoprenaline induced acute myocardial necrosis in rats
Male Sprague Dawley rats were treated for 30 days as per the above protocol. At the end of the treatment period, isoprenaline (150 mg /kg, s.c)\textsuperscript{[127]} was administered to all the animals (except the normal control) for two consecutive days. Forty eight hours after the first dose of ISO, the animals were anesthetised with ketamine (75mg/kg, i.p) and xylazine (8mg/kg, i.p)\textsuperscript{[128,129]} and blood was withdrawn by retro-orbital puncture. Serum was separated by centrifugation for the estimation of biomarkers (LDH, CK-MB). Thereafter the animals were sacrificed; three hearts were used for the preparation of homogenate to estimate biomarkers (LDH and CK-MB) and antioxidants (SOD and Catalase). Remaining three hearts were embedded in 10%v/v formalin solution in saline for histological examination.

The parameters estimated were
1. LDH and CK-MB activity in serum and heart tissue homogenate.
2. SOD, Catalase and TBARS activity in heart tissues homogenate.
3. Histopathological Studies: Scoring in myocardial tissue was determined from H-E transverse stain.

Isoprenaline induced chronic myocardial necrosis in rats.
Female Sprague Dawley rats were treated with pineapple for 30 days, 10 days of losartan and incorporation of losartan in the last week of 30 days of pineapple treatment in their respective groups, animals in all groups except group I will receive isoprenaline 3 mg/kg/day subcutaneously.\textsuperscript{[130]} At the end of the treatment period, the animals were anesthetised with ketamine (75mg/kg, i.p) and xylazine. (8mg/kg, i.p) and blood was withdrawn by retro-orbital puncture. Serum was separated by centrifugation for the estimation of biomarkers (LDH, CK-MB). ECG recordings were made for each animal using lead II method. Thereafter the animals were sacrificed; three hearts were used for the preparation of homogenate to estimate biomarkers (LDH and CK-MB) and antioxidants (SOD and Catalase). Remaining three hearts were embedded in 10%v/v formalin solution in saline for histological examination.

The parameters estimated were
1. LDH and CK-MB activity in serum and heart tissue homogenate.
2. SOD, Catalase and TBARS activity in heart tissues homogenate.
3. Histopathological Studies: Scoring in myocardial tissue was determined from H-E transverse stain.
Note
1. Animals were injected ISO approximately the same time each day.
2. Animals were kept under cool conditions, to prevent death of the animals due to hyperthermia and respiratory failure.

**Preparation of Heart Tissue Homogenate**[129]

The hearts removed after the ECG recording was made free of the adjacent vessels and fatty tissue mass with the help of scissors. Hearts were then cut open, rinsed with saline (0.9% NaCl) and dried using filter paper. The weight of the heart was recorded. Then the heart homogenate was prepared in ice cold 0.25M sucrose solution using a mortar and pestle. The homogenate thus obtained was centrifuged at 5000 rpm for 15 min. The supernatant was decanted and used for the estimation of CK-MB, LDH, SOD, catalase and TBARS.

**Procedure for Estimation of Biomarkers**

Biomarkers like CKMB and LDH were estimated using CKMB (NAC act) Kit and LDH (P-L) Kit respectively.

**Estimation of CK-MB**

CK is a dimeric molecule composed of M and B subunits which are immunologically distinct. It exists as CK-MM, CK-MB and CK-BB isoenzymes form and found in muscles, myocardial cells and brain respectively. Rise in the CK-MB level is an appropriate indicator of MI as its level does not increase in chest pain caused by angina or pulmonary embolism. The levels return to normal, in case there is no further myocardial damage.[131]

**PRINCIPLE**

CK-M fractions of CK-MM and CK-MB in the sample are completely inhibited by an anti CK-M antibody in the reagent. The activity of CK-B fraction is measured by the CK (NAC Act.) method.

**SAMPLE MATERIAL**

Serum (should be free from haemolysis) and heart tissue homogenate.

**PROCEDURE**

<table>
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<th>U.V kinetic</th>
<th>Interval:</th>
<th>60 sec</th>
</tr>
</thead>
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<td></td>
</tr>
<tr>
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<td>Sample Volume:</td>
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<tr>
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</tbody>
</table>
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Read Time: 180sec  
Linearity: 100U/L  
No. Of read: 4 

For the sample start assays 0.8 ml of enzyme reagent (L₁) and 0.05 ml of sample (serum/homogenate) are mixed in a clean dry Eppendorffs tube and incubated for 5 minutes following 0.5ml of starter reagent (L₂) was added. Then the mixture was shaken and fed into the auto analyser.

Estimation of LDH
LDH is found in many body tissues particularly heart, liver, skeletal muscles, kidney and erythrocytes. LDH is found in the form of isoenzyme. Levels of LDH increase in myocardial infarction, pulmonary diseases, hepatic diseases, haemolytic anaemia, renal diseases and muscular dystrophy.

PRINCIPLE
Lactate dehydrogenase catalyses the reduction of pyruvate with NADH to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the LDH activity in the sample.

