Final Report

Title of the Project

"To study the cardioprotective effect of a herbomineral formulation Bruhat Vata Chintamani Rasa in experimental model of Isoproterenol induced cardiotoxicity in Male *Sprague Dawley* rats"

Sponsored by

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Report Submitted by

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1. INTRODUCTION

Coronary Artery Disease (CAD) prevalence continues to rise in India with rapid epidemiological transition, as per the World Health Organization (WHO). The incidence of CAD in young population in Western countries is 2–5%, whereas it is 11–16% in Asian Indians. In a study of ethnic differences in patients with Myocardial Infarction (MI) in England, it was observed that young Indians had ten times more risk of developing MI as compared to the white population (Guha et al., 2017). CAD has already surpassed communicable diseases as the major cause of mortality in India. The prevalence of CVDs in India was estimated to be 54.5 million in 2016. Ischemic heart disease (IHD) and stroke constitute the majority of CVD mortality in India (83%), with IHD being predominant. These diseases tend to affect patients in the most productive years of their lives and result in catastrophic social and economic consequences. (Prabhakaran et al., 2016)

Acute myocardial infarction is a severe condition of ischemic heart disease which is a key contributor worldwide to the mortality caused within the population which suffer from coronary heart disease (Fan, 2019). It is well known that ischemic heart tissue forms oxygen-derived free radicals that are responsible for oxidative damage of membrane lipids, proteins and carbohydrates. This abnormality develops the quantitative and qualitative alterations of the myocardium (Burton et al., 1984). If cardiac ischemia remains for a longer duration it leads to a myocardial infraction which is nothing but the death of heart muscle tissue.

Isoprenaline or isoproterenol (ISO), a catecholamine derivative and β-adrenergic agonist by its positive inotropic and chronotropic action, increases myocardial oxygen demands when administered in high dose and develop ischemic necrosis of myocardium in rats. Auto-oxidation of ISO generates cytotoxic free radicals which causes peroxidation of phospholipids of the myocardium and alters its permeability and ultimately damages myocardium. A higher dose of catecholamine also deplete the reserved energy of cardiac muscle and develop biochemical and structural abnormality in the myocardium (Rona, 1985). Various regimens of reactive oxygen species scavengers are now is a developing phase and will be considered as newer therapeutic interventions for ischemic diseases.

Ayurved medicines (herbs, minerals and their formulations) can be beneficial in the reducing the risk of IHD. Bruhat Vata Chintamani Rasa is a classical gold containing cardioprotective and neuroprotective formulation offering the benefits of Suvarna Bhasma (Processed Gold),

Raupya Bhasma (Processed Silver), Abhrak Bhasma (Processed Mica), Loha Bhasma (Processed Iron), Pravala Bhasma (Processed Coral), Mouktik Bhasma (Processed Pearl), Rasasindoor and the herb Kumari (*Aloe barbadensis*). It is widely used by Ayurved physicians in the management of ischemic heart and brain disorders. It is documented for its cardioprotective / antiarrhythymic activity in experimental model of ouabain-induced arrhythmia (Data on file – Unpublished data). It is reported to offer neuroprotection in experimental model of cerebral ischemia induced by occlusion of both common carotid arteries. Its neuroprotective attribute may be due to its anti-oxidant and lipid peroxidation attenuating actions (Goshan et al., 2015). It has the potential to improve the cardiac circulation, whereby increasing myocardial oxygen requirement and functioning of the heart. Therefore, in the present study, a herbomineral formulation Bruhat Vata Chintamani (BVC) Rasa was tested for its activity against ISO induced cardiotoxicity in rats.

2. PRODUCT DESCRIPTION

Product Name: Bruhat Vata Chintamani Rasa
Manufactured by: Shree Dhootapapeshwar Limited, Mumbai
Marketed by: Shree Dhootapapeshwar Limited, Mumbai
Batch Number: P19100206
Manufacturing Date: 10/2019
Expire Date: 09/2024

Composition of Bruhat Vata Chintamani Rasa (BVC) Tablets:

Each tablet (62.5 mg) of **Bruhat Vata Chintamani Rasa** contains:

Suvarna Bhasma [Premium] (Processed Gold)	7.5 mg
Rajata Bhasma (Processed Silver)	5.0 mg
Abhraka Bhasma (Processed Mica)	5.0 mg
Loha Bhasma (Processed Iron)	12.5 mg
Pravala Bhasma (Processed Coral)	7.5 mg
Mouktik [Mukta] Bhasma (Processed Pearl)	7.5 mg
Rasasindoor (Generic Ayurvedic Formulation)	17.5 mg
Processed in: Kumari (*Aloe barbadensis*)	q.s.

Dosage: One to two tablets once or twice a day with honey, goghrut (cow's ghee), Dashamoolarishta, Arjunarishta, Maharasnadi Kadha, lukewarm water or as directed by the Physician.

3. MATERIALS AND METHODS:

Male *Sprague Dawley* rats (180-220 g) were procured from the National Institute of Biosciences, Pune, Maharashtra, India and housed in the animal facility. The temperature of 22 ± 2°C, relative humidity 75 ± 5%, and a 12 h light/dark cycle was maintained in an animal facility throughout the study. Animals received a basal multi-nutritional diet and purified water, *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (Approval Number & Date: CPCSEA/IAEC/P-35/2019, 10th August 2019).

Chemicals and Kits:

Isoproterenol (ISO) 2-Thiobarbituric acid, Reduced glutathione, 1,1,3,3-tetramethoxypropane, and Nitrobluetetrazolium were procured from Sigma (St. Louis, MO, USA). Diagnostic kits like Aspartate transaminase (AST), Lactate dehydrogenase (LDH), Creatinine kinase (CK-MB) were purchased from Transasia Biomedicals Ltd., India. ELISA kits of 5' adenosine monophosphate-activated protein kinase (AMPK) and Silent information regulator 1 (SIRT1) were procured from Bioassay Technology Laboratory, Birmingham, United Kingdom.

Dose calculation:

Formulation	Weight of tablet	Human Recommended dose	Animal dose calculated from human dose
Bruhat Vata Chintamani (BVC) Rasa	157 mg	**Dose 1 (Low Dose):** 1 tab/day	**Dose 1 (Low Dose):** 14.13 mg/kg/day
		Dose 2 (High Dose): 2 tab/day	**Dose 2 (High Dose):** 28.26 mg/kg/day

Stock solution preparation:

Stock solution of BVC for low dose treatment was prepared by suspending 14.13 mg of BVC powder in 10 mL of 0.5% Carboxymethyl cellulose (CMC).

Stock solution of BVC for high dose treatment was prepared by suspending 28.26 mg of BVC powder in 10 mL of 0.5% Carboxymethyl cellulose (CMC).

Experimental design:

Male *Sprague Dawley* rats after acclimatization (7 days) were randomly divided into four groups of ten animals each. The treatment regimen was as follows:

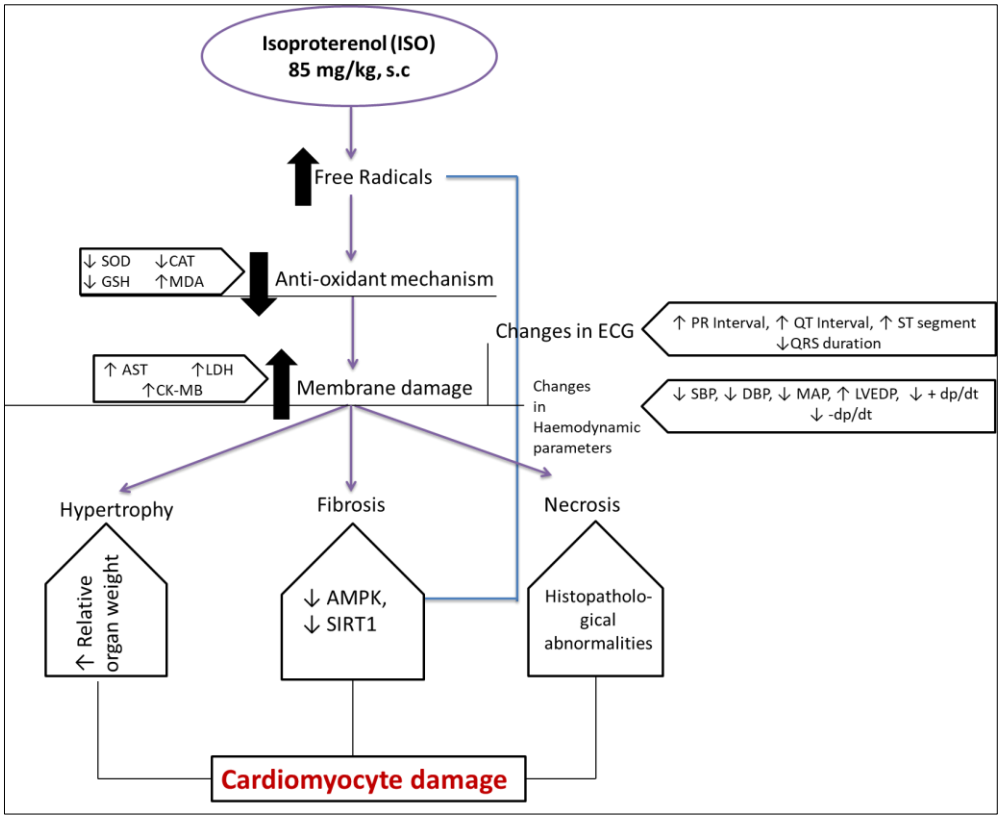
Group I (Normal Control) - Animals in this group received vehicle [0.5% Carboxymethylcellulose (CMC)] for 30 days.

Group II (Disease Control) - Animals in this group received 0.5% CMC for 30 days and additionally, received ISO (85 mg/kg. s.c.) on the 28th and 29th day at an interval of 24 h (Panda et al., 2016).

Group III (BVC, Low Dose) - Animals in this group received BVC (14.13 mg/kg p.o.) for 30 days and additionally, received ISO (85 mg/kg. s.c.) on the 28th and 29th day at an interval of 24 h.

Group IV (BVC, High Dose) - Animals in this group received BVC (28.26 mg/kg p.o.) for 30 days and additionally received ISO (85 mg/kg. s.c.) on the 28th and 29th day at an interval of 24 h.

Mechanism of ISO in cardiotoxicity:



Parameters assessed:

Assessment of electrocardiogram:

Electrocardiogram (ECG) was recorded with the help of Power Lab data acquisition system (AD Instruments, Australia) by canulating the left carotid artery (Oza and Kulkarni, 2020). After 24 h of the last dose of ISO, animals were anesthetized using an intraperitoneal injection of urethane at a dose (1.2 g/kg, *i.p.*). The changes in PR and QT intervals, QRS duration, and ST-segment were recorded.

Assessment of haemodynamic studies:

After the ECG recording, the neck portion was opened with a ventral midline incision, and the left carotid artery of each rat was canulated for measurement of left ventricular end-diastolic pressure (LVEDP), rate of ventricular contractility (+dp/dt and –dp/dt), systolic blood pressure, diastolic blood pressure and mean arterial blood pressure (MABP) (Kulkarni and Laddha, 2020).

Estimation of cardiac marker enzymes:

Blood was withdrawn by cardiac puncture and serum was separated by centrifugation at 5000 RPM for 15 min.

The marker enzymes AST, LDH and CK-MB were assayed in serum using ERBA chem 7 biochemical analyzer (Germany) as per manufacture's protocol provided by Transasia Biomedical Ltd. India.

Assessment of AMPK and SIRT1 levels:

Levels of AMPK and SIRT1 were determined in serum samples of animals from different treatment groups using 96 well-plate using rat AMPK and sirt1 ELISA kit procured from Bioassay Technology Laboratory, Birmingham, UK (Feng et al., 2019).

Determination of cardiac hypertrophy:

After 30 days of treatment, animals were sacrificed and heart tissue was isolated and weighed. Relative organ weight was determined by considering the percentage of heart to body weight ratio.

Determination of oxidative stress parameters:

Isolated heart tissues were washed with ice-cold saline and divided into two equal parts. One part of heart tissue was used for the determination of oxidative stress parameters and the other part was used for histopathology.

