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SALBUTAMOL: A SUBSTITUENT OF ISOPROTERENOL TO ESTABLISH AN EXPERIMENTAL ANIMAL MODEL TO INDUCE MYOCARDIAL INFARCTION

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ABSTRACT

Isoproterenol, a catecholamine acting as a β adrenergic agonist, is commonly used to establish myocardial infarction (MI) in animal models for drug development. Although cheaper than isoproterenol, salbutamol is another type of catecholamine, may be potentially and equally used for the onset of MI in animal model. However the concentrations of salbutamol for inducing MI need to be optimized and standardized. In current work, following the treatment with different concentrations of salbutamol, the blood samples of rats were taken at different time intervals according to "Central Composite Design" (CCD) for measuring the level of cardiac markers, CK-MB, LDH and SGOT. Response Surface Methodology (RSM) indicated an optimal salbutamol dosage of 80 mg/kg for inducing MI, which resulted in elevated level of SGOT after 20 hr with CK-MB and LDH level changes in 23 hr following salbutamol (80 mg/kg) administration. The level changes of cardiac marker Trop I and ECG also confirmed the onset of myocardial infarction at the optimal concentration of salbutamol. Current work obtained the optimal dose and the feasibility of salbutamol as a substituent of isoproterenol for inducing MI.

Key words: Salbutamol, CCD, MI, RSM.

INTRODUCTION

The cardiovascular disease (CVD) is a global life threat (Srivastav *et al.,* 2013). 17.3 million People died from CVDs in 2008, this number will rise up to 23.6 million in 2030 (Cao *et al*., 2013). Among CVDs, myocardial infarction (MI) is increasing with frequent proportion all over the world (Clarke *et al*., 2014). As the number of cardiac patients is increasing therefore to combat heart diseases new drugs with new concepts is needed. The animal models impart crucial role to discover new drugs for management and treatment of various diseases in human being (Ahsan *et al*., 2014). The chemically provoked MI in animals is a well recognized typical model to examine the valuable outcomes of medicinal plants on cardiac dysfunction (Sahreen *et al*., 2014).

Catecholamine is potent inducer of myocardial contractility in animal models at its high dose and may lead to ischemia, hypoxia, myocardial hyperactivity and coronary hypertension (Jahan *et al*., 2012; Yousefi *et al*., 2014; Beaulah *et al*., 2014). Among various catecholamine, the isoproterenol [1-(3,4-dihydroxyphenyl)-2-isopropylamino ethanol hydrochloride] is documented to cause severe oxidative stress in the myocardium (Beaulah *et al.,* 2014; Kumar and Gurusamy 2014; Prabha *et al.,* 2014). The necrosis of cardiomyocytes causes the alteration in membrane permeability and leads to the loss of functions and integrity of myocardial membranes (Ramadoss *et al*., 2012).

Salbutamol is also synthetic catecholamine that brings about severe myocardium stress and necrosis due to its structural similarity and mode of action with isoproterenol (Aslam *et al*., 2015). Salbutamol elevates intracellular concentration of cyclic AMP (cAMP) by activating adenylate cyclase. The elevated level of cAMP also inhibits the release of inflammatory mediators from mast cells and eosinophils (Rahman *et al*., 2012). High dose of salbutamol resulted in tachycardia which may lead to myocardial infarction (Hina *et al*., 2010). As the salbutamol is economical, easily available as compared to isoproterenol but very few literature citations with varying concentrations of salbutamol to induce myocardial infarction are available.

A variety of cardiotoxic agents are available for preclinical trials and the most commonly reported toxin used in experimental animals is isoproterenol. The salbutamol may be used as an alternative drug to induce MI because of its accessibility and low price. In this research the animal model was established to optimize the concentration of salbutamol to induce MI. statistically the animal model was designed by applying Central Composite Design (CCD) of Response Surface Methodology (RSM) to get the optimal concentration that may induce myocardial infarction.

MATERIALS AND METHODS

Selection of animals: The rats having 150-250 g weight were kept in animal house, Department of Clinical Medicine and Surgery, University of Agriculture,

Faisalabad to acclimatize for one week under laboratory conditions. They were fed with standard rats' feed throughout the experimental period. The husk in the cages was renewed three times a week to ensure hygiene and maximum comfort for rats.

Experimental design for dose optimization: The rats were orally administered with varying concentrations of salbutamol as suggested by "Central Composite Design" of Response Surface Methodology (RSM-CCD) for two consecutive days. The blood sampling was also performed at different time intervals from 0 to 116 hr, also proposed by RSM-CCD (Table. 1).

Table 1. Experimental design suggested by Central Composite Design to optimize the dose of salbutamol.

Sr. No.	Time of blood sampling (hr) X_1	Conc. of salbutamol (mg/kg) X_2
1	0	50
2	96	50
3	0	125
4	96	125
5	0	88
6	116	88
7	48	34
8	48	141
9	48	88
10	48	88
11	48	88
12	48	88
13	48	88

Biochemical analysis: The rats were anesthetized and the blood sample was drawn by aortic puncture. The serum was used to analyze the effect of different concentrations of salbutamol on cardiac markers (CK-MB, SGOT and LDH) by using Biomed kits through chemistry analyzer. After biochemical analysis the statistical tool "RSM" was applied to predict the optimal dose of salbutamol at which it may induce MI.

Endorsement of optimized dose: To confirm the optimal dose of salbutamol, rats were administered with optimized concentration of salbutamol orally for two consecutive days. The blood sample was taken at optimal time to confirm the indication of MI by performing Trop I test. The ECG was also performed for the corroboration of myocardial infarction. Standard lead II was recorded by power Lab and needle electrodes were placed subcutaneously in the extended limbs of the supine animals.

Statistical analysis: The dose optimization of salbutamol was executed by applying Central Composite Design (CCD) of Response Surface Methodology (RSM).

RESULTS AND DISCUSSION

The response of cardiac markers was predicted by applying multiple regression analysis on the experimental data. The quadratic models obtained from regression analysis for Y_1 (CK-MB), Y_2 (SGOT) and Y_3 (LDH) were as follows.

$$Y1=85.70810+1.39235\ X_1+1.81761\ X_2+6.11111E\text{-}003\ X_1X_2\ -0.018873\ X_1^2\ -8.87704E\text{-}003\ X_2^2$$
$$Y2=-48.13573+1.43497X_1+2.42125X_2+5.46484E\text{-}003X_1X_2\ -0.017539X_1^2\ -0.012166X_2^2$$
$$Y3=201.45349+1.50376X_1+2.10444X_2+0.010833X_1X_2\ -0.022602X_1^2\ -0.011076X_2^2$$

Where the X_1 and X_2 are the coded variables for time of blood sampling (hr) and concentrations of salbutamol (mg/kg) respectively.

The relationship between dependant (cardiac markers) and independent variables (concentrations of salbutamol and time intervals) was graphically presented by 3D Response surface plots in Fig. 1-3.

The interaction of different concentrations of salbutamol and time intervals of blood sampling on the level of CK-MB has been given in Fig. 1. The increased in concentration of salbutamol from 34 to 88 mg/kg b.wt. after 48 hr of salbutamol administration resulted in elevation in CK-MB level from 152 to 297 IU/L. Further increase in concentration from 88 to 141 mg/kg of salbutamol showed comparatively low elevation in the CK-MB level. However after 116 hr of salbutamol administration (88 mg/kg.b.wt), there was considerable decline in enzyme level from 293 to 184 IU/L. The possible explanation could be that the CK-MB level fall to normal with passage of time intervals after 48 to 72 hr of MI development (Dianita *et al*., 2015). In case of the rats administered the salbutamol at the concentrations of 125 and 50 mg/kg did not show the considerable elevation in the level of CK-MB after intervals of 96 hr. Thus the quantity of enzymes released, depends upon the degree of cellular damage (Khan *et al*., 2014).

The Fig. 2 described the relation between dependent (SGOT) and independent variables (conc. and time intervals). The salbutamol at the concentrations of 34, 88 and 141 mg/kg b.wt depicted the inclined in the level of SGOT after 48 hr of salbutamol administration. This might be due to the reason that salbutamol causes leakage of SGOT from cardiomyocytes into the blood stream. It occurs as a result of collapse of cellular and subcellular compartments that reflect pathological alterations in myocardium (Jaffe *et al*., 2006). After 96 hr of salbutamol administration at the concentration of 50 and 125 mg/kg, the SGOT level was comparatively low.

This decline in SGOT level might be due to the increase in time intervals as the SGOT level shifted towards normal with passage of time (Dianita *et al*., 2015).

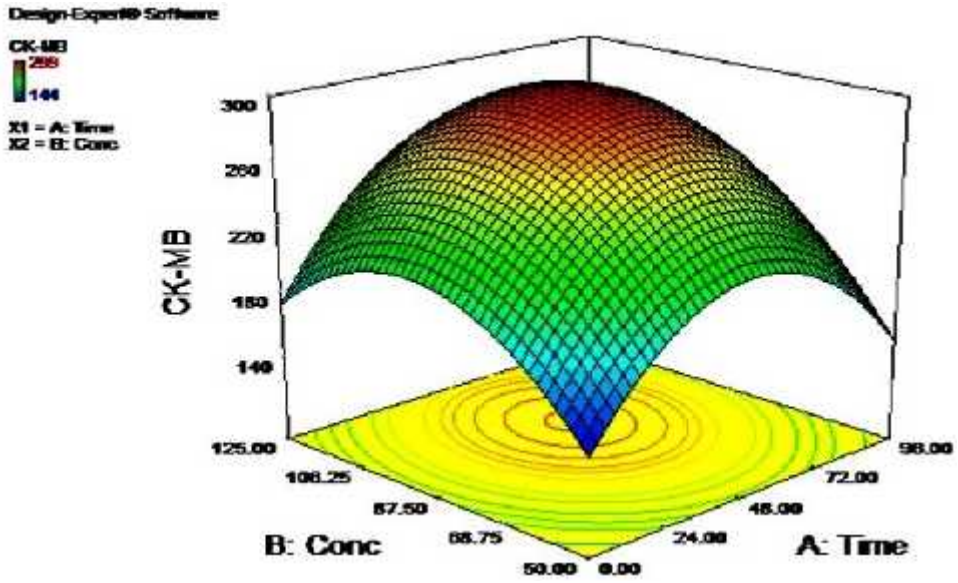


Fig. 1. Response surface plot of CK-MB vs. time and concentration

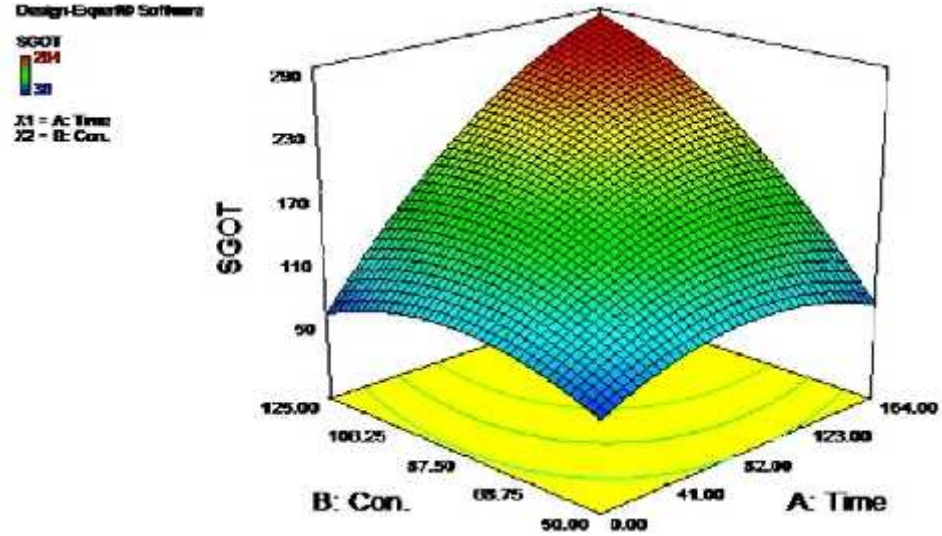


Fig. 2. Response surface plot of SGOT vs time and concentration

In case of LDH, the increase in concentrations (34 to 88 mg/kg b.wt) resulted in increase in level of LDH after 48 hr interval of salbutamol administration as the LDH is detectable from 8 to12 hours of post MI and reached at its peak at 24-72 hours (Rosenblat *et al*., 2012). The rats treated with salbutamol at the concentration of 88 mg/kg depicted the considerable fall in the level of LDH with further increase in time interval of 116 hr after salbutamol administration (Fig. 3). This decline in LDH level supported the fact that serum levels of LDH approaches to normal value after 96 hours after MI (Wang *et al*., 2006). The level of LDH was 278 and 357 IU/L in response to the corresponding concentrations of 50 and 125 mg/kg b.wt. after 96 hr intervals of salbutamol administration. This increase in the level of cardiac markers in serum indicated the altered membrane permeability and leakage of these enzymes into blood stream.

The salbutamol increased the enzyme level by inducing oxidative stress and myocardial cell necrosis (Dianita *et al*., 2015; Zafar *et al*., 2015). The extent of leakage of cardiac markers indicated the onset of myocardial infarction and act as sensitive markers of myocyte injury (Nandave *et al*., 2007). Previous researchers also indicated the cardiotoxicity due to administration of salbutamol which resulted in increased

cardiac marker, lipid profile and alleviated antioxidant enzymes as compared to normal control group (Zafar *et al*., 2015). The histopathological examination of heart after salbutamol administration also indicated the development of myocardial infarction in another animal trial (Aslam *et al*., 2015).

The analysis of variance (ANOVA) presented the statistical significance of the fitted quadratic polynomial model (Table. 2). The F values of quadratic model were, 48.18, 27.27 and 122.30 for CK-MB, SGOT and LDH respectively which suggested the significance of this model. The model is adequate to present the relationship between the response of dependent (cardiac markers) and independent variables (concentrations of salbutamol and time intervals). The value of determination coefficient (R^2) for studied cardiac markers with non significant lack of fit at P>0.05 depicted that the calculated model was able to explain more than 97.18 % of the results. Meanwhile, a relatively lower value of coefficient of variation showed a better precision and reliability of the experiment.

The Table. 3. presented the predicted optimal concentration and time at which salbutamol elevated the level of cardiac markers. The cardioprotective potential of *Coriandrum sativum* against salbutamol induced cardiac injury in another experimental model also favored the increased in cardiac markers after administration of two doses of salbutamol (Kousar *et al*., 2012).

Depletion in myocardial LDH and CK-MB isoenzymes levels due to salbutamol administration indicated the altered membrane permeability and leakage of these enzymes into blood stream (Nandave *et al*., 2007). Increase in cardiac enzymes was due to unnecessary formation of free radicals which activate membrane permeability variations, causing the failure of functions and integrity of myocardial membranes (Barman *et al*., 2013).

Endorsement of optimized dose: The onset of MI was confirmed by administrating the optimized dose (80 mg/kg) of salbutamol for two consecutive days and blood sample was taken after 23 hr of salbutamol administration to perform the Trop I test. The positive indication of Trop-I confirmed the onset of myocardial infarction (Fig. 4). Troponin is a protein found in cardiac tissue and locates in the thin filament of striated muscles consisting of the three subunits Trop T, Trop I and Trop C. Out of the three troponins; Troponin T and I are being used as the biochemical markers for the diagnosis of myocardial injury (Subashini *et al*., 2011).

The significant increased level of Trop I might be due to the leakage of Trop I from the damaged heart tissues into the blood stream as a result of necrosis induced by optimized dose of salbutamol in rats. Electrocardiograph abnormalities are the main criteria generally used for the definite diagnosis of MI. The ECG showed the significant elevation of ST-segment in rats to which the salbutamol was administered.

The characteristic findings were reductions in the P wave intensity, increase in QRS amplitude, QT interval and prolongation of cardiac cycle along with ST segment elevation. These alterations could be due to the consecutive loss of cell membrane in injured myocardium (Thippeswamy *et al*., 2009). These alterations depicted the onset of MI by optimized dose of salbutamol (80 mg/kg).

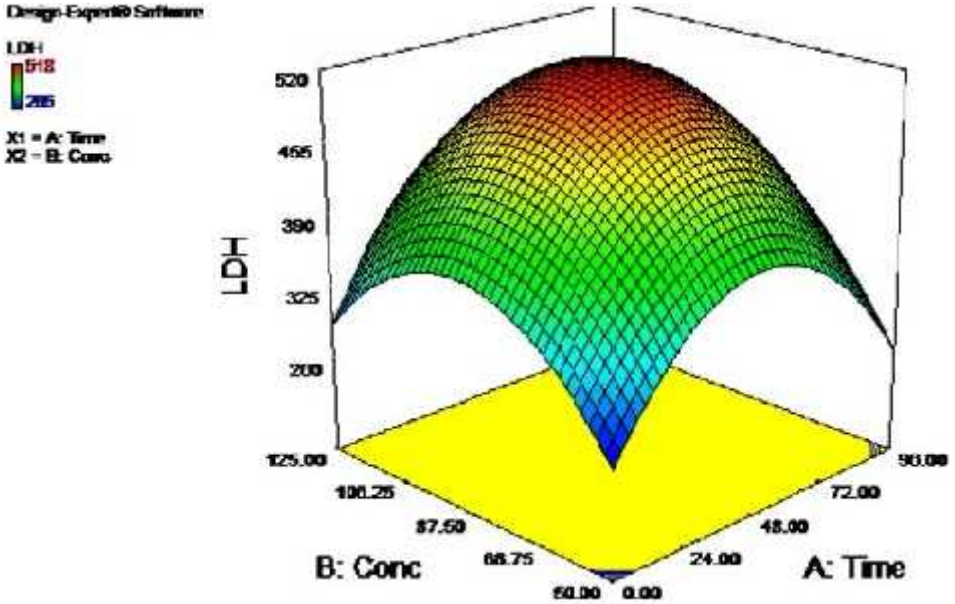


Fig. 3. Response surface plot of LDH vs. time and concentration

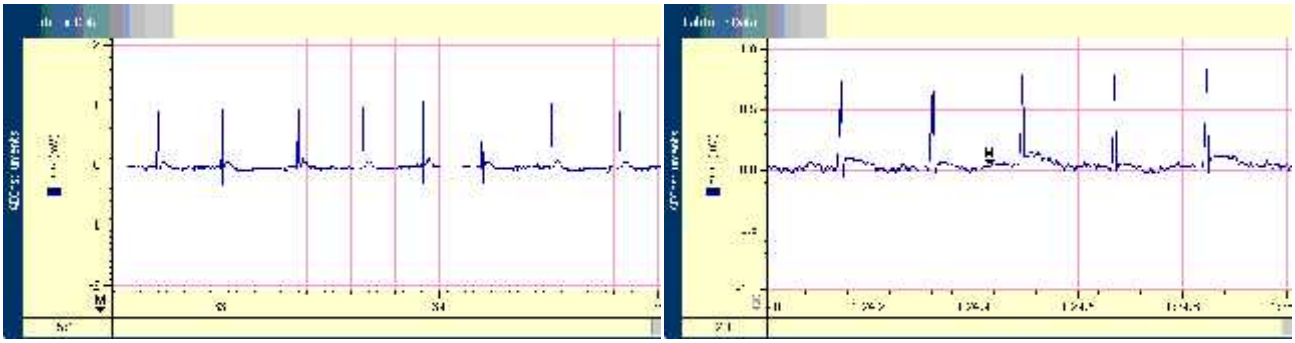
Table 2. Analysis of variance (ANOVA) for the fitted quadratic model of CK-MB, LDH and SGOT activity as a function of independent variables.

Parameter	Source	SS	Df	MS	F Value	P>F
	Model	54546.23	5	10909.25	48.18	<0.0001
	A-Time	1188.22	1	1188.22	5.25	0.0557
	B-Conc	4585.08	1	4585.08	20.25	0.0028
CK-MB	AB	400.00	1	400.00	1.77	<0.0001
R^2=0.9718	A^2	34111.31	1	34111.31	150.65	<0.0001
CV=6.86	B^2	20304.00	1	20304.00	89.67	<0.0001
	Residual	1585.00	7	226.43		
	Lack of Fit	1564.20	3	521.40	100.27	<0.0521
	Pure Error	20.80	4	5.20		
	Model	17167.89	5	3433.58	27.27	0.0002
	A-Time	969.95	1	969.95	7.7	0.0275
	B-Con	3459.58	1	3459.58	27.48	0.0012
SGOT	AB	387.04	1	387.04	3.07	0.123
R^2=0.9512	A2	11359.67	1	11359.67	90.22	<0.0001
CV=12.96	B2	2036.11	1	2036.11	16.17	0.0051
	Lack of Fit	731.36	3	243.79	6.5	0.0541
	Residual	881.36	7	125.91		
	Pure Error	150	4	37.5		
	Model	1.571E+005	5	31423.93	122.30	<0.0001
	A-Time	2680.09	1	2680.09	10.43	<0.0145
	B-Conc	7444.98	1	7444.98	28.97	<0.0010
LDH	AB	1521.00	1	1521.00	5.92	
R^2=0.9887	A^2	99216.21	1	99216.21	386.13	
CV=4.20	B^2	64680.21	1	64680.21	251.72	
	Residual	1798.64	7	256.95		
	Lack of Fit	1777.44	3	592.48	111.79	0.0613
	Pure Error	21.20	4	5.30		

Table 3. Effects of optimized dose of salbutamol on different cardiac markers suggested by Response Surface Methodology.

Parameter	Time (hr)	Concentration(mg/Kg)	Optimized dose (IU/L)	Desirability
CK-MB	23	80	265	0.907
SGOT	20	80	99	0.786
LDH	23	80	467	0.912

Fig. 4. Salbutamol induced MI group showing positive Troponin I indicating the onset of MI.



(Normal control) (Salbutamol induced Group)

Fig. 5. The Electrocardiograph pattern in normal control and salbutmol administered rats.

Conclusions: The research concluded that salbutamol being economic and effective agent might be used as an alternate of isoproterenol to induce MI successfully with an optimal concentration of 80 mg/kg.

Author's contribution: Nadia Afsheen (NA), Ph.D research scholar performed the optimization of salbutamol as a substituent of isoproterenol to standardize an experimental animal model to induce MI. All the research work was planned under the kind supervision of Prof. Khalil-ur-Rehhman (KR). Nazish Jahan (NJ) and Anjum Zia (AZ) also interpreted the data. All the authors read and approved the manuscripts carefully.

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Compliance with ethical guidelines:

Competing interests: The authors declare that they have no competing interests.

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