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightarrow \text{Lactate} + \text{NAD}^+$$

PROCEDURE

<table>
<thead>
<tr>
<th>System Parameters</th>
<th>U.V Kinetics</th>
<th>Interval</th>
<th>60sec</th>
<th>Sample Volume</th>
<th>10µl</th>
<th>Reagent Volume</th>
<th>10µl</th>
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</thead>
<tbody>
<tr>
<td>Reaction</td>
<td>U.V Kinetics</td>
<td>Interval</td>
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<td>Sample Volume</td>
<td>10µl</td>
<td>Reagent Volume</td>
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<tr>
<td>Wavelength</td>
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<tr>
<td>Blank</td>
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<tr>
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<tr>
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<td>Units</td>
<td>U/L</td>
<td></td>
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</tr>
</tbody>
</table>

For the substrate start assay 0.8 ml of buffer reagent (L₁) and 0.5 ml of sample (serum/homogenate) were incubated for 1 minute. Then 0.2ml of starter reagent (L₂) was added. Thereafter the mixture was shaken and fed into auto analyser.

Note
RBC’s have a very high LDH content and hence haemolysed samples should not be used.
Enzyme oxidants

Superoxide Dismutase (SOD)\(^{[132]}\)

**PRINCIPLE**

Estimation of SOD was done by detecting O\(_2^-\) by oxidation of hydroxylamine hydrochloride yielding nitrite, which was measured colorimetrically at 560 nm. O\(_2^-\) is also detected during auto oxidation of hydroxylamine at pH 10.2, Nitro blue tetrazolium is reduced and nitrite is produced in the presence of EDTA, which can be detected colorimetrically.

SOD acts in following way

\[
O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2
\]

**Reagents needed**

1. *Sodium carbonate solution*: 1.06 gm of sodium carbonate was dissolved in 100 ml of distilled water.

2. *Nitro Blue Tetrazolium (NBT)*: 10 mg NBT was dissolved in 100 ml water.

3. *EDTA solution*: 37 mg di sodium EDTA salt was dissolved in 100 ml of distilled water.

4. *Hydroxylamine Hydrochloride*: 16.5 mg of hydroxylamine hydrochloride was dissolved in 100 ml distilled water.

**PROCEDURE**

1. To 100µl of 10% heart tissue homeogenate in 0.25 M sucrose, a mixture containing 1 ml sodium carbonate, 0.4 ml NBT and 0.2 ml EDTA was added and zero minute reading was taken at 560 nm.

2. By addition of 0.4 ml of 1 mM hydroxylamine hydrochloride, the reaction was initiated.

3. The reaction mixture was incubated at 25\(^\circ\)C for 5 minutes, the reduction of NBT was measured at 560 nm.

4. Without the tissue homogenate, parallel control was also made in 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 24 mM NBT by 50% and is expressed as units/mg of protein.

The concentration of the enzyme is calculated using following formula\(^{[133]}\)

\[
\text{Unit/mg} = \frac{1000}{\mu \text{gm of enzyme resulting in } \frac{1}{2} \text{ max. inhibition}}
\]
**Estimation of total proteins**

Protein estimation is based on the formation of a coloured complex of proteins with Folin catechu reagent, which was measured colorimetrically at 610 nm. Bovine albumin was used as a standard.

**Reagents**

1. **Alkaline reagent:** 2 gm of Sodium carbonate was dissolved in 100 ml of 0.1N NaOH solution.

2. **Alkaline mixture:** To 100 ml of alkaline reagent, 1 ml of 4 % aqueous copper sulphate solution was added. This was prepared freshly.

3. **Phenol reagent (Folin Ciocalteau reagent) (Loba chemie, Mumbai, India):** Commercially available reagent was diluted by adding equal volume of distilled water before use and stored in the refrigerator.

4. 0.1N NaOH solution.

5. 90 % alcohol.

6. Preparation of standard bovine albumin solution: 8 mg (0.08 %) of standard bovine albumin (S.D. fine chemicals, Mumbai, India) was dissolved in 10ml of distilled water.

**PROCEDURE**

1. To 0.1 ml of heart homogenate, 0.9 ml of 90 % alcohol was added and centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded and precipitate was dissolved in 1 ml of 0.1N NaOH.

2. 0.05 ml of the above solution was taken in a test tube, to which 4 ml of freshly prepared alkaline mixture was added and allowed to stand for 10 minutes.

3. To the above reaction mixture 0.4 ml of phenol reagent was added and allowed to stand for further 10 minutes for the reaction to complete.

4. The absorbance was measured at 610 nm using distilled water as blank.

5. 0.1 ml of standard bovine solution was taken in a test tube and processed as mentioned in step 2 and 3.

**The amount of protein was calculated using the formula**

\[
\text{Protein} = \frac{\text{O.D of sample} \times \text{Final Volume} \times \text{Dilution Factor} \times \text{Concentration of Std}}{(\text{Mg/ml})} \times \frac{\text{O.D of Standard}}{\text{O.D of Standard}}
\]
Catalase\textsuperscript{[134]}

**PRINCIPLE**

This enzyme is found in the peroxisomes and converts hydrogen peroxide to water and oxygen.

\[ 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \]

\[ \text{ROOH} + \text{AH}_2 \rightarrow \text{H}_2\text{O} + \text{ROH} + \text{A} \]

Catalase was estimated by determining the decomposition of \( \text{H}_2\text{O}_2 \) at 240 nm in an assay mixture containing phosphate buffer (0.25 M, pH 7).

**Reagents**

1. Phosphate buffer (0.25 M, pH 7): 3.22 gm potassium dihydrogen orthophosphate and 7.268 gm disodium hydrogen orthophosphate was dissolved in 1000 ml distilled water.
2. Hydrogen peroxide (0.042 ml = 12.3 M): 0.34 ml of 30% hydrogen peroxide was diluted to 100 ml with distilled water.

**PROCEDURE**

1. To 100µl of 10% tissue homogenate in 0.25 M sucrose, 1.9 ml phosphate buffer was added and absorbance was measured at 240 nm.
2. 1 ml hydrogen peroxide was added to above reaction mixture and it was allowed to stand for 1 minute. The absorbance was measured at 240 nm using phosphate buffer as blank solution.

One international unit of catalase utilised is that amount which catalyzes the decomposition of 1mM hydrogen peroxide per minute at 37\textdegree C and expressed in terms of units/mg of protein. Catalase activity can be calculated using the following formula.

\[
\text{Units/mg} = \frac{\text{Absorbance}_{240\text{ nm}} \times 1000}{43.6 \times \text{mg of enzyme/ml reaction mixture}}
\]

**Thiobarbituric acid reactive substances\textsuperscript{[135,136]}**

**PRINCIPLE**

Plasma concentration of thiobarbituric acid reactive substance is the index of oxidative stress and lipid peroxidation. Elevated levels of TBARS are associated with increased risk of cardiovascular diseases. Oxidative stress during reperfusion leads to lipid peroxidation. Direct measurement of liberated ROS is difficult due to their instability, malondialdehyde...
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(MDA), a stable lipid peroxidation end-product, is frequently used as a marker of ROS production and measured at 532 nm.

**Reagents**

1. Phosphoric acid (1%): 1ml phosphoric acid was diluted to 100 ml with distilled water.
2. Thiobarbituric acid (TBA) (0.6%): 0.6 gm Thiobarbituric acid was dissolved in 100 ml of distilled water.
4. Malondialdehyde (MDA).

**PROCEDURE**

1. 3ml phosphoric acid and 1ml of 0.6% TBA was added 0.5ml of heart tissue homogenate. This mixture was boiled over the water bath for 45 minutes.
2. Upon cooling, 4 ml n-butanol was added to the above mixture and mixed for 1 minute followed by centrifugation at 20000 rpm for 20 minutes.
3. The organic layer formed, was transferred to a fresh test tube and the absorbance was measured at 532nm.

**Procedure for standard curve**

1. Primary stock: 100mm MDA was prepared by dissolving 3.135 gm MDA in 100ml of distilled water.
2. From the primary stock solution 1,2,3 and 4 ml was taken and diluted to 10 ml to get the concentration of 10,20 30 40 mm of MDA.
3. 0.5ml of solution was taken from each of the above concentration and steps 1-3 were repeated without the addition of heart homogenate.
4. Graph was plotted between absorbance Vs concentration and amount of TBARS in heart homogenate was read by interpolation method.

**Histopathological examination**

Heart tissue obtained from all groups were washed immediately with saline and then fixed in 10% v/v formalin solution in saline. The ventricular mass from the left ventricular location was sectioned from heart in order to obtain 0.4 cm thick transverse section and dehydrated with alcohol followed by embedding with paraffin wax. These sections were stained with hematoxylin and eosin (H&E).

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\[136\]
The myocardial damage was determined by giving scores depending on the intensity as follows; no changes-score 00; mild-score 01 (focal myocytes damage or small multifocal degeneration with slight degree of inflammatory process); moderate-score 02 (extensive myofibrillar degeneration and/or diffuse inflammatory process); marked-score 03 (necrosis with diffuse inflammatory process).\[80\]

**Statistical analysis:** The statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey multiple comparison tests. The values were expressed as mean ± SEM where P<0.05 was considered significant.

**RESULTS**

**Preparation of Ananas comosus homogenate**

Ananas comosus fruits were peeled, sliced, grounded in to paste and suspended in distilled water. three different concentrations of ananas comosus homogenate were prepared, 0.1g/ml, 0.25g/ml and 0.5g/ml, corresponding to 100mg/kg body weight, 250mg/kg body weight and 500mg/kg body weight of animal.

**Phytochemical investigation**

**Table. 1: Phytochemical investigation of various compound.**

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<th>TEST</th>
<th>INFEREN CE</th>
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<td>-</td>
</tr>
<tr>
<td>1b</td>
<td>Mayer’s test</td>
<td>Cream precipitate</td>
<td>-</td>
</tr>
<tr>
<td>1c</td>
<td>Dragendorff’s test</td>
<td>Orange precipitate</td>
<td>-</td>
</tr>
<tr>
<td>1d</td>
<td>Wagner’s test</td>
<td>Red-brown precipitate</td>
<td>-</td>
</tr>
<tr>
<td>2a</td>
<td>Molisch’s test</td>
<td>Violet colour</td>
<td>+</td>
</tr>
<tr>
<td>2b</td>
<td>Fehling’s test</td>
<td>Brick red colour</td>
<td>+</td>
</tr>
<tr>
<td>2c</td>
<td>Barfoed’s test</td>
<td>Red colour</td>
<td>+</td>
</tr>
<tr>
<td>2d</td>
<td>Benedict’s test</td>
<td>Red colour</td>
<td>+</td>
</tr>
<tr>
<td>3a</td>
<td>Liebermann-Burchard test</td>
<td>Reddish- violet colour</td>
<td>+</td>
</tr>
<tr>
<td>3b</td>
<td>Salkowski test</td>
<td>Red colour</td>
<td>+</td>
</tr>
<tr>
<td>3c</td>
<td>Baljet’s test</td>
<td>Orange colour</td>
<td>+</td>
</tr>
<tr>
<td>3d</td>
<td>Keller-Killani test</td>
<td>Red colour</td>
<td>+</td>
</tr>
<tr>
<td>4a</td>
<td>Froth test</td>
<td>1 cm foam</td>
<td>+</td>
</tr>
<tr>
<td>4b</td>
<td>Haemolytic test</td>
<td>No precipitate</td>
<td>+</td>
</tr>
<tr>
<td>5a</td>
<td>Ferric chloride test</td>
<td>Blue colour</td>
<td>+</td>
</tr>
<tr>
<td>5b</td>
<td>Lead acetate test</td>
<td>Yellow colour</td>
<td>+</td>
</tr>
</tbody>
</table>
6. Test for proteins and Amino acids

| 6.a | Millon’s test | Red colour | + |
| 6.b | Biuret test | Violet colour | + |
| 6.c | Ninhydrin test | Violet colour | + |

7. Test for flavanoids

| 7.a | Ferric chloride test | Blackish red colour | + |
| 7.b | Lead acetate test | Yellow precipitate | + |

Toxicity studies: Acute toxicity studies carried out according to OPPTS guidelines\textsuperscript{[122]}, demonstrated that extract was not toxic at doses 2 g/kg body weight, 3 g/kg body weight and 5 g/kg body weight P.O, Hence 1/10\textsuperscript{th}, 1/25\textsuperscript{th} and 1/50\textsuperscript{th} of maximum safe dose corresponding to 500mg/kg, 250 mg/kg and 100mg/kg orally were selected as high, intermediate and low doses respectively.

Isoprenaline induced myocardial infarction acute model

Effect on haemodynamic parameters

A) Endogenous biomarker enzymes

Effect on CK-MB and LDH activity: Administration of ISO through subcutaneous route significantly elevated the CK-MB and LDH levels in ISO control but decreased in all pre-treated groups compared to NC. A significant fall in the serum CK-MB and LDH was found in all treated group compared to ISO control. LTN treatment alone and combination showed significant reduction of CK-MB and LDH activity in serum. ACHD+LTN combination was showing better effect in the reduction of CK-MB and LDH levels than LTN alone.

In HTH all the treated groups were showing significant decline in the CK-MB and LDH levels compared to NC. There was a significant rise in the CK-MB and LDH levels in all treated groups compared to ISO control. Concurrent administration of LTN with different doses of AC was found to have significant effect on CM-MB and LDH levels compared to LTN alone. (Table:2).

Table 2: Effect on CK-MB and LDH activities in serum and heart tissue homogenate (units/gm) against isoprenaline induced acute myocardial damage.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>CKMB ACTIVITY</th>
<th>LDH ACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SERUM (Unit/Lit)</td>
<td>HTH (Unit/gm)</td>
</tr>
<tr>
<td>NORMAL</td>
<td>193.1±4.956</td>
<td>66.65±4.110</td>
</tr>
<tr>
<td>ISO CONTROL</td>
<td>1023.86±8.903</td>
<td>10.58±1.76c</td>
</tr>
<tr>
<td>ACLD</td>
<td>709.86±6.55cl</td>
<td>26.4±1.47cl</td>
</tr>
<tr>
<td>ACID</td>
<td>650.716±11.9cl</td>
<td>52.68±2.031cl</td>
</tr>
<tr>
<td>ACHD</td>
<td>608.01±12.8cl</td>
<td>53.58±1.73cl</td>
</tr>
</tbody>
</table>
LTN 509.86±10.036\textsuperscript{c} 31.05±1.003\textsuperscript{f} 29.3±1.083\textsuperscript{f} 6.71±0.233\textsuperscript{f}
ACLD+LTN 373.4±9.82\textsuperscript{d} 50.5±1.445\textsuperscript{b} 21.466±0.833\textsuperscript{f} 7.25±0.206\textsuperscript{a}
ACID+LTN 281.6±9.247\textsuperscript{c} 60.5±0.641\textsuperscript{f} 21.466±0.833\textsuperscript{f} 8.11±0.116\textsuperscript{f}
ACHD+LTN 269.63±11.03\textsuperscript{c} 62.38±1.008\textsuperscript{f} 13.09±0.981\textsuperscript{f} 8.5±0.171\textsuperscript{f}

All values are mean ± SEM, n=6, \textsuperscript{a}P<0.05, \textsuperscript{b}P < 0.01, \textsuperscript{c}P < 0.001 when compared to normal control; \textsuperscript{d}P < 0.05, \textsuperscript{e}P < 0.01, \textsuperscript{f}P < 0.001 compared to ISO control, \textsuperscript{g}P < 0.05, \textsuperscript{h}P < 0.01, \textsuperscript{i}P < 0.001 compared to ACLD/ACID/ACHD respectively, \textsuperscript{j}P < 0.05, \textsuperscript{k}P < 0.01, \textsuperscript{l}P < 0.001 compared to LTN. ACLD (ananas comosus low dose 100mg/kg), ACID (ananas comosus intermediate dose 250mg/kg), ACHD (ananas comosus highdose 500mg/kg) and LTN (30mg/kg).

B) SOD, Catalase AND TBARS

Effect on SOD and catalase: SOD and catalase activities were reduced significantly in the ISO control compared to NC where as all the pre treated groups were showed significant rise in the SOD and catalase activities compared to ISO control. LTN alone and combinations with different doses of AC were found to have significant effect on reversing the ISO induced elevation of SOD and catalase.

Effect on TBARS: Significant elevation of TBARS levels were found in ISO control and decline in all treated groups compared to NC. All the treated groups were showing a significant fall in TBARS activity compared to ISO control. ACLD, LTN and ACID+LTN were found to have significant fall in TBARS levels.(table:3).

Table. 3: Effects on SOD, CATALASE and TBARS activity in heart tissue homogenate against isoprenaline induced acute myocardial damage.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>SOD (Units/mg protein)</th>
<th>CATALASE (Units/mg protein)</th>
<th>TBARS (ng/mg protein)</th>
<th>Histological score</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL</td>
<td>6.75±0.38</td>
<td>17.15±0.4463</td>
<td>19.6±1.67</td>
<td>0</td>
</tr>
<tr>
<td>ISO CONTROL</td>
<td>0.303±0.021\textsuperscript{c}</td>
<td>6.3±0.339\textsuperscript{c}</td>
<td>27.8±3.59\textsuperscript{a}</td>
<td>3</td>
</tr>
<tr>
<td>ACLD</td>
<td>1.383±0.1662\textsuperscript{d}</td>
<td>8.667±0.1909\textsuperscript{e}</td>
<td>2.3±0.34\textsuperscript{f}</td>
<td>2</td>
</tr>
<tr>
<td>ACID</td>
<td>2.88±0.088\textsuperscript{f}</td>
<td>9.416±0.1778\textsuperscript{f}</td>
<td>13.8±0.93\textsuperscript{f}</td>
<td>2</td>
</tr>
<tr>
<td>ACHD</td>
<td>3.3±0.1633\textsuperscript{f}</td>
<td>10.66±0.2539\textsuperscript{f}</td>
<td>14.0±0.17\textsuperscript{f}</td>
<td>2</td>
</tr>
<tr>
<td>LOSARTAN</td>
<td>5.3±0.3260\textsuperscript{f}</td>
<td>10.61±0.3422\textsuperscript{f}</td>
<td>9.8±0.25\textsuperscript{f}</td>
<td>1</td>
</tr>
<tr>
<td>ACLD+LTN</td>
<td>5.61±0.1493\textsuperscript{h}</td>
<td>11.766±0.3422\textsuperscript{h}</td>
<td>13.7±0.75\textsuperscript{f}</td>
<td>2</td>
</tr>
<tr>
<td>ACID+LTN</td>
<td>6.7±0.2366\textsuperscript{h}</td>
<td>13.21±0.1922\textsuperscript{h}</td>
<td>9.9±8.75\textsuperscript{f}</td>
<td>2</td>
</tr>
<tr>
<td>ACHD+LTN</td>
<td>7.233±0.2060\textsuperscript{h}</td>
<td>13.21±0.1922\textsuperscript{h}</td>
<td>10.2±0.20\textsuperscript{f}</td>
<td>1</td>
</tr>
</tbody>
</table>

All values are mean ± SEM, n=6, \textsuperscript{a}P<0.05, \textsuperscript{b}P < 0.01, \textsuperscript{c}P < 0.001 when compared to normal control; \textsuperscript{d}P < 0.05, \textsuperscript{e}P < 0.01, \textsuperscript{f}P < 0.001 compared to ISO control, \textsuperscript{g}P < 0.05, \textsuperscript{h}P < 0.01, \textsuperscript{i}P < 0.001 compared to ACLD/ACID/ACHD respectively, \textsuperscript{j}P < 0.05, \textsuperscript{k}P < 0.01, \textsuperscript{l}P < 0.001 compared to LTN.
compared to LTN. ACLD (ananas comosus low dose 100mg/kg), ACID (ananas comosus intermediate dose 250mg/kg), ACHD (ananas comosus highdose 500mg/kg) and LTN (30mg/kg).

**Effect on histological score:** Isoprenaline injections caused necrosis of cells with degeneration of myofibril and increased interstitial space. In ISO induced acute M.I. by administration of isoprenaline for two consecutive day’s myocardial integrity was disturbed evident with increased interstitial space and necrosis of cells with degeneration of myofibril (Fig. 5.2.3). Pre-treatment of ACLD, ACID and ACHD with or without Carvedilol to rats before subjected to Isoprenaline induced myocardial damage showed significant fall in histological scores compared to ISO control.

Groups treated with ACLD, ACID and ACHD has shown less diffuse necrosis, mild inflammation and fibrosis. Recovery from necrosis, mild inflammation with less interstitial space and least multifocal degeneration was observed in the groups treated with LTN and different doses of AC combinations.

**Histopathological scores**
0-normal cardiac muscle architecture,
1-mild focal necrosis/degeneration,
2-mild diffuse/moderate necrosis+/- mild inflammation,
3-moderate diffuse necrosis+/- mild inflammation and fibrosis,
4-sever necrosis+/-inflammation+/-fibrosis.

**Haematoxylin and eosin (H&E) stained section of heart in Isoprenaline induced acute myocardial damage. Photographed at magnification 400X.**

![Fig.5.4.3a Heart tissue of Normal group.](image1)

![Fig.5.4.3b Isoprenaline Treated group.](image2)
Isoprenaline induced chronic myocardial infarction model

The biomarkers such as LDH and CK-MB were estimated in serum and homogenate while SOD, Catalase and TBARS estimated in heart tissue homogenate.
Effect on haemodynamic parameters

A) Endogenous biomarkers

The LDH and CK-MB activities were significantly increased and decreased in serum and in heart tissue homogenate (HTH) respectively in ISO treated group when compare to Normal group. Groups treated with ACLD, ACID, ACHD, LTN, ACLD+LTN, ACID+LTN and ACHD+LTN showed significant fall in LDH and CK-MB activities in serum and elevation in HTH compared to ISO control. (Table:4).

Table 4: Effects on LDH and CKMB level in serum and heart tissue homogenate against Isoprenaline induced chronic myocardial infarction.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ckmb Activity</th>
<th>LDH Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum (Unit/Lit)</td>
<td>HTH(Unit/gm)</td>
</tr>
<tr>
<td>NORMAL</td>
<td>161.94±5.2</td>
<td>75.33±1.4</td>
</tr>
<tr>
<td>ISO CONTROL</td>
<td>451.833±6.571\textsuperscript{ct}</td>
<td>7.15±0.32\textsuperscript{ct}</td>
</tr>
<tr>
<td>ACLD</td>
<td>269.483±11.936\textsuperscript{ct}</td>
<td>36.3±1.429\textsuperscript{ct}</td>
</tr>
<tr>
<td>ACID</td>
<td>243.45±4.505\textsuperscript{ct}</td>
<td>53.7±0.756\textsuperscript{ct}</td>
</tr>
<tr>
<td>ACHD</td>
<td>228.66±2.535\textsuperscript{ct}</td>
<td>60.46±3.99\textsuperscript{ct}</td>
</tr>
<tr>
<td>LOSARTAN</td>
<td>210±1.597\textsuperscript{mt}</td>
<td>62.33±1.26\textsuperscript{mt}</td>
</tr>
<tr>
<td>ACLD+LTN</td>
<td>190.86±2.69\textsuperscript{n}</td>
<td>61.766±0.368\textsuperscript{ctji}</td>
</tr>
<tr>
<td>ACID+LTN</td>
<td>175.70±3.203\textsuperscript{mi}</td>
<td>67.433±0.6561\textsuperscript{mi}</td>
</tr>
<tr>
<td>ACHD+LTN</td>
<td>163.833±3.477\textsuperscript{mi}</td>
<td>73.11±0.808\textsuperscript{mi}</td>
</tr>
</tbody>
</table>

All values are mean ± SEM, n=6, \textsuperscript{a}P<0.05, \textsuperscript{b}P<0.01, \textsuperscript{c}P<0.001 when compared to normal control; \textsuperscript{d}P<0.05, \textsuperscript{e}P<0.01, \textsuperscript{f}P<0.001 compared to ISO control. \textsuperscript{g}P<0.05, \textsuperscript{h}P<0.01, \textsuperscript{i}P<0.001 compared to ACLD/ACID/ACHD respectively, \textsuperscript{j}P<0.05, \textsuperscript{k}P<0.01, \textsuperscript{l}P<0.001 compared to LTN. ACLD (ananas comosus low dose 100mg/kg), ACID (ananas comosus intermediate dose 250mg/kg), ACHD (ananas comosus highdose 500mg/kg) and LTN (30mg/kg).

B) SOD, Catalase AND TBARS

Effect on SOD and Catalase

In ISO control group there were decreased in SOD and Catalase activity when compared to normal control. All treated groups were found to have significant effect on increase in SOD and catalyse values. ACID+LTN and ACHD+LTN groups were having more significant effect on SOD and catalase values.
Effect on TBARS

A significant elevation in TBARS levels were found in ISO control compared to normal control. All the treated groups were found to have significant effect on TBARS values. ACLD, ACID+LTN treated group was found to more fall in TBARS level.

Effect on histological score

Administration of ISO caused necrosis of cells with degeneration of myofibril and increased interstitial space. In ISO induced chronic M.I. by administration of Isoprenaline for 21 days (3mg/kg/day) myocardial integrity was disturbed evident with increased interstitial space and necrosis of cells with degeneration of myofibril (Figure 5.5.3). Pre-treatment of ACLD, ACID and ACHD with or without LTN to rats before subjected to Isoprenaline induced myocardial damage showed significant fall in histological scores compared to ISO control.

ISO treated group shows moderate diffuse necrosis+/- mild inflammation and fibrosis. Groups treated with ACLD, ACID and ACHD has shown less diffuse necrosis, mild inflammation and fibrosis as compare to ISO group. Group treated ACLD, ACID and ACHD with LTN showed more effective than individual treatment. ACLD+LTN showed significant fall in TBARS levels. (Table no. 5).

Table no. 5: Effects on SOD, Catalase and TBARS activity in heart tissue homogenate against isoprenaline induced chronic myocardial damage.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>SOD</th>
<th>CATALASE</th>
<th>TBARS</th>
<th>Histological score</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL</td>
<td>6.91±0.174</td>
<td>15.64±6.19</td>
<td>17.8±0.72</td>
<td>0</td>
</tr>
<tr>
<td>ISO CONTROL</td>
<td>0.571±0.030&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.81±0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.9±4.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3</td>
</tr>
<tr>
<td>ACLD</td>
<td>2.946±0.2810&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>7.233±0.170&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>10.3±1.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2</td>
</tr>
<tr>
<td>ACID</td>
<td>3.683±0.2242&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>8.416±0.132&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>13.6±0.34&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>2</td>
</tr>
<tr>
<td>ACHD</td>
<td>3.766±0.1892&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>9.033±0.2892&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>12.9±3.29&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td>LOSARTAN</td>
<td>3.466±0.1622&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>9.98±0.284&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>12.8±0.129&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td>ACLD+LTN</td>
<td>4.166±0.2212&lt;sup&gt;st&lt;/sup&gt;</td>
<td>11.93±0.2872&lt;sup&gt;st&lt;/sup&gt;</td>
<td>10.9±1.89&lt;sup&gt;st&lt;/sup&gt;</td>
<td>2</td>
</tr>
<tr>
<td>ACID+LTN</td>
<td>6.566±0.1082&lt;sup&gt;st&lt;/sup&gt;</td>
<td>14.9±0.2622&lt;sup&gt;st&lt;/sup&gt;</td>
<td>8.8±0.9&lt;sup&gt;st&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>ACHD+LTN</td>
<td>6.56±0.1362&lt;sup&gt;st&lt;/sup&gt;</td>
<td>14.86±0.2172&lt;sup&gt;st&lt;/sup&gt;</td>
<td>9.1±2.38&lt;sup&gt;st&lt;/sup&gt;</td>
<td>0</td>
</tr>
</tbody>
</table>

All values are mean ± SEM, n=6, <sup>a</sup>P<0.05, <sup>b</sup>P<0.01, <sup>c</sup>P<0.001 when compared to normal control; <sup>d</sup>P<0.05, <sup>e</sup>P<0.01, <sup>f</sup>P<0.001 compared to ISO control, <sup>g</sup>P<0.05, <sup>h</sup>P<0.01, <sup>i</sup>P<0.001 compared to ACLD/ACID/ACHD respectively, <sup>j</sup>P<0.05, <sup>k</sup>P<0.01, <sup<l>P<0.001 compared to LTN. ACLD (ananas comosus low dose 100mg/kg), ACID (ananas comosus intermediate dose 250mg/kg), ACHD (ananas comosus highdose 500mg/kg) and LTN (30mg/kg).
**Histopathological scores**

0-normal cardiac muscle architecture,  
1-mild focal necrosis/degeneration,  
2-mild diffuse/moderate necrosis+/- mild inflammation,  
3-moderate diffuse necrosis+/- mild inflammation and fibrosis,  
4-severe necrosis+/-inflammation+/-fibrosis.

---

**Fig.5.5.3a Heart tissue of Normal group.**  
**Fig.5.5.3b Isoprenalin treated group.**

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**Fig.5.5.3c ACLD (200 mg/kg) Treated group.**  
**Fig.5.5.3d AID (500 mg/kg) Treated group.**

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**Fig.5.5.3e ACHD (500 mg/kg) treated group.**  
**Fig.5.5.3e Losartan (30 mg/kg) Treated group.**
DISCUSSION

The present investigation was to elucidate the role of AC during myocardial dysfunction and metabolic derangement induced by ISO in rat heart and to explore its pharmacodynamic interaction with conventional cardioprotective drug LTN. The findings of investigation revealed the beneficial role of Ananas Comosus (AC) when treated concurrently with LTN in conditions of anticipated cardiac injury. ISO, a synthetic catecholamine and β-adrenoceptor stimulant, is known to cause myocardial damage at higher concentration.\[96\] Disturbances in coronary microcirculation, anoxia and formation of catecholamine oxidative products apart from intracellular calcium load have been postulated to induce cardiac toxicity.\[137\] ISO administration results in increase in calcium uptake and energy consumption leading to cell death.\[138\] Different biomarkers enzymes elevation in serum is due to leakage of enzymes from the heart as a result of ISO- induced necrosis.\[105\] ISO has increases the production of cytotoxic free radicals through its auto-oxidation. It has been suggested that the oxidative products of catecholamines produce changes in the myocardium by stimulating lipid peroxidation and cause irreversible damage to the myocardial membrane this alters membrane permeability, leading to the loss of function and integrity of myocardial
membranes. Hence, leakage of endogeneous biological markers and free radical formation are attributed for myocardial distress in post isoprenaline administration.[86]

In acute model, administration of two high doses of ISO in consecutive days was found to induce myocardial damage which is evident from our observation.[138] In chronic model, administration of ISO (3mg/kg/day) led to cardiac hypertrophy with increased collagen content. In the chronic phase of ISO infusion, increased ROS play important roles in extracellular matrix biosynthesis, which may lead to the alteration of wall stiffness and affect cardiac function by involving in the progression of cardiac hypertrophy and heart failure.[130]

Ananas comosus, contains an enzyme complex called Bromelain, contains peroxidase, acid phosphate, several protease inhibitors and organically bound calcium which is found to have cardioprotective activity.[2] Bromelain has the capacity to reduce angina[139], exert antihypertensive action[140], and significantly reduce the incidence of coronary infarct when administered with potassium and magnesium orotate.[141] Bromelain decreases the severity of angina pectoris and the infarct size.[44, 45] Bromelain decreases platelet aggregation[46,43], is an effective fibrinolytic agent[47], and inhibits thrombus formation.[48] Bromelain have the protective action on the myocardial ischemia-reperfusion by the upregulation of survival kinases known to attenuate the process of apoptosis particularly the serine or threonine kinase Akt is well established to play an important role in endothelial and cardiomyocyte cell biology that activates an antiapoptotic or prosurvival signaling cascade.[28] Apoptosis is main reason that contributes to many cardiac dysfunctions and it is the dominant form of myocardial cell death in the infarct area, bromelain acts as antiapoptotic by phosphorylating the protein kinases like Akt and FOXO proteins which inturn linked to the reduction of the apoptotic process.[49] Angiotensin receptor blockers (ARB) displace angiotensin II from the angiotensin I receptor and produce their blood pressure lowering effects by antagonizing angiotensin II induced vasoconstriction, aldosterone release, catecholamine release, arginine vasopressin release, water intake and hypertrophic response. LTN is a competitive and selective Ang II receptor antagonist. It has selective inhibition of angiotensin II by competitive antagonism of the angiotensin II receptors, has been spectualted to reduce adverse effects and possibly improve clinical efficacy.[30]

The presence of diagnostic biomarker enzymes like CKMB and LDH in heart tissue homogenate (HTH) is indicative of myocardial integrity and their release in serum signifies myocardial injury. In our study, there was substantial fall and rise in activities of marker
enzymes in HTH and serum respectively upon ISO administration. These effects could be secondary events following ISO induced lipids peroxidation of cardiac membranes, with a consequent increase in enzyme leakage from cardiac myocytes. Pretreatment with ACLD, ACID, ACHD, LTN or combination with LTN with different doses of AC prevented the inclination of CK-MB and LDH in serum. Our biochemical findings were supported by the improvement in histopathological 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 ISO induced cardiac damage. It can be speculated that the damage caused to myocardium during ISO might be irreversible due to release of free radicals and hence we also measured the endogenous anti oxidant activities in HTH. Our findings on antioxidant estimation explain the well established assumption of involvement of OFRs in myocardial injury. Among number of OFRs associated with myocardial contractile and rhythmic disturbances, contribution of superoxide to myocardial damage is believed to be the highest and this radical is combated by elevated activities of endogeneous anti oxidant enzyme – the superoxidase (SOD). In addition to this, measurement of catalase activity was carried out as elevation in SOD dimutes superoxide but results in accumulation of H₂O₂ which could further precipitate the myocardial infarction. Pretreatment of animals with AC (100mg/kg, 250mg/kg and 500mg/kg) alone or along with LTN produced remarkable elevation in SOD and catalase activities when compared to ISO control indicating cardioprotective effect.

AC decreases the apoptosis of the cardiac myocytes, which inturn decreases the release of biomarkers from the myocyte in to the serum, and thus retains the antioxidant activity of the myocytes, and protects the myocardium from cell death. The cardioprotective and antiapoptotic activity of ananas comosus was already reported in earlier studies. The potential of protective effect may be due to the rich source of Bromelain present as a main constituent. AC cardioprotection with or without LTN by inhibiting the apoptosis process of the myocytes.

CONCLUSION

From the results it may be concluded that three doses of AC (100, 250 and 500 mg/kg) possess cardioprotective efficacy when given prophylactically against ISO induced acute and chronic myocardial necrosis in rats. In both the models high dose was found more effective. the manifestations induced by isoprenaline mediated stress were further alleviated with the introduction of conventional cardioprotective drug Losartan. The efficacy of Ananas comosus
could be attributed to presence of complex enzyme bromelain, which is responsible for the increase in the biomarkers in heart homogenate, while the ARB blocking property of losartan could be responsible for its cardioprotective nature. However for the interaction it could be synergetic effect of Ananas comosus and losartan for cardioprotection. Further studies should be carried out, to understand mechanism of interaction between Ananas comosus and Losartan with extensive evaluation of histological and ultra structural changes.

SUMMARY
The current investigation dealt with the pharmacodynamic interaction of pineapple juice with losartan on experimentally induced myocardial infarction (MI) in rats. The study was carried out using two different animal models like ISO induced acute and chronic myocardial damage in Sprague dawley rats. Myocardial damage was either induced by administration of isoprenaline (ISO) 150mg/kg s.c for two consecutive days or by daily dosage of 3mg/kg s.c for 21 days. Animals were treated with pineapple juice orally for 21 days and for interactive groups last 10 days with LTN for both the models. Three doses of AC showed the significant protection against acute and chronic administration of ISO induced myocardial damage by indicating a significant decrease in biomarkers such as LDH and CKMB in serum and increase in heart homogenate with respect to ISO control.

In this study high, medium and low dose of AC treatment along with conventional drug LTN inclined the activity of antioxidants with decline in TBARS activity in HTH indicating the structural integrity and protection to the myocardium. The cardioprotection offered by AC could be attributed to the presence of enzyme complex bromelain that shows anti apoptic activity effect by inhibiting the progressed cell death of myocytes. The protective activity of AC was further augmented when used along with LTN. Above findings were further conformed by decrease in the histological scores.

REFERENCES