For oxidative stress parameters, 10% (w/v) tissue homogenate was prepared in phosphate buffer (50mM, pH 7.4). Oxidative stress parameters like malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were estimated in heart tissue. GSH and MDA levels were determined in heart tissue homogenate. CAT was estimated in the post-nuclear fraction of homogenate whereas SOD was estimated in the post mitochondrial fraction of homogenate (Ellman, 1959; Ohkawa et al., 1979; Paoletti et al., 1990).

Histopathology of heart tissue:

For histopathology, heart tissue was fixed in 10% buffered formalin. Sections of heart tissue were taken using a microtome and stained with Hematoxylin-Eosin (H & E) and Masson Trichrome stain as per previously reported method for determination of tissue necrosis and collagen deposition under a photomicroscope (Oza and Kulkarni, 2018).

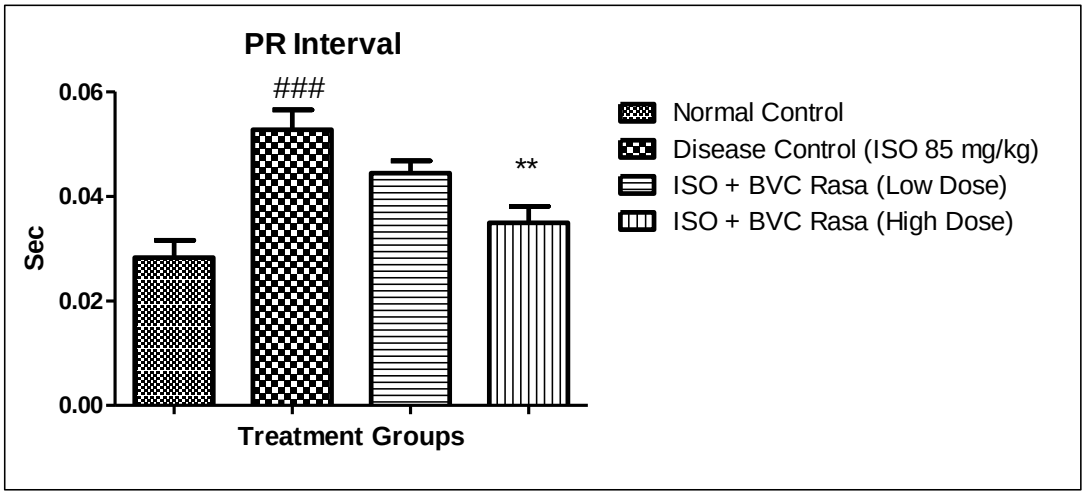
Statistical analysis:

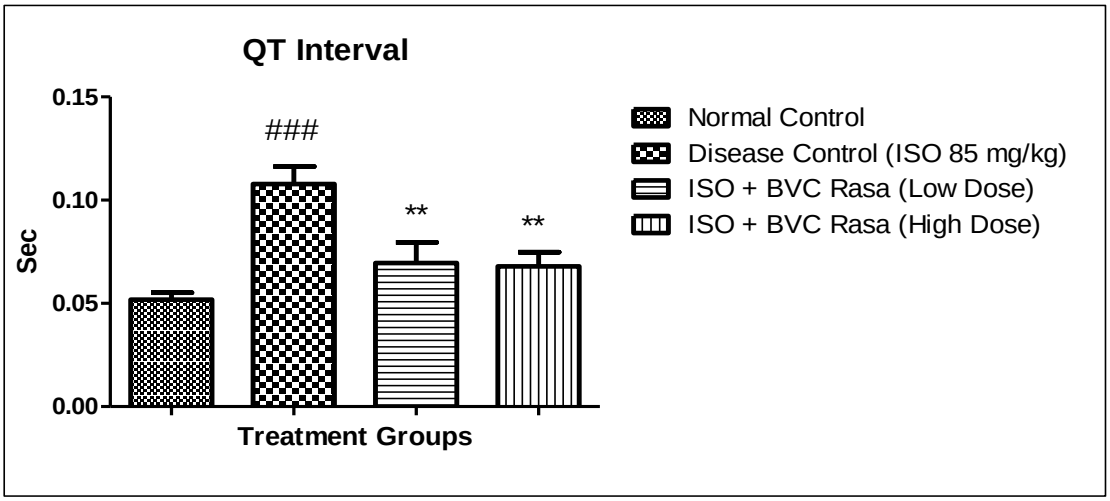
All the data were expressed in Mean ± SEM. Statistical analysis was carried out using Graph pad prism 5 software. One way ANOVA followed by Bonferroni's multiple comparison tests was carried out to determine the level of significance. $P<0.05$ were kept as the level of significance.

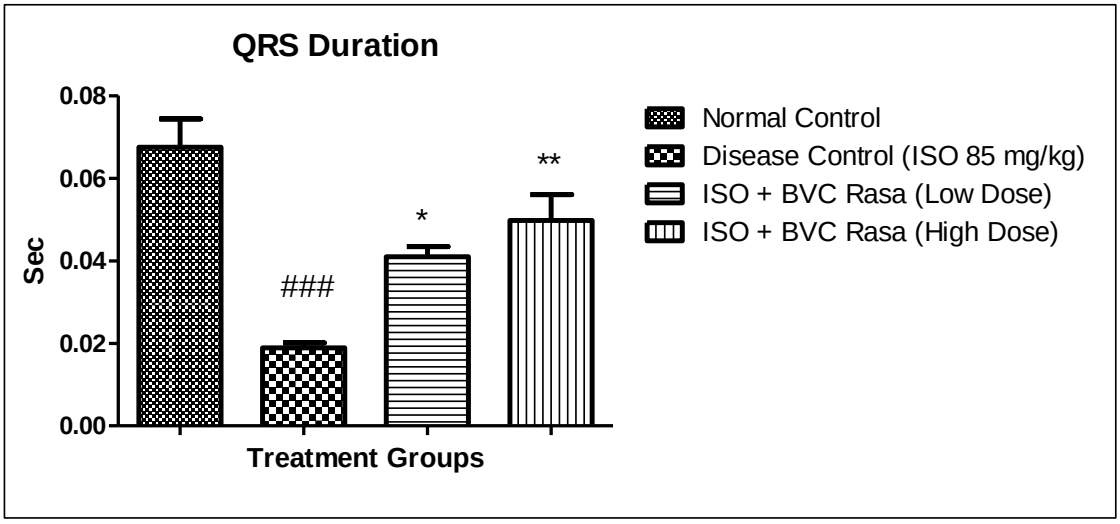
4. RESULTS

***Effect of BVC Rasa electrocardiogram*:**

Significant increase in PR interval, QT interval and ST-segment were observed in disease control animals when compared with normal control animals ($p<0.001$). Treatment with BVC at high dose significantly reduced ($p<0.01$) the elevated PR interval, QT interval and ST-segment. Treatment with BVC at low dose significantly reduced and QT interval ($p<0.01$) and ST segment ($p<0.05$) but did not exhibit any significant reduction in PR interval. Disease control animals showed a significant reduction in QRS duration as compared to normal control animals ($P<0.001$). Treatment with BVC at low and high dose showed significant improvement ($p<0.05$ and $p<0.01$) in QRS duration when compared with disease control animals (Figure 1, Table 1).







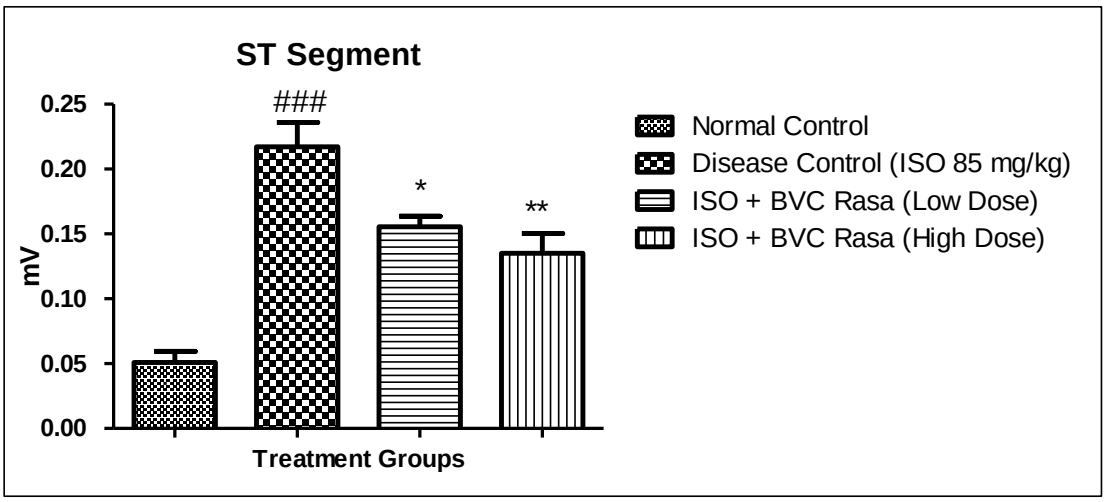
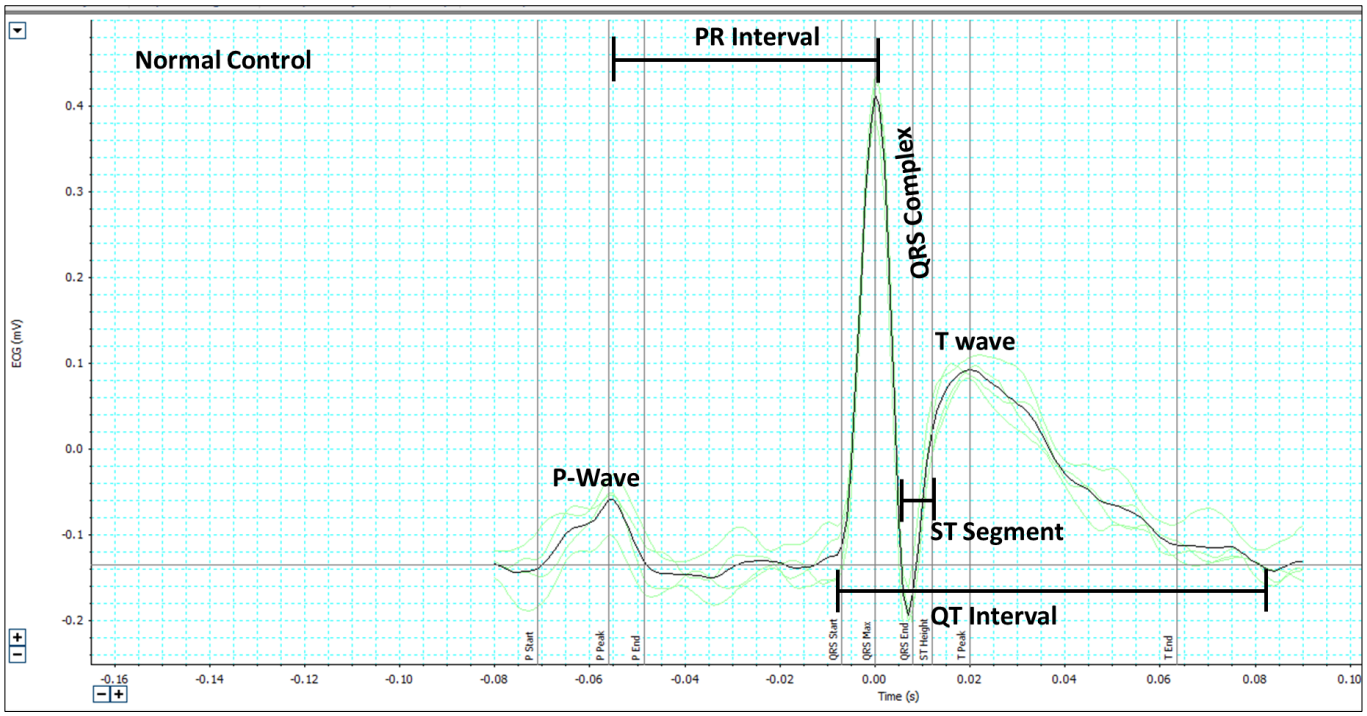
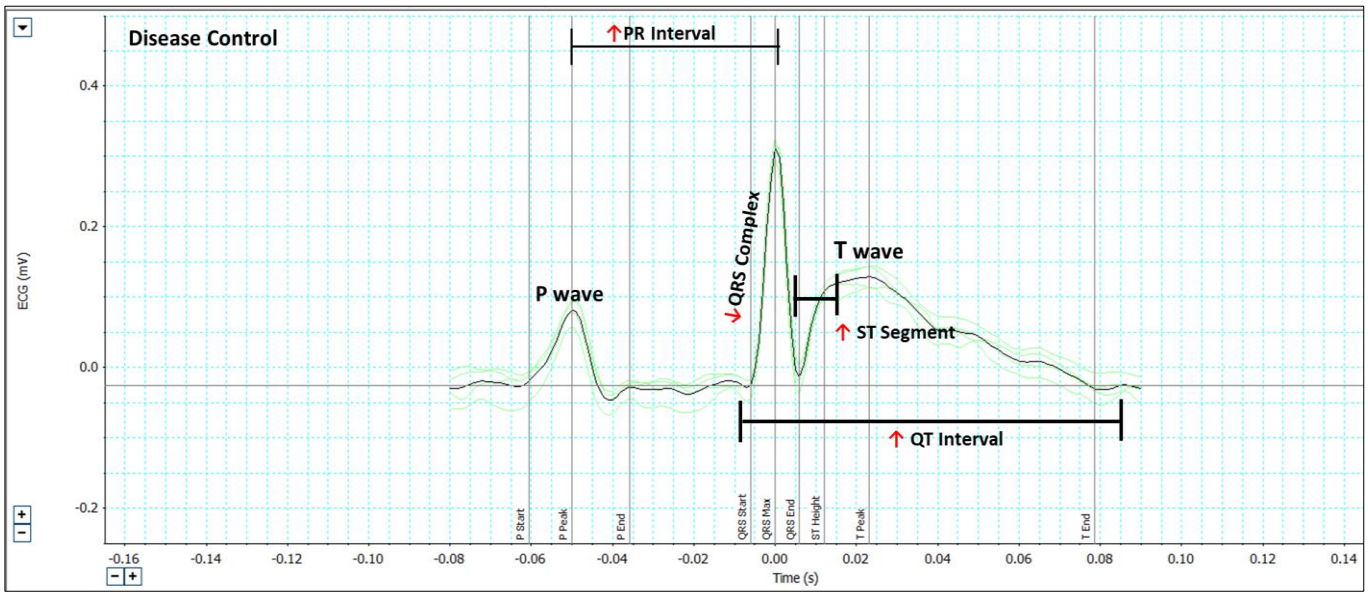
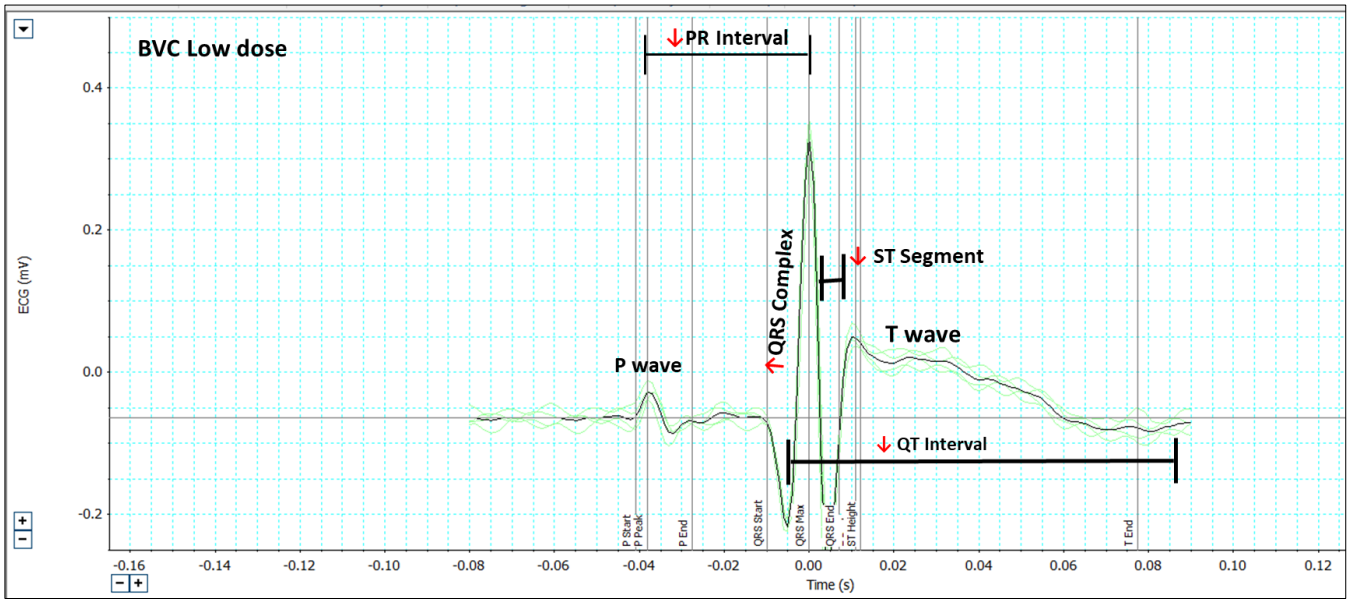


Figure 1 (a): Effect of BVC Rasa on electrocardiogram. All values are expressed as Mean ± S.E.M. (n=6). **p<0.01 and *p< 0.05 when treatment groups compared with disease control. ###p<0.001 when disease control group compared with normal control.


Normal Control
PR Interval
QRS Complex
T wave
P-Wave
ST Segment
QT Interval
ECG (mV)
Time (s)


Disease Control
PR Interval
QRS Complex
T wave
P wave
ST Segment
QT Interval
ECG (mV)
Time (s)



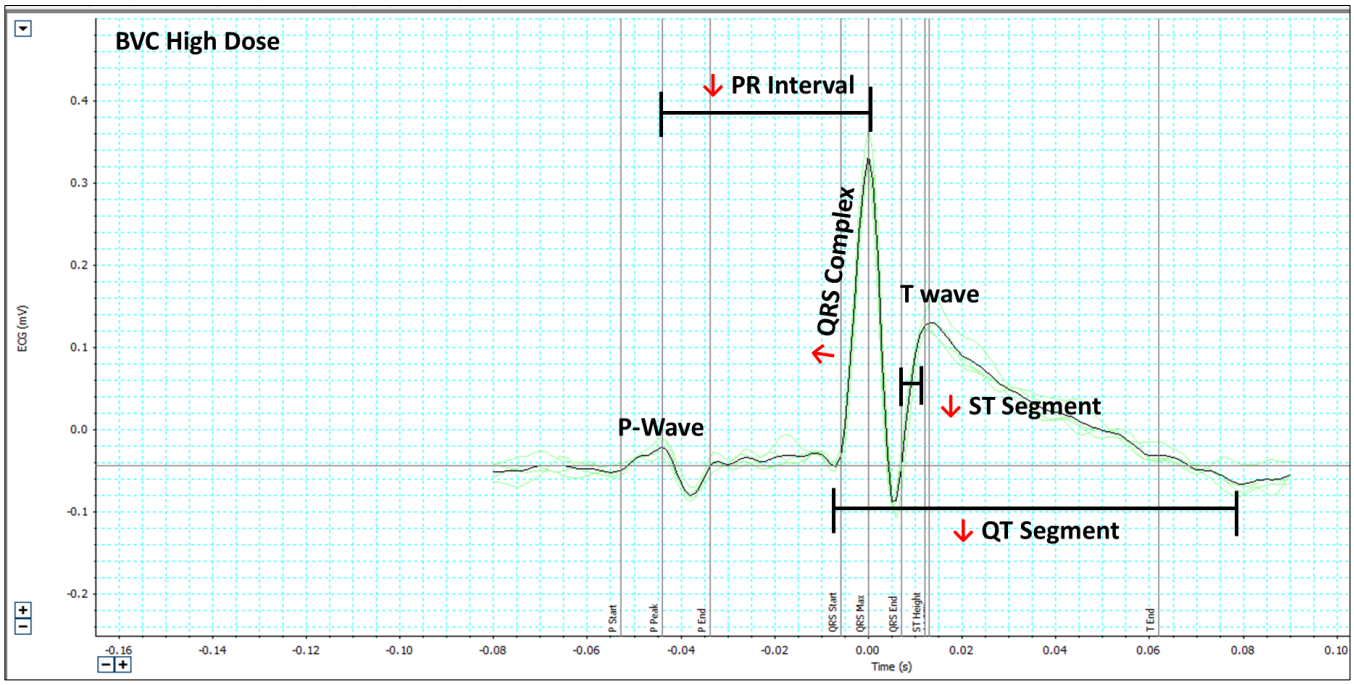


Figure 1 (b): Effect of BVC Rasa on electrocardiogram.

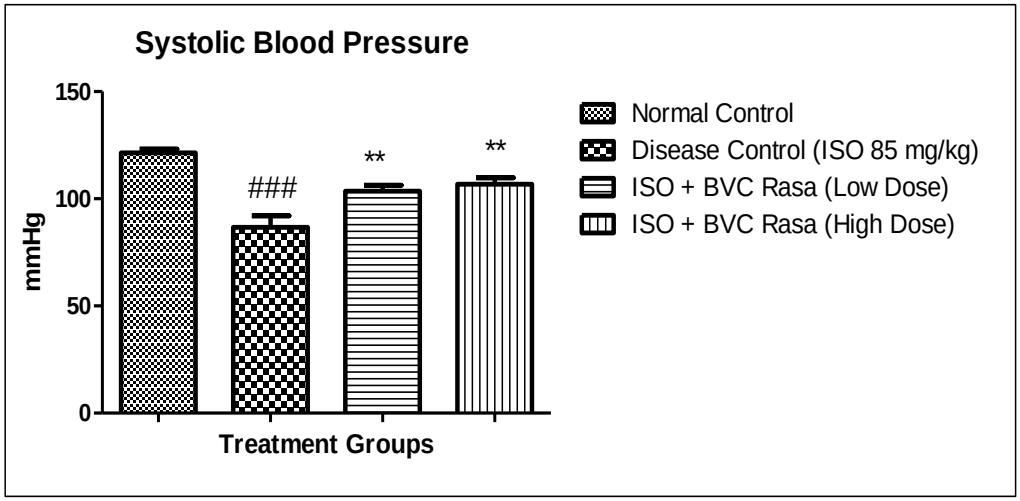
Table 1: Effect of BVC Rasa on electrocardiogram

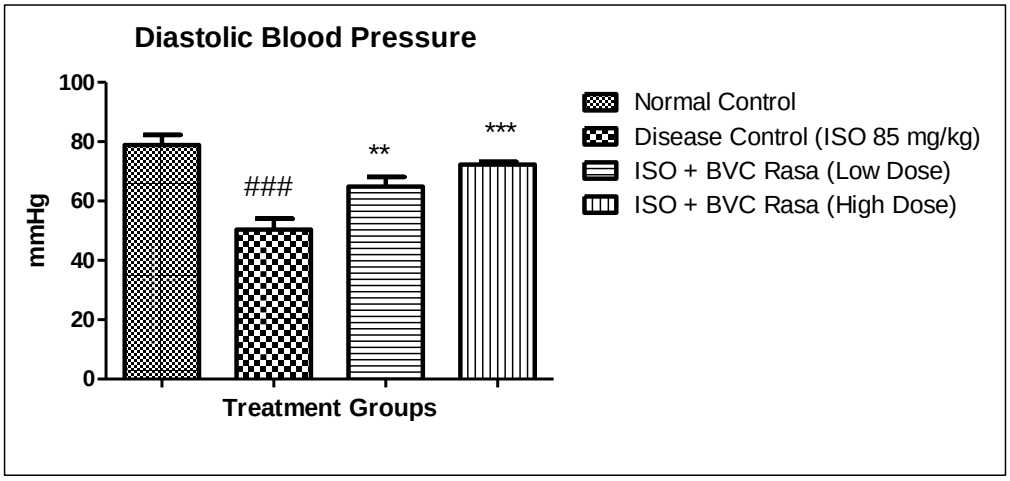
Treatment Groups / Parameters	Normal Control	Disease Control (ISO 85 mg/kg)	ISO + BVC Rasa (Low Dose)	ISO + BVC Rasa (High Dose)
PR Interval (Sec)	0.02826±0.0033	0.05273±0.0038$^{###}$	0.04442± 0.0023	0.03493± 0.0031**
QT Interval (Sec)	0.05176±0.0033	0.1079±0.0084$^{###}$	0.06957± 0.010**	0.06794± 0.006**
QRS Duration (Sec)	0.06748±0.0068	0.01893±0.0011$^{###}$	0.04098± 0.0024*	0.04974± 0.0062**
ST Segment (mV)	0.06765± 0.0182	0.2171± 0.0188$^{###}$	0.1556 ± 0.0080*	0.1351 ± 0.015**

All values are expressed as Mean ± S.E.M. (n=6). **p<0.01 and *p< 0.05 when treatment groups compared with disease control. $^{###}$p<0.001 when disease control group compared with normal control.

***Effect of BVC Rasa on haemodynamic parameters*:**

Disease control animals showed a significant reduction in systolic blood pressure, diastolic blood pressure and mean arterial blood pressure when compared with normal control animals (p<0.001). Treatment with BVC at high dose significantly improved systolic blood pressure (p<0.01), diastolic blood pressure (p<0.001) and mean arterial blood pressure (p<0.01) when compared with disease control animals. Treatment with BVC at low dose significantly improved systolic blood pressure (p<0.01), diastolic blood pressure (p<0.01) and mean arterial blood pressure (p<0.05) when compared with disease control animals (Figure 2, Table 2).





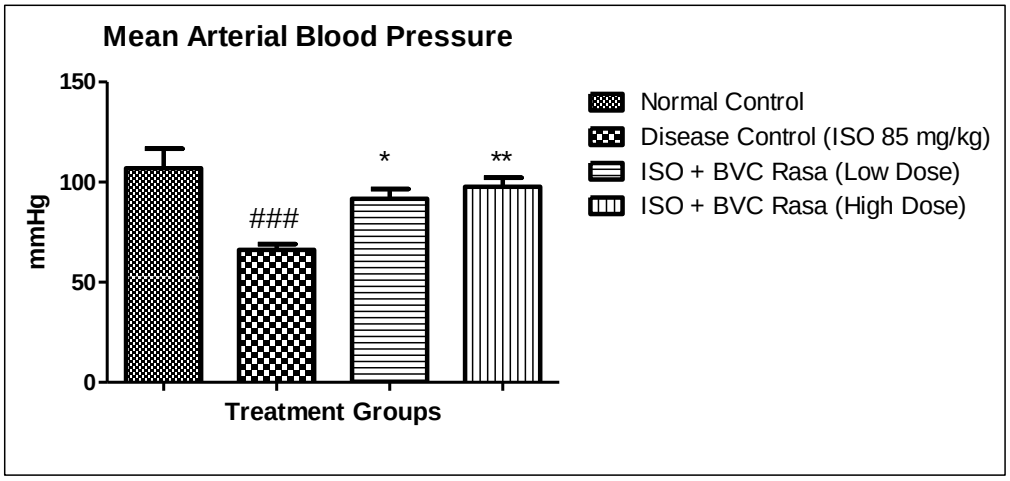


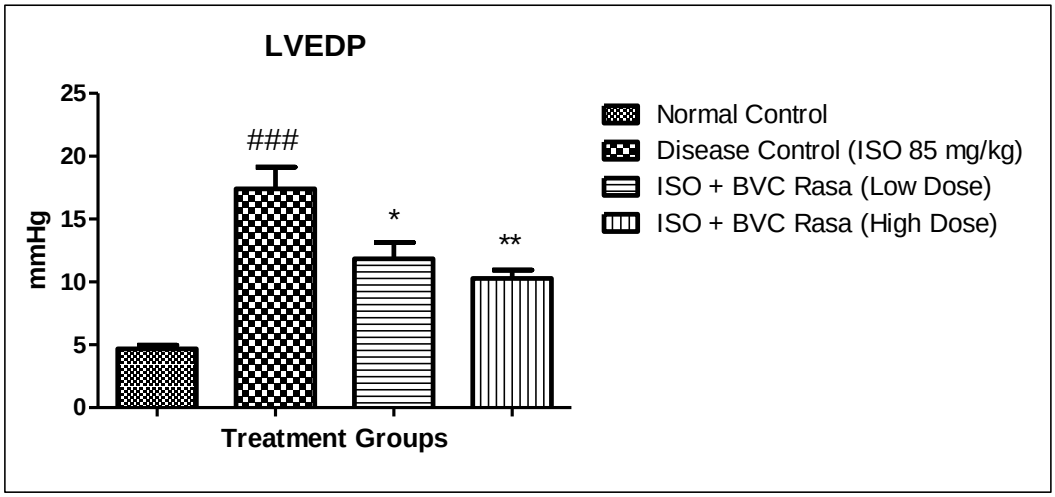
Figure 2: Effect of BVC Rasa on systolic blood pressure, diastolic pressure and mean arterial blood pressure. All values are expressed as Mean ± S.E.M. (n=6)., ***p<0.001, **p<0.01 and *p< 0.05 when treatment groups compared with disease control. ### p < 0.001 when disease control group compared with normal control.

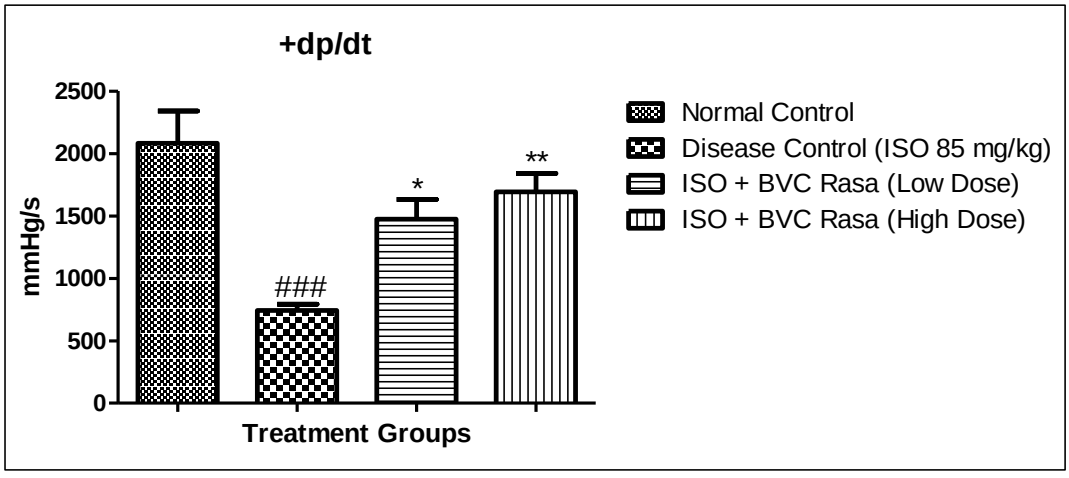
Table 2: Effect of BVC Rasa on systolic blood pressure, diastolic pressure and mean atrial pressure

Treatment Groups / Parameters	Normal Control	Disease Control (ISO 85 mg/kg)	ISO + BVC Rasa (Low Dose	ISO + BVC Rasa (High Dose)
Systolic BP (mmHg)	121.5 ± 1.69	86.73 ± 5.27$^{###}$	103.6 ± 2.72**	106.8 ± 2.98**
Diastolic BP (mmHg)	78.85 ± 3.47	50.4± 3.60$^{###}$	64.84 ± 3.24**	72.27 ± 0.97***
Mean Arterial BP (mmHg)	107 + 9.69	66.21 + 2.88$^{###}$	91.64 ± 4.87*	97.68 ± 4.48**

All values are expressed as Mean ± S.E.M. (n=6). ***p<0.001, **p<0.01 and *p< 0.05 when treatment groups compared with disease control. $^{###}$p < 0.001 when disease control group compared with normal control.

Left ventricular end diastolic pressure (LVEDP) was also found to be elevated in disease control animals when compared with normal control animals ($p<0.001$). Treatment with BVC at high and low dose significantly reduced ($p<0.05$ and $p<0.01$) the elevated LVEDP when compared with disease control animals. A significant reduction in maximum and minimum rate of ventricular contraction was observed in disease control animals when compared with normal control animal ($p<0.001$). Treatment with BVC at high dose significantly improved the maximum ($p<0.01$) and minimum ($p<0.001$) rate of ventricular contraction when compared with disease control animals. Treatment with BVC at low dose significantly improved ($p<0.05$) the maximum and minimum rate of ventricular contraction when compared with disease control animals (Figure 3, Table 3).





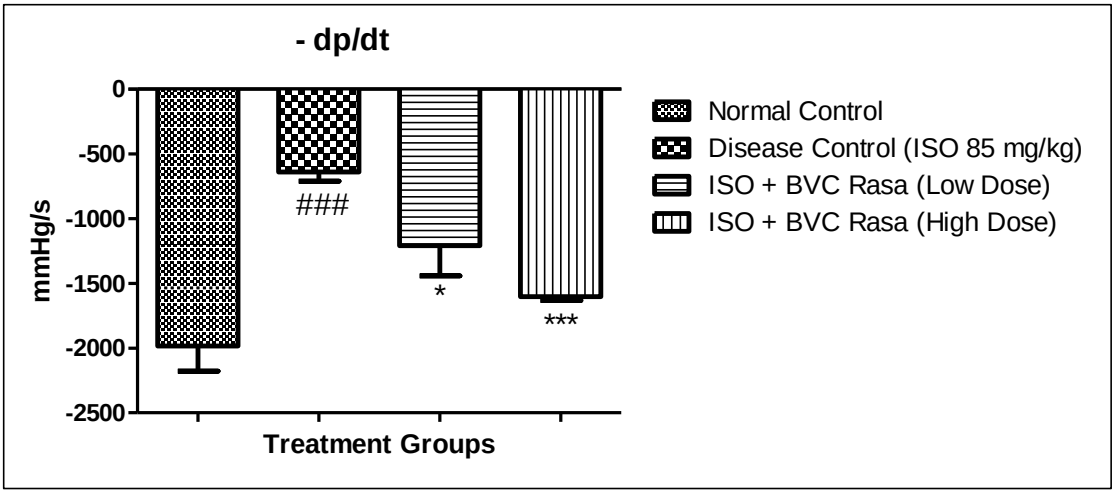


Figure 3: Effect of BVC Rasa on LVEDP, +dp/dt and -dp/dt. All values are expressed as Mean ± S.E.M. (n=6). ***p<0.001, **p<0.01 and *p< 0.05 when treatment groups compared with disease control. ###p<0.001 when disease control group compared to normal control.

Table 3: Effect of BVC Rasa on LVEDP, +dp/dt and -dp/dt

	LVEDP (mmHg)	**+dp/dt (mmHg/s)**	**-dp/dt (mmHg/s)**
Normal Control	4.682±0.2880	2082±257.5	-1985±192.5
Disease Control (ISO – 85 mg/kg)	17.40±1.726###	741.8±50.05###	-639.9±71.17###
ISO + BVC Rasa (Low Dose)	11.84±1.28*	1475±158.3*	-1210±232.3*
ISO + BVC Rasa (High Dose)	10.28±0.6587**	1693±147.7**	-1603±129.26***

All values are expressed as Mean ± S.E.M. (n=6). ***p<0.001, **p<0.01 and *p< 0.05 when treatment groups compared with disease control. ###p<0.001 when disease control group compared with normal control.

***Effect of BVC Rasa on biochemical parameters*:**

Significantly increased levels of serum AST (p<0.001), LDH (p<0.01) and CK-MB (p<0.001) were observed in disease control animals when compared with normal control animals. Treatment with BVC at high dose significantly reduced serum AST (p<0.01), LDH (p<0.05) and CK-MB (p<0.001) when compared with disease control animals. Treatment with BVC at

low dose significantly reduced ($p<0.05$) CK-MB level but did not exhibit significant reduction in AST and LDH levels when compared with disease control animals. (Figure 4, Table 4)

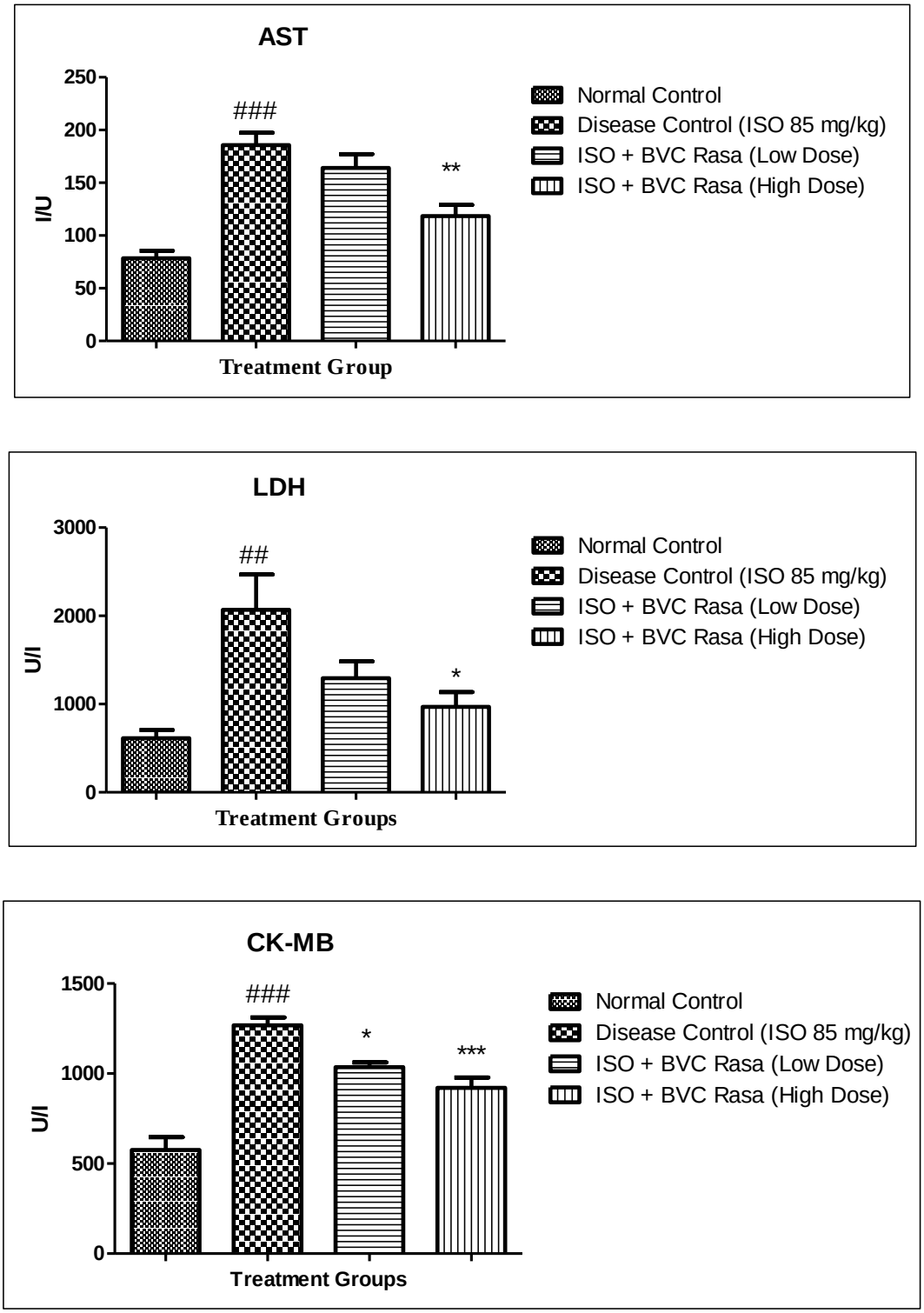


Figure 4: Effect of BVC Rasa on biochemical parameters. All values are expressed as Mean ± S.E.M. (n=6), ***p<0.001**p<0.01 and *p< 0.05 when treatment groups compared with disease control. ###p<0.001 when disease control group compared with normal control.

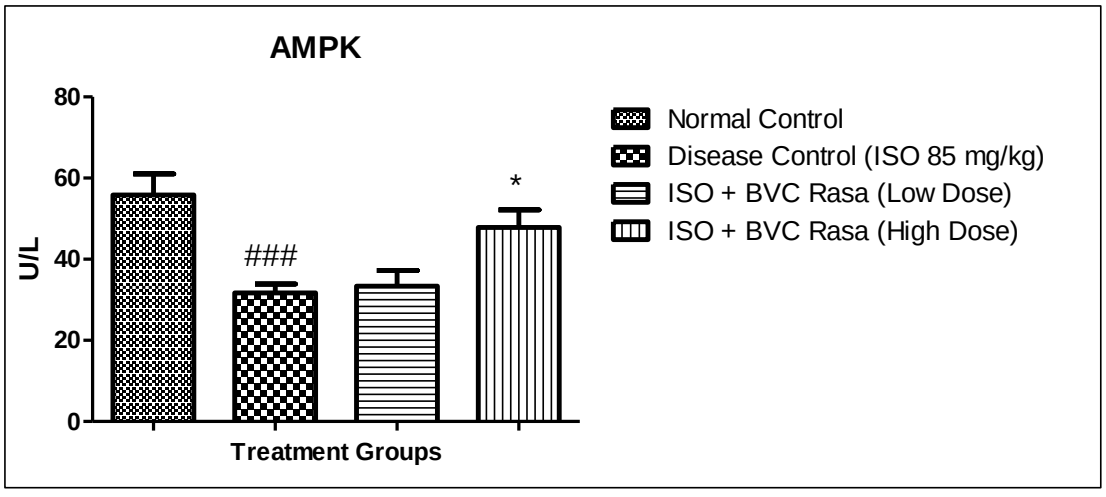
Table 4: Effect of BVC Rasa on biochemical parameters

Treatment Groups / Parameters	Normal Control	Disease Control (ISO 85 mg/kg)	ISO + BVC Rasa (Low Dose	ISO + BVC Rasa (High Dose)
AST (I/U)	78.39 ± 7.06	185.7 ± 11.71$^{###}$	164.1 ± 13.17	118.3 ± 10.62**
LDH (I/U)	613.7 ± 92.71	2068 ± 401.1$^{###}$	1293 ± 191.1	969.6 ± 166.1*
CK-MB (I/U)	575.7 ± 71.66	1268 ± 43.49$^{###}$	1035 ± 27.07*	920.8 ± 57.16***

All values are expressed as Mean ± S.E.M. (n=6), ***p<0.001, **p<0.01 and *p< 0.05 when treatment groups compared with disease control. $^{###}$p<0.001 when disease control group compared with normal control.

Effect of BVC Rasa on AMPK and SIRT1 levels:

Disease Control animals showed significantly reduced serum levels of AMPK (p<0.001) and SIRT1 (p<0.001) when compared with normal control animals. Treatment with BVC at high dose significantly improved (P<0.05 and p<0.01) the levels of AMPK and SIRT1 when compared with disease control animals. Treatment with BVC at low dose did not exhibit significant improvement in AMPK and SIRT1 when compared with disease control animals (Figure 5, Table 5)



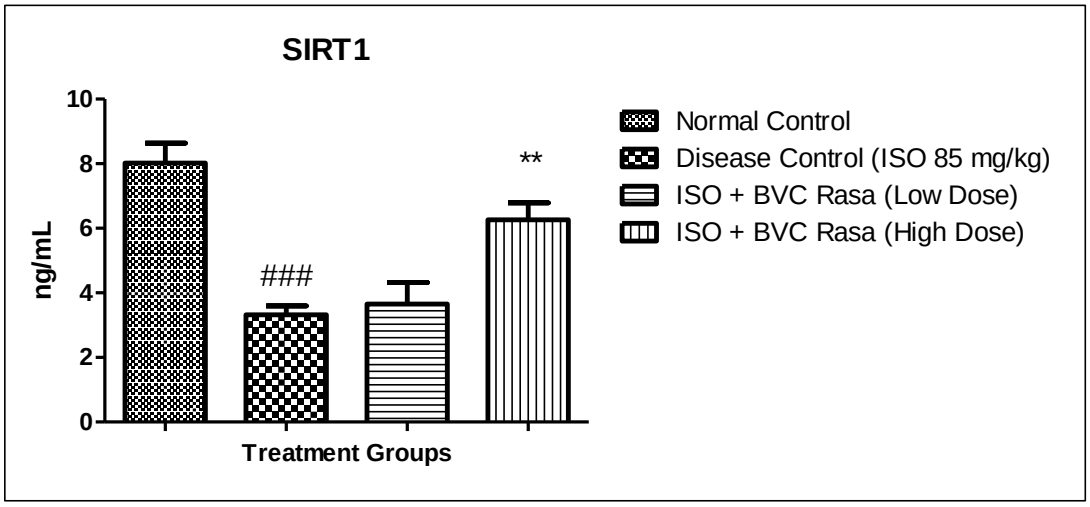


Figure 5: Effect of BVC Rasa on AMPK and SIRT1 level. All values are expressed as Mean ± S.E.M. (n=6). **p<0.01 and *p< 0.05 when treatment groups compared with disease control. ###p<0.001 when disease control group compared with normal control.

Table 5: Effect of BVC Rasa on AMPK and SIRT1 level

Treatment Groups / Parameters	Normal Control	Disease Control (ISO 85 mg/kg)	ISO + BVC Rasa (Low Dose	ISO + BVC Rasa (High Dose)
AMPK (U/L)	55.86 ± 5.17	31.73 ± 2.17###	33.39 ± 3.89	47.81 ± 4.38*
SIRT1 (ng/mL)	8.023 ± 0.61	3.32 ± 0.27###	3.655 ± 0.66	6.265 ± 0.52**

All values are expressed as Mean ± S.E.M. (n=6). **p<0.01 and *p< 0.05 when treatment groups compared with disease control. ###p<0.001 when disease control group compared with normal control.

Effect of BVC Rasa on cardiac hypertrophy:

Disease control animals showed a significant increase in percentage heart to bodyweight ratio when compared with normal control animals. Treatment with BVC at high dose significantly prevented ($p<0.001$) the rise in percentage heart to bodyweight ratio. (Figure 6, Table 6)

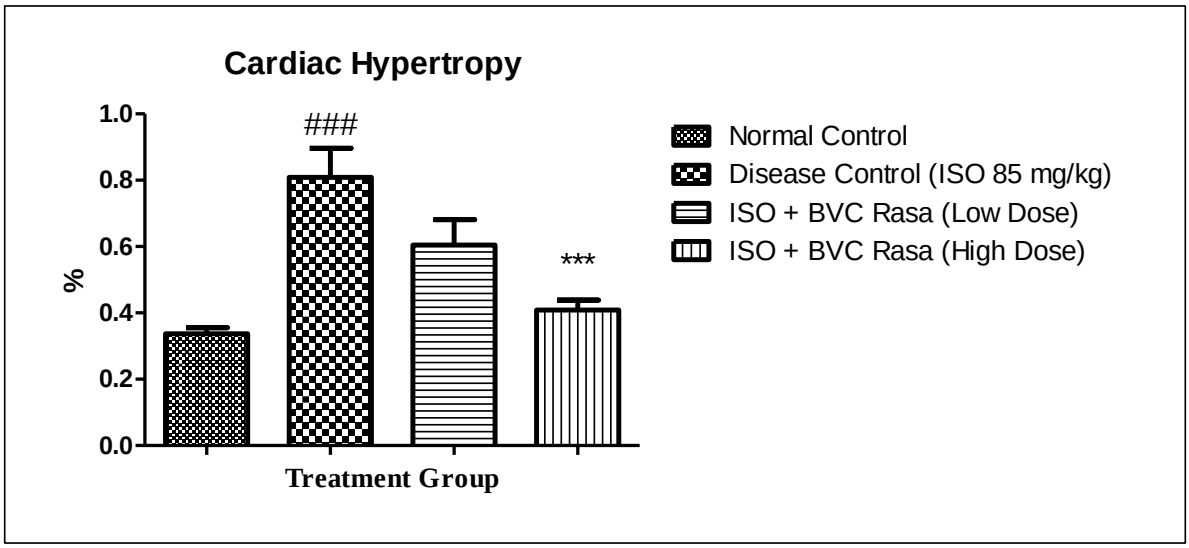


Figure 6: Effect of BVC Rasa on Cardiac Hypertrophy. All values are expressed as Mean ± S.E.M. (n=6). ***p<0.01, when treatment groups compared with disease control. ###p<0.001 when disease control group compared with normal control.

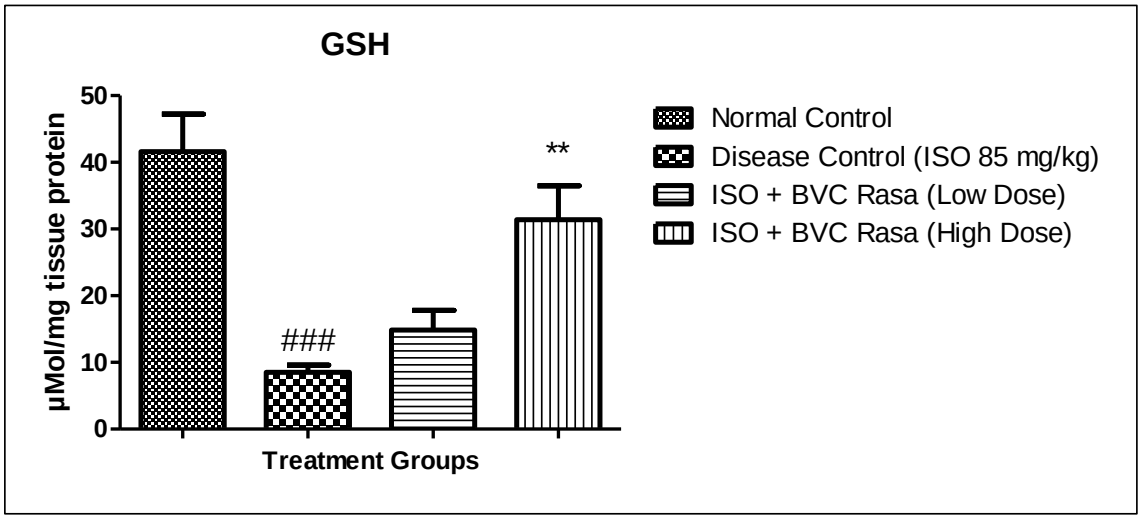
Figure 6: Effect of BVC Rasa on Cardiac Hypertrophy

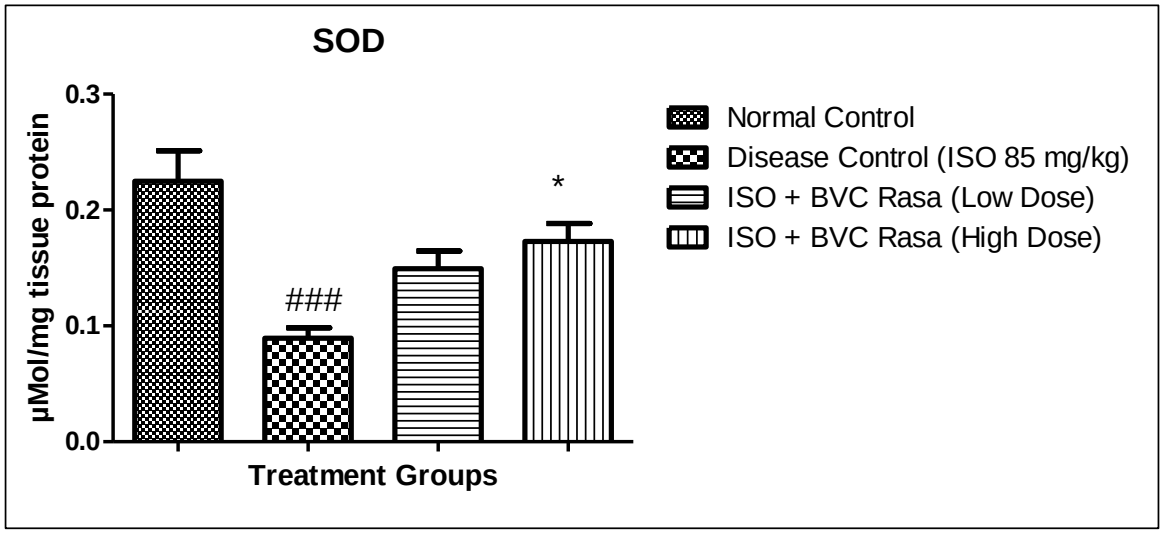
Treatment Groups / Parameters	Normal Control	Disease Control (ISO 85 mg/kg)	ISO + BVC Rasa (Low Dose	ISO + BVC Rasa (High Dose)
Cardiac Hypertrophy (%)	0.3368 ± 0.019	0.8091 ± 0.086###	0.6045 ± 0.07711	0.408 ± 0.03800***

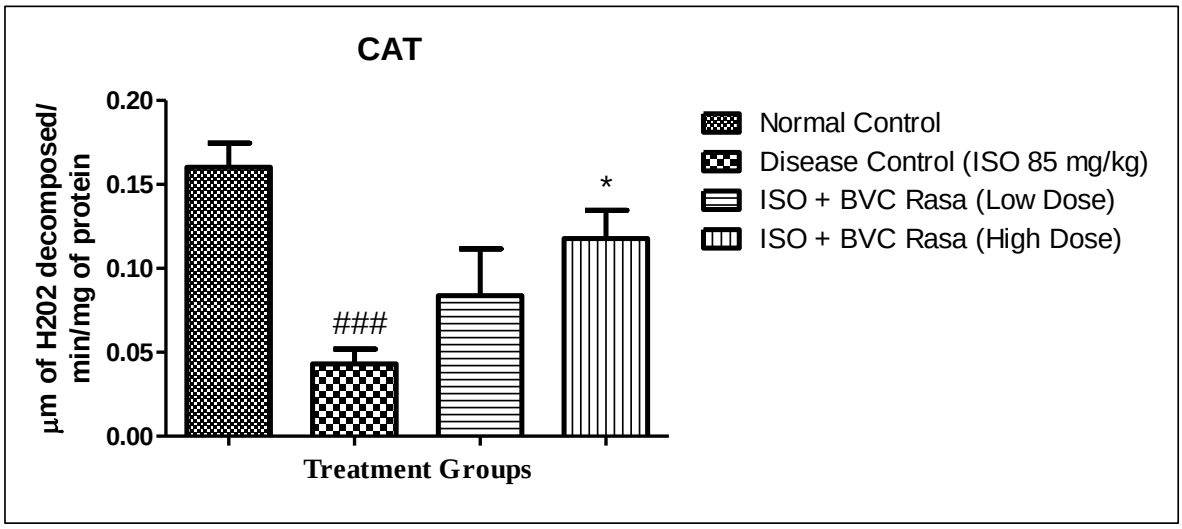
All values are expressed as Mean ± S.E.M. (n=6). ***p<0.01, when treatment groups compared with disease control. ###p<0.001 when disease control group compared with normal control.

***Effect of BVC Rasa on oxidative stress parameters*:**

Disease control animals showed a significantly reduced level of anti-oxidant enzymes like GSH, SOD, CAT and a significantly increased level of MDA in heart tissue homogenate when compared with normal control animals ($p<0.001$). Treatments with BVC at high dose significantly prevented the loss of GSH ($p<0.01$), SOD ($p<0.05$) and CAT ($p<0.05$) when compared with disease control animals. Low dose treatment of BVC did not show significant prevention in the loss of GSH, SOD and CAT levels. Treatment with BVC Rasa at high and low dose significantly reduced ($p<0.01$ and $p<0.001$) the level of MDA when compared with disease control animals (Figure 7, Table 7)







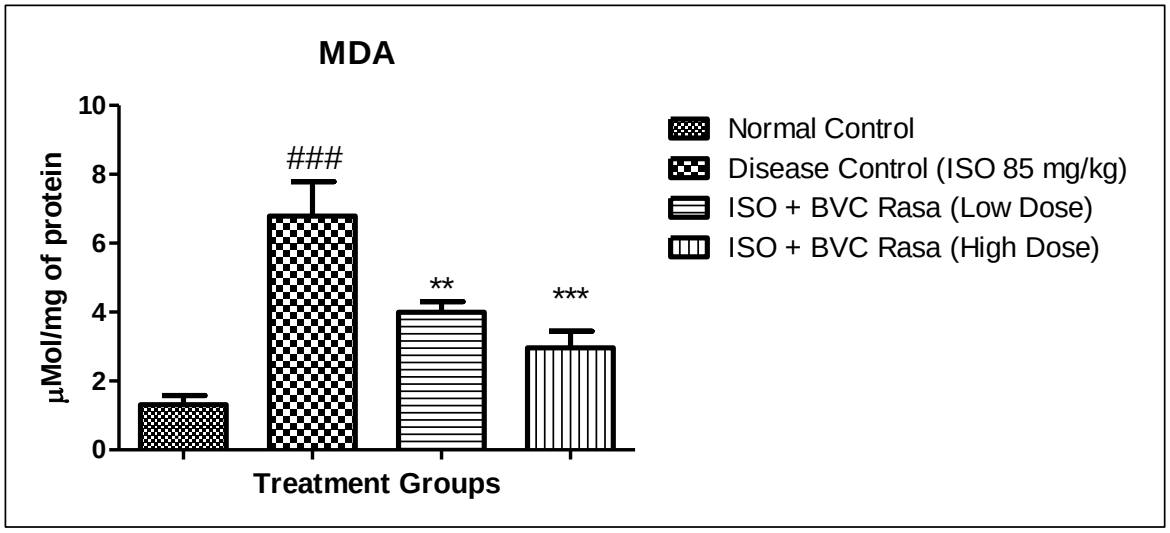


Figure 7: Effect of BVC Rasa on oxidative stress parameters. All values are expressed as Mean ± S.E.M. (n=6). ***p<0.001, **p<0.01 and *p< 0.05 when treatment groups compared with disease control. ### p< 0.001 when disease control group compared with normal control.

Table 7: Effect of BVC Rasa on oxidative stress parameters

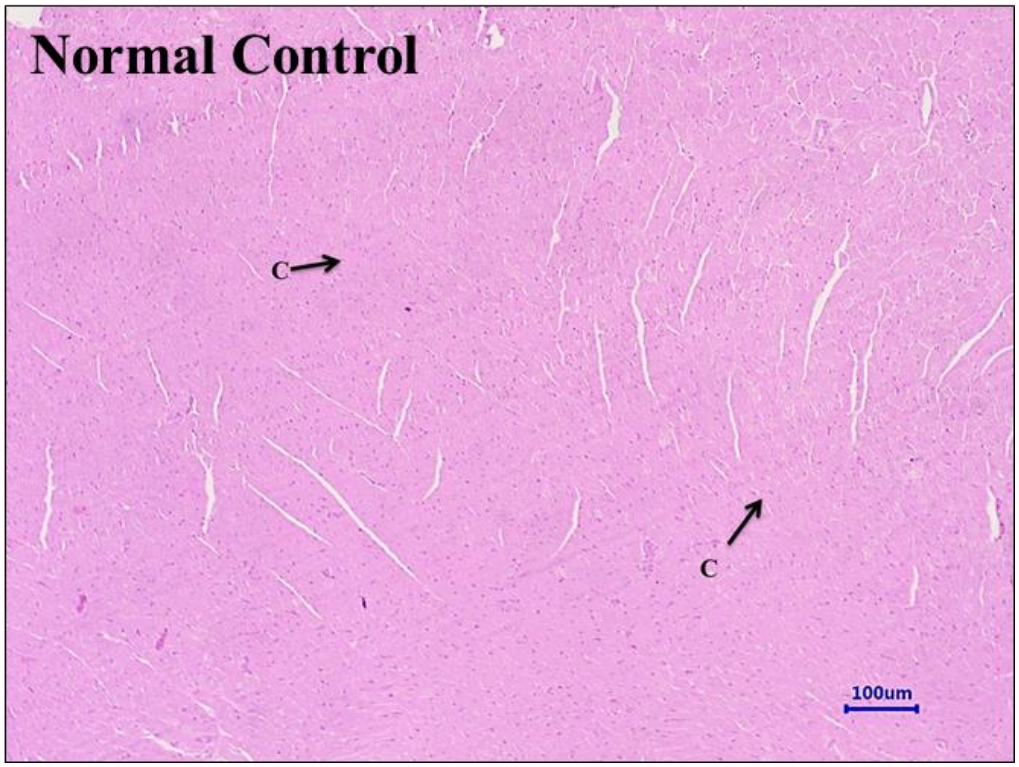
Parameter / Groups	GSH (µMol/mg tissue protein)	SOD (IU/mg of protein)	CAT (µM of H202 decomposed/min/mg of protein)	MDA (µMol/mg of protein)
Normal Control	41.62±5.597	0.2249±0.02626	0.1603±0.01431	1.314±0.2669
Disease Control (ISO- 85 mg/kg)	8.519±1.090###	0.08932±0.009076###	0.04310±0.008809###	6.790±0.9959###
ISO+ BVC Rasa (Low Dose)	14.86± 2.948	0.1495±0.01527	0.08369±0.02789	3.997±0.3033**
ISO+ BVC Rasa (High Dose)	31.38±5.111**	0.1495±0.01548*	0.1177±0.01684*	2.959±0.4834***

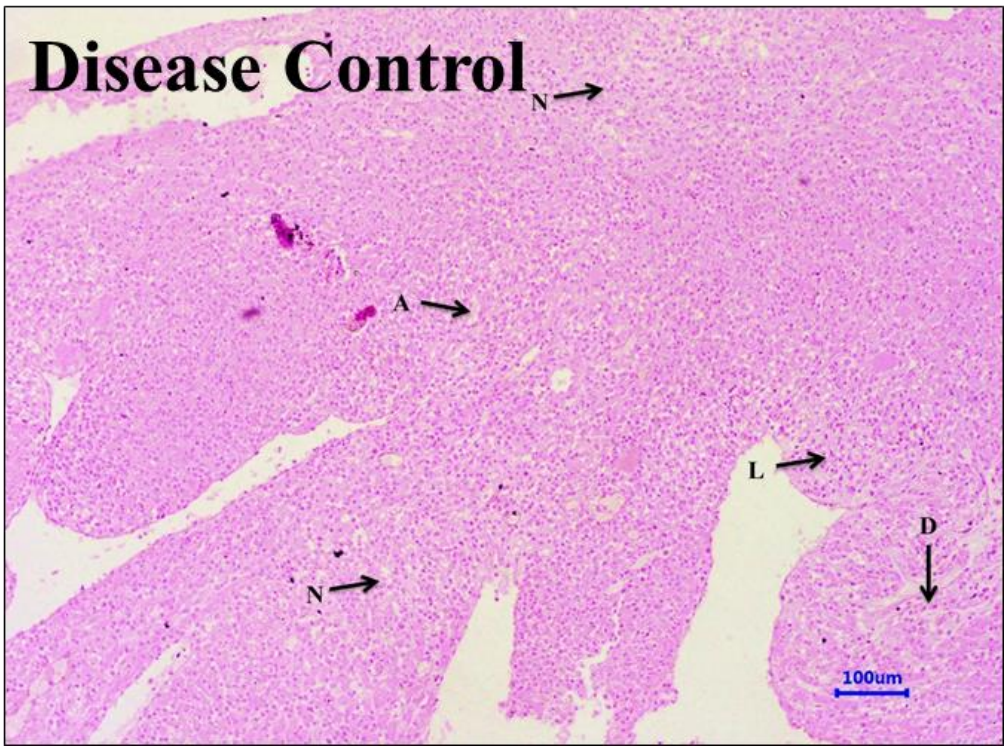
All values are expressed as Mean ± S.E.M. (n=6). ***p<0.001, **p<0.01 and *p< 0.05 when treatment group compared with disease control. ### p < 0.001 when disease group compared to normal control.

***Effect of BVC Rasa on cardiac tissue histology*:**

H&E staining of cardiac tissue of disease control animals showed a significant increase in tissue necrosis (N), degeneration (D), angiogenesis (A) and lymphocytic infiltration (L) in the myocardium when compared with normal control animals. Treatment with BVC at high reduced the severity of the damage in myocardium (Figure 8). Masson Trichrome staining of cardiac tissue of disease control animals showed markedly increased deposition of collagen in cardiac muscle as compared to normal control animals (C). Treatment with BVC at a high dose inhibited the deposition of collagen in cardiac muscle (C) (Figure 9). BVC treatment at high dose showed better improvement in histopathology as compared to BVC low dose.

H & E staining:





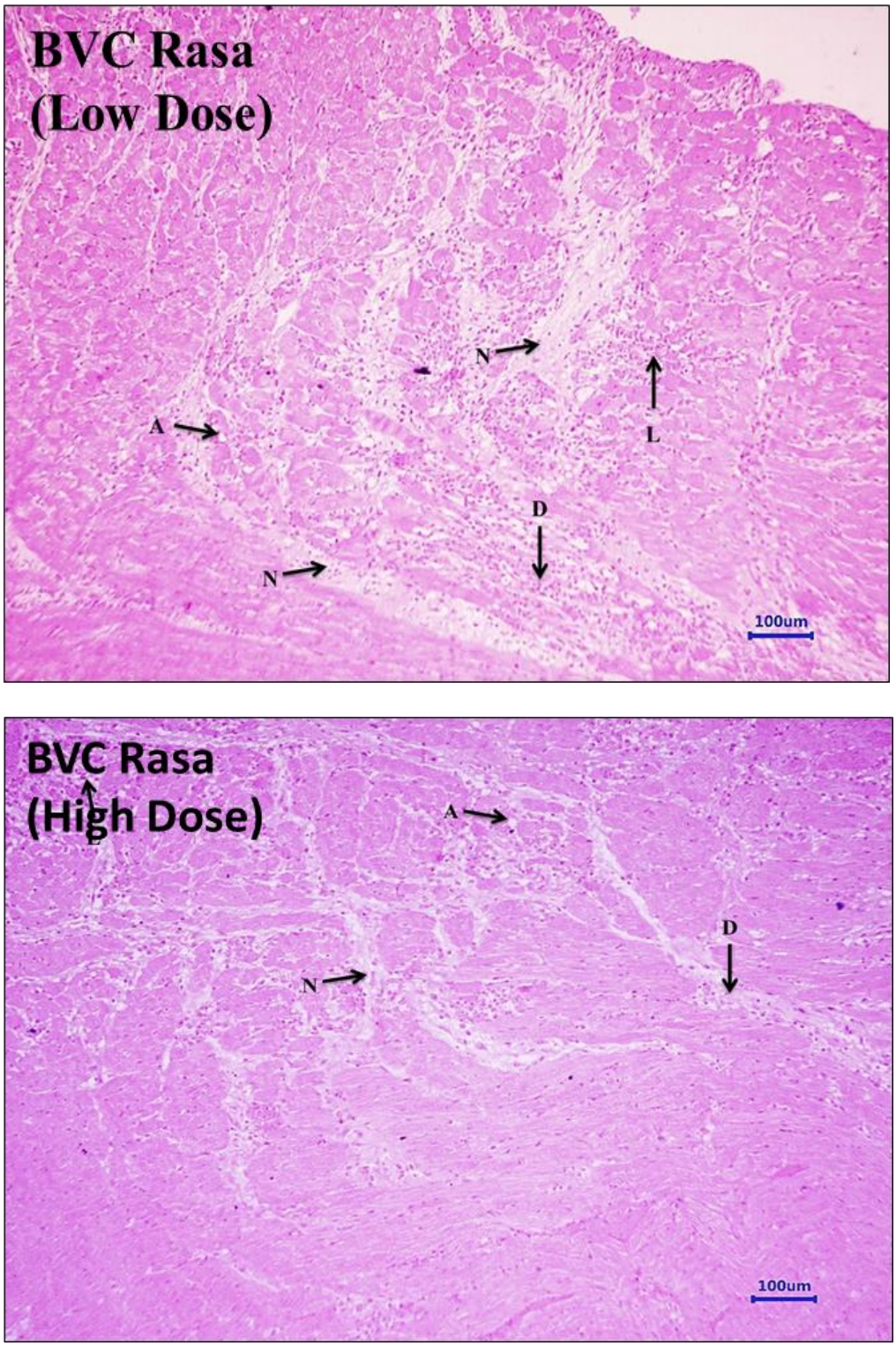
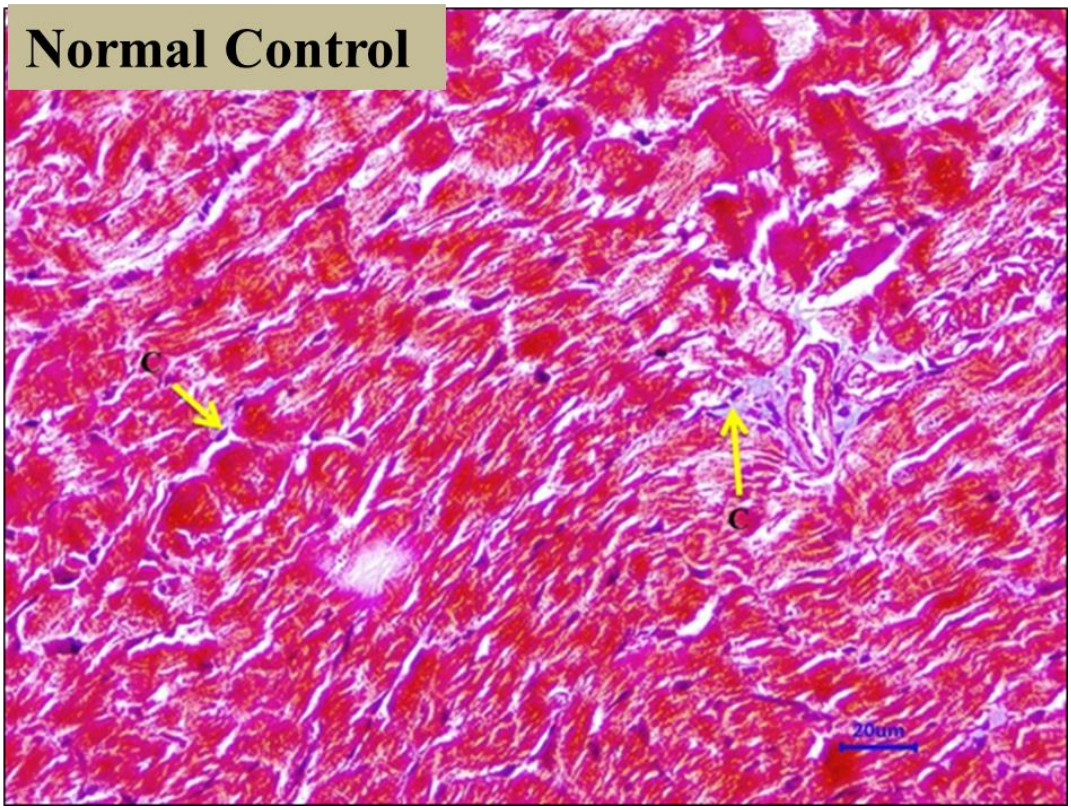
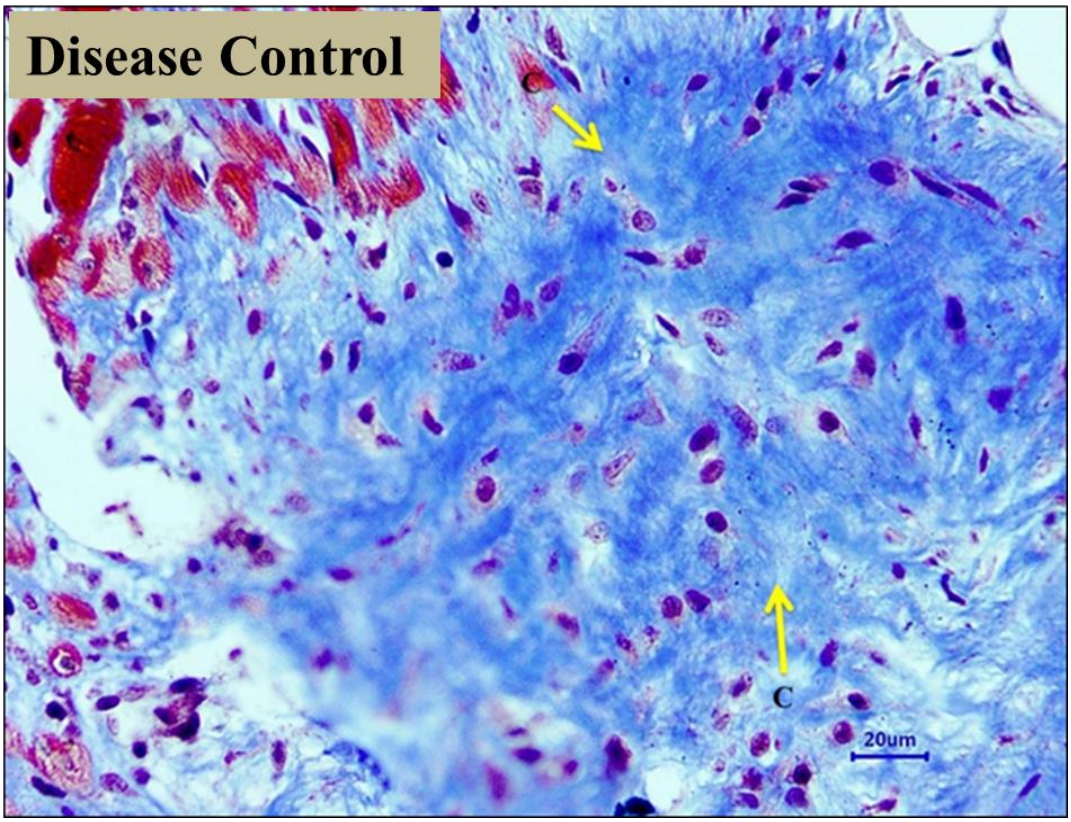


Figure 8: Effect of BVC Rasa on cardiac histopathology (H& E Staining, 100X).
N: Tissue necrosis, D: Degeneration, A: Angiogenesis, L: Lymphocytic infiltration, C: Collagen cardiac muscle.

Masson Trichrome staining:





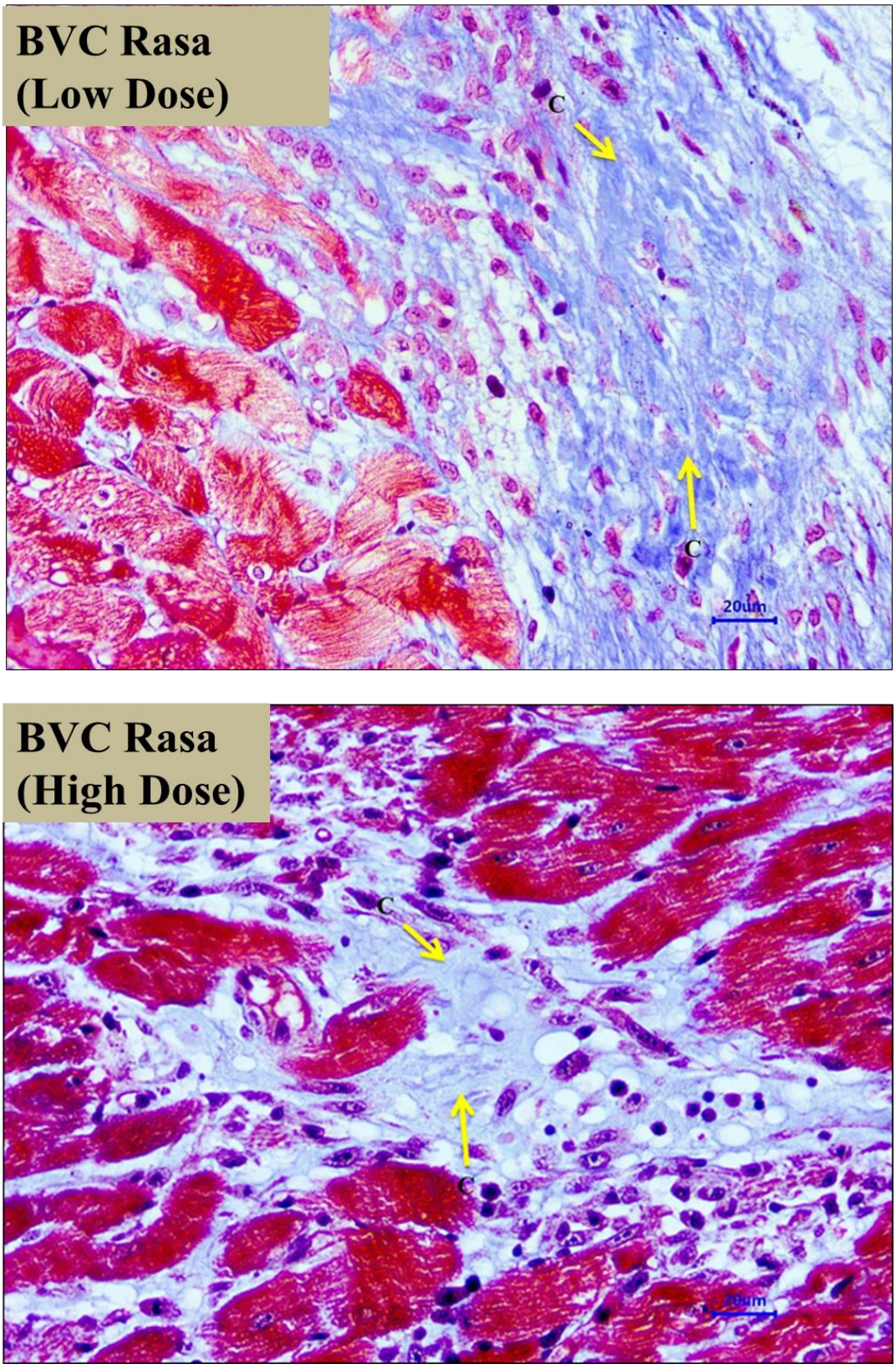


Figure 7: Effect of BVC Rasa on cardiac histopathology (Masson-Trichrome Staining, 400X).

5. DISCUSSION:

Administration of β-adrenergic agonist, ISO at high dose is a standardized animal model for a myocardial infarction in rats. ISO has been known to exert abnormality in both inotropic and chronotropic cardiac function which leads to necrosis and cell infiltration because of inflammation, cardiac hypertrophy and fibrosis.

Ayurved medicines in the form of herbs, minerals and their formulations are advocated by Ayurved practitioners in the management of ischemic heart and brain disorders. Bruhat Vata Chintamani Rasa is a widely used, leading classical herbomineral cardioprotective and neuroprotective formulation.

The biochemical and histological changes developed after ISO administration in rats resembles with those observed in human myocardial infarction (Milei et al., 1978). Hence, ISO-induced cardiotoxicity in a rat model has been used in this study for better understanding of pathogenesis of ischemic heart disease and the role of herbomineral formulation containing traditional herbs in the prevention of progression of ischemic heart diseases.

ECG abnormalities are the primary and the most important criteria used for definitive diagnosis of cardiotoxicity. ISO treatment showed significant alteration in ECG as compared to normal animals that could be because of the loss of cell membrane integrity in damaged cardiomyocytes due to free radical damage (Králová et al., 2008). The PR interval is the time from the onset of the P wave to the start of the QRS complex. PR interval represents conduction time between beginnings of atrial depolarization to the beginning of ventricular depolarization. Whereas, QT interval indicates the time between ventricular depolarisation to ventricular repolarisation. Both the intervals are prolonged in case of myocardial infarction, ischemia and abnormality in the conduction system (Upaganlawar et al., 2012). ISO treated animals showed significant elevation in PR and QT interval. Treatment with BVC significantly shortened the PR and QT intervals. The ST segment is the isoelectric section of the ECG between the end of S wave and the beginning of T wave. The ST segment represents the time when ventricular contractile fibers are depolarized during the plateau phase of the action potential (Moradi-Arzeloo et al., 2016). ISO treated animals showed significant elevation in ST-segment which is an indication of myocardial infarction. Treatment with BVC prevented the elevation in ST-segment. QRS duration represents how fast ventricles depolarize. ISO-treated animals showed fast ventricular depolarization which is because of the positive inotropic effect that results in a

decrease in QRS duration(Mohan et al., 2010). Treatment with BVC prevented rapid depolarization and showed a significant increase in QRS duration.

Reduction in atrial pressure i.e SBP, DBP and MABP are indications that represent deranged sympathetic and parasympathetic inputs to the heart (Ojha et al., 2010). Deterioration in myocardial contractility followed by ISO treatment is responsible for reduction in MABP which is a marker of after-load. Similarly deterioration in myocardial relaxation after ISO treatment is responsible for an increase in LVEDP which is a key marker for pre-load (Goyal et al., 2009). Treatment with BVC prevented the myocardial necrosis by ISO and improved the decreased SBP, DBP, MABP and significantly decreased ISO induced increased LVEDP.

The myocardium contains a large number of lysosomal marker enzymes like AST, LDH and CK-MB. These markers are important which help in assessing the degree of necrosis to the myocardium. Free radicals generated by ISO causes lipid peroxidation of membrane-bound polyunsaturated fatty acid and damages the structural and functional integrity of the myocardium. Damaged myocardium releases its lysosomal marker enzymes into the blood (Mythili and Malathi, 2015). ISO treated animals showed a significant increase in serum levels of AST, LDH and CK-MB. BVC treatment protects the myocardium from damage by its anti-oxidant property and decreased levels of AST, LDH and CK-MB.

AMP-activated protein kinase (AMPK) is an enzyme that regulates many physiological processes in the body. It also plays a major role in cardiac energy metabolism. For efficient working healthy heart (cardiomyocytes) require large energy supply (ATP), which comes from fatty acid oxidation and glucose oxidation. Under stress AMPK in the heart increases glucose uptake as a cardioprotective and adaptive response of the heart and it can also increase fatty acid oxidation. This ultimately increases the ATP production to ameliorate the imbalance between energy supply and demand to the heart (Li et al., 2019). AMPK activation is also reported to have a protective role in the process of cardiac fibrosis (Qi et al., 2017).

Sirtuin 1 (SIRT1) protein, is a member of Silent Information Regulator 2 (Sir2) protein family and has a role in regulating cellular health. Evidences suggest that SIRT1 is involved in the initiation and progression of a number of diseases, particularly fibrotic diseases such as liver fibrosis, cardiac fibrosis, and renal fibrosis (Chou et al., 2017). Jie et al. have reported that upregulated SIRT1 expression inhibits the formation and development of cardiac fibrosis in a rat myocardial infarction model (Xiao et al., 2016). In our study, significant reduced levels of

AMPK and SIRT1 were observed in disease control animals. Treatment with BVC prevented a reduction in levels of AMPK and SIRT1 and protects myocardium form fibrotic damage.

Oxidative damage to the myocardium by ISO also affects the antioxidant enzyme status in the myocardium. GSH is most abundant anti-oxidant present in the body. Together with SOD and CAT, GSH protect myocardium from superoxide, alkoