Cardioprotective Effects of Bovine Colostrum Against Isoproterenol-induced Myocardial Infarction in Rats

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ABSTRACT

Cardiovascular diseases like hypertension and Myocardial Infarction (MI) are the most important determinants for high mortality rate globally with a prevalence of 12.2% deaths as estimated by the World Health Organization. An Angiotensin Converting Enzyme (ACE) inhibitor, such as enalapril is the commonly used synthetic drug for treating high blood pressure, but may cause side effects like kidney toxicity or diarrhea. Therefore, there exists a need for alternative therapy. Bovine Colostrum (BC) containing several growth and immune factors triggers nearly fifty beneficial processes in newborns showing no side effects. Besides many clinical applications, there is no credible evidence of colostrum showing cardio-protective effect against MI. This study was designed to evaluate the effects of BC alone or in combination with enalapril against isoproterenol-induced myocardial infarction in rats. A dose finding toxicity study with BC (500, 1,000 or 2,000 mg kg⁻¹, p.o.) did not reveal any overt adverse symptoms or mortality in rats dosed for 14 consecutive days. Adult Wistar albino rats were weighed and randomly divided in seven groups where each group consists of six rats. Isoproterenol-induced myocardial infarcted group and group VII were fed with ACE inhibitor enalapril 0.25 mg kg⁻¹. Group III and V were given different doses of colostrum 250 and 500 mg kg⁻¹, respectively, whereas group IV and VI received various doses of colostrum along with ACE inhibitor enalapril to observe the pharmacodynamic interaction. Results obtained from the *in vitro* DPPH free radical scavenging method and *ex vivo* lipid peroxidation inhibitory activity by TBARS method indicated that BC possesses significant free radical scavenging activity and causes marked inhibition of lipid peroxidation. In biochemical serum estimation, the activity of various cardiac enzymes such as Lactate Dehydrogenase (LDH) and Creatine Kinase (CK) were determined. The serum levels altered by isoproterenol showed significant restoration by the administration of BC along with enalapril as compared with enalapril alone, suggesting that colostrum seems to have beneficial cardio-protective effects in rats. Additional studies are warranted to verify the results of these preliminary findings.

Key words: Colostrum-induced cardioprotection, isoproterenol-induced myocardial infarction in rat, cardioprotective actions of colostrum and enalapril, antioxidant effects of colostrum
INTRODUCTION

The hidden cause of arteriosclerosis and cardiovascular disease may be the alteration of immunity (Jensen, 1992). Anti-bodies are created by the immune system, once heart tissue is damaged. Heart disease occurs due to the immune sensitization to cardiac antigens (Lange and Schreiner, 1994). The acute necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand gives rise to a clinical condition called myocardial infarction or most commonly known as heart attack (Boudina et al., 2002). Several biochemical alterations leading to dysfunction of cardiovascular system may cause ultimately cell death because ischemic tissue constantly generate free radicals leading to degradation of tissue defense system and thereby causing damage to myocardium and necrosis (Ferrari et al., 1990). The pathogenesis of myocardial infarction becomes a dynamic process with the widespread existence of coronary atherosclerosis and implication of oxidative stress in mankind (Ojha et al., 2011).

One fourth of a million people die every year from heart failure. Death due to heart failure has increased six-folds over the last 40 years. It is the leading cause of hospitalization in those over 65 years of age (Rich, 1997). Many developing countries like India are struggling to bring about the impact of infectious diseases concurrently with growing burden caused by non-communicable diseases such as myocardial infarction (Srivastav et al., 2013). Nearly 29.8 million people in India are suffering from coronary heart disease (Goyal and Yusuf, 2006).

Also, with the known mechanism of cardiovascular diseases like myocardial infarction, epidemiological, experimental and clinical studies have shown evidence that myocardial infarction is largely preventable by suppression of oxidative stress occurring in the body due to the free radical generation (Filippo et al., 2006). Today there are many synthetic drugs available in the markets, which are used for the treatment of heart diseases, but they also carry a risk of side effects. ACE inhibitors like enalapril are widely used in the treatment of hypertension, congestive heart failure and post ischemic myocardial dysfunction (Pahor et al., 2000). There is a need for natural products, which possesses beneficial effects with almost no side effects. The present investigation was promoted in the purview of the claims of a nutraceutical drug having no side effect. The nutrient-rich, colostrum is the first secretion produced by mammals in 24 to 48 h after parturition. It has various vitamins, minerals, protein, and growth factors and carbohydrates that are vital for the nourishment of a developing neonate (Uruakpa et al., 2002). Colostrum also contains bioactive constituents like, antimicrobial peptides, growth factors and immunoglobulins (Pakkanen and Aalto, 1997). The beneficial effect of colostrum is because of the several immune factors and growth factors present in it (Thapa, 2005). During the myocardial attack the heart muscles gets damaged (Thygesen et al., 2007). Colostrum contents like growth hormone and IGF-1 can decrease LDL-cholesterol whereas increasing HDL cholesterol concentrations (Rona, 1988). The growth factors present in colostrum help in reparation and regeneration of heart muscle and also for new blood vessels for collateral coronary circulation (Gauthier et al., 2006). As heart disease bears a resemblance to an autoimmune response in this way, Proline Rich Polypeptides (PRP) in colostrum can help limit the disease severity by toning down the attack of immune on damaged heart tissue. Though proved to be this valuable, there has been no recent credible research claiming the cardio-protective effect of colostrum on myocardial infarction. A synthetic catecholamine and beta-adrenergic agonist, isoproterenol causes a severe stress in the myocardium which results in infarct like necrosis of heart muscle and also generates free radicals and stimulate lipid peroxidation which might be a causative factor for irreversible damage to the myocardium (Prabhu et al., 2006).
The aim of this investigation was to determine the cardioprotective action of bovine colostrum alone as well as to study its pharmacodynamic interaction with the Angiotension Converting Enzyme (ACE) inhibitor enalapril. The study was carried out on Wistar albino rats with myocardial infarction induced by isoproterenol (Srivastav et al., 2013).

**MATERIALS AND METHODS**

**Preparation of colostrum powder:** Bovine colostrum milk was obtained from dairy source (Mumbai), after the birth of calf (within 24-72 h). In order to preserve its biological activity, colostrum must be processed at low temperature. This milk was subjected to spray drying. The inlet temperature was 40°C and outlet temperature was 35°C. Colostrum powder was stored at 2-8°C in the refrigerator and used within 60 days.

**Acute toxicity study of colostrum:** The acute toxicity of combination consisting of group I control and group II test was evaluated in mice using the OECD guidelines 423. Swiss albino mice were randomly divided into two groups, each containing 6 animals. The colostrum was administered orally at doses of 500, 1000 and 2,000 mg kg⁻¹ of body weight. Distilled water was administered to control group. The general behavior of the mice was continuously monitored for 1 h after dosing, periodically during the first 24 h with special attention given during the first 4 h and daily thereafter, for a total of 14 days. Changes in the normal activity of mice and their body weights were monitored and the time at which signs of toxicity or death appeared was recorded.

**Estimation of antioxidant activity by DPPH (1, 1-diphenyl-2-pieryl hydrazyl) method, reducing power assay and lipid peroxidation inhibitory activity by TBARS (Thio Barbituric Acid Reacting Substances) method (Mehta et al., 2012):** Evaluation of free radical scavenging activities of colostrum was carried out using DPPH Method. The *in vitro* antioxidant activity was evaluated keeping ascorbic acid as standard at different concentrations and its radical scavenging activity was compared with that of colostrum. The graph was plotted using percentage inhibition vs., concentration and IC₅₀ value was determined.

The reducing power of Colostrum was determined by the slight modification of the method of Oyaizu (1986). Various concentrations of the extract in corresponding solvents were mixed with phosphate buffer (2.5 mL) and potassium ferricyanide (2.5 mL). This mixture was kept at 50°C in water bath for 20 min. After cooling, 2.5 mL of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 min whenever necessary. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared ferric chloride solution (0.5 mL). The absorbance was measured at 700 nm. Control was prepared in similar manner excluding samples. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power. Reducing power was measured by varying the concentration of the extract and the contact time.

Evaluation of lipid per oxidation inhibitory activity of colostrum was evaluated on rat liver homogenate using Thiobarbituric Acid Reacting Substances (TBARS) method and the graph was plotted to determine average IC₅₀ value.

**Induction of myocardial infarction:** Isoproterenol was be dissolved in normal physiological saline and injected subcutaneously to the rats (200 mg kg⁻¹) daily for 2 consecutive days according to the method of Srivastav et al. (2013).
**Experimental design:** Animals (male rats) were divided into 7 groups each group containing 6 rats (150-250 g b.wt.) to receive the following treatment:

**Group I:** Rats received 1.0 mL water as a vehicle. Group I rats were referred as control rats

**Group II:** Rats were administered the dose of isoproterenol 200 mg kg\(^{-1}\) subcutaneously twice daily at an interval of 24 h. Group II rats were referred as isoproterenol myocardial infarcted rats

**Group III:** Rats were dosed with the test agent colostrum 250 mg kg\(^{-1}\) alone

**Group IV:** Rats were treated with colostrum 250 mg kg\(^{-1}\) plus 0.25 mg kg\(^{-1}\) enalapril

**Group V:** Rats treated the test agent colostrum 500 mg kg\(^{-1}\) alone

**Group VI:** Rats received with colostrum 500+0.25 mg kg\(^{-1}\) enalapril

**Group VII:** Rats received dose of standard ACE inhibitor enalapril 0.25 mg kg\(^{-1}\) alone

**Biochemical serum estimation of CKMB and LDH:** Blood serum collection for CKMB and LDH value calculation.

The following biochemical estimation was carried out in the SPP SPTM NMIMS CIL laboratory. The Creatine Kinase MB (CKMB) and Lactase Dehydrogenase (LDH) were evaluated using ERBA kits.

**Statistical analysis:** The results were analyzed using one-way factorial analysis of variance (ANOVA) followed by Tukey's multiple comparison test using Graphpad Prism 5 software. The value of p<0.05 was considered as statistically significant. The results are expressed as Mean±SEM.

**RESULTS**

**Acute toxicity study of colostrum:** Colostrum was found to be non-toxic up to the dose of 2.0 g kg\(^{-1}\) and did not cause any mortality or overt symptoms of toxicity through the 14 day dosing period. According to Organization for Economic Cooperation and Development (OECD, clause 423) guidelines for acute oral toxicity, the LD\(_{50}\) dose of 2000 mg kg\(^{-1}\) and above is categorized as "unclassified" and hence the drug or test substance is considered to be safe. Hence, further dosing escalation to find out LD\(_{50}\) of colostrum was not performed.

**Estimation of antioxidant activity by DPPH method, reducing power assay and lipid peroxidation inhibitory activity by TBARS method:** In DPPH method, the radical scavenging activity of ascorbic acid was used as a standard. The *in vitro* antioxidant activity was evaluated keeping ascorbic acid as standard at different concentrations. The results obtained from the colostrum showed less per cent inhibition compared to the standard antioxidant ascorbic acid. The graph was plotted using percentage inhibition vs concentration and IC\(_{50}\) value was determined. The IC\(_{50}\) for ascorbic acid and colostrum was found to be 80 and 200 ppm, respectively (Fig. 1).

In the reducing power assay, higher absorbance of the reaction mixture indicates higher reductive potential which reflects the antioxidant activity. The observed results are summarized in Table 1. The absorbance values of standard and colostrum were found to be 0.4 at 100 ppm and 0.2 at 100 ppm, respectively. This indicates the reducing power ability of the compound as it has exhibited dose dependent increase in the antioxidant activity at different concentrations. The concentration of colostrum needs to be increased from 200 ppm to around 230 ppm to exhibit approx, the 0.4 absorbance value. The test compound showed absorbance in an increasing order of the dose.
Table 1: Reducing power of ascorbic acid standard solution and colostrum (absorbance values of ascorbic acid vs. test sample)

<table>
<thead>
<tr>
<th>Concentration ascorbic acid/test sample (ppm)</th>
<th>Absorbance of ascorbic acid</th>
<th>Absorbance of colostrum powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.058</td>
<td>0.062</td>
</tr>
<tr>
<td>40</td>
<td>0.125</td>
<td>0.063</td>
</tr>
<tr>
<td>80</td>
<td>0.360</td>
<td>0.169</td>
</tr>
<tr>
<td>160</td>
<td>0.482</td>
<td>0.250</td>
</tr>
<tr>
<td>200</td>
<td>0.794</td>
<td>0.329</td>
</tr>
</tbody>
</table>

Fig. 1: Comparison of the percentage inhibition of antioxidant activity of colostrum and standard ascorbic acid (ppm = parts per million)

![Graph showing radical scavenging activity of ascorbic acid and colostrum](image)

Fig. 2: Lipid peroxidation percentage inhibitory activity of colostrum

![Graph showing inhibition of lipid peroxidation](image)

Evaluation of lipid per oxidation inhibitory activity of colostrum was evaluated on rat liver homogenate using Thiobarbituric Acid Reacting Substances (TBARS) method. In acidic condition, pink colored complex was formed, absorbance of which was measured at 532 nm spectrophotometrically. As depicted in Fig. 2, colostrum showed concentration-dependent increase in the antioxidant activity. And average IC₅₀ value was found to be 180 ppm. The percent inhibition was found to be in increasing order of the concentration of the test compound.

**Biochemical serum estimation of CKMB and LDH:** The increase in the CK-MB values and LDH indicate the extent of isoproterenol-induced cardiotoxicity. As shown in Fig. 3 and 4, the isoproterenol-induced myocardial infarcted control group had the highest CK-MB and LDH values indicating cardiac damage. Colostrum produced a dose-dependent decrease in the toxicity. There
Fig. 3: Creatine kinase MB, Bars represent as Mean±SEM, one way ANOVA followed by Dunnett’s multiple comparison test, significantly differ from control group 1, **p<0.01 and ***p<0.001

Fig. 4: Lactase dehydrogenase, Bars represent as Mean±SEM, one way ANOVA followed by Dunnett’s multiple comparison test, significantly differ from control group 1, ***p<0.001 was a significant reduction in cardiotoxicity after the combined administration of 500 mg kg⁻¹ of colostrum and 0.25 mg kg⁻¹ enalapril as shown by the CK-MB and LDH values compared with the isoproteranol myocardial infarcted group (Group II rats).

DISCUSSION

It is widely recognized that cardiovascular diseases like Myocardial Infarction (MI) threatens to become the foremost cause of deaths occurring worldwide (Choudhury and Marsh, 1999). MI can lead to secondary disorders, such as arteriosclerosis, congestive heart failure and arrhythmia (Tataru et al., 2000). In spite of surfeit of research data available on cardiovascular diseases, there is no apt treatment for MI that is vital to avert permanent heart damage and to save lives. At present, dietary interventions and nutraceuticals are widely recommended as a major substitute for synthetic drugs for their well-known health benefits with no known side effects. Colostrum is one of the promising nutraceutical possessing various growth factors and immunoglobulins that are responsible for enhancing the immunity in newborn infants as well as adults (Korhonen and Pihlanto, 2007). The immunoglobulin present in colostrum can bind to all of the cell receptors in human body. Proline Rich Polypeptide (PRP), the essential component present in colostrum is widely anticipated to possess cardio-protective effect for patients diagnosed with myocardial complications.
In this context, there is a need to assess the cardio-protective activity of colostrum against MI. Isoproterenol (beta-1 and beta-2 adrenergic agonist) inflicts damage to the myocardium and endocardium and significant increases the levels of serum enzymes such as LDH, ALT, AST and CKMB. Isoproterenol is used for creating fatal arrhythmias in experimental animals and myocardial infarction of variable size and location. The myocardium contains an abundant concentration of diagnostic serum enzymes like Lactate Dehydrogenase (LDH) and Creatine Kinase (CKMB), which have proven highly beneficial as biochemical markers for proper diagnosis of ischemic myocardial necrosis in patients when measured within 24-36 h following MI attack.

The present study demonstrates the protective effects of colostrum along with standard ACE inhibitor enalapril against isoproterenol-induced MI in rats. The colostrum administration not only inhibited lipid peroxidation (Fig. 2), but also decreased the serum levels of LDH and CKMB (Fig. 3 and 4), thereby suggesting the curtailed disturbance in heart tissue damage.

The in vitro antioxidant method based on the principle of DPPH was performed because of the importance it has achieved and the ease of use. There is a preference for antioxidants from natural rather than synthetic sources. From the results obtained in this study, it could be said that there was a corresponding increase in the antioxidant activity of colostrum with its increasing concentration (Table 1). The results of the assay are expressed in percentage (%) of inhibition. There is a concentration-dependent increase in the activity of IC_{50} value. While colostrum exhibits antioxidant activity but requires a 10% increase in the concentration to achieve the standard IC_{50} value of ascorbic acid. The results of in vitro study suggests that colostrum has antioxidant property of scavenging free radicals (Fig. 1). This method has shown effectiveness compared to standard ascorbic acid.

Reducing power of a substance is associated with its antioxidant activity. The increase in the absorbance value indicates the reducing ability of the test compound. Since, the reducing power measures the electron donating capacity of an antioxidant, the reducing power of the compound is increased with increase in its concentration. The presence of antioxidants in colostrum resulted into reduction of the ferric cyanide complex (Fe^{3+}) to the ferrous cyanide form (Fe^{2+}). However, the reducing power of reference compound (ascorbic acid) was found to be greater than that of colostrum (Table 1). It has been reported that the reducing power of substances is related to their hydrogen-donating ability, which was found to be present in our test compound colostrum.

Correlation was found between the two antioxidant methods. The IC_{50} value of colostrum was found to be 180 ppm. So, as we compare the two in vitro DPPH and ex vivo TBARS methods, it can be argued that in the DPPH method, we require approximately 220 ppm to achieve IC_{50} value as compared to TBARS 180 ppm. Thus it may be concluded that the bovine colostrum has a nutraceutical potential as was shown by its good antioxidant activity both in the in vitro as well as ex vivo findings.

It is well established that damage to the myocardium results in increased circulating levels of both CKMB and LDH isoform enzymes in the blood stream. Highest concentrations of CKMB and LDH were seen in the standard group of myocardial infarction induced rats. Due to the various beneficial components present in the colostrum, there was a significant decrease in CKMB value with the 500 mg kg^{-1} dose of colostrum along with 0.25 mg kg^{-1} enalapril (Fig. 3). Correspondingly,
biochemical diagnostic serum enzyme LDH effect was studied in seven groups of rats and the
colostrum with the dose 500 mg kg\(^{-1}\) along with 0.25 mg kg\(^{-1}\) enalapril showed significantly
declined concentrations of LDH in myocardial-infarction-induced rats (Fig. 4).

CONCLUSION

Based on the results of current study, it may be concluded that the bovine colostrum possesses
concentration-related antioxidant activity as revealed by the various in vitro tests. In addition, the
combined administration of 500 mg kg\(^{-1}\) colostrum plus 0.25 mg kg\(^{-1}\) enalapril showed marked
cardioprotective effects in rats after 28 days dosing. Colostrum itself was also cardioprotective
against isoprotearenol-induced myocardial infarction. Overall, the results indicated that colostrum
in combination with enalapril exhibited far greater cardioprotective activity when compared with
enalapril or colostrum alone. Further studies are needed to evaluate the nutraceutical potential of
colostrum before it can be used for treating cardiovascular diseases in humans.

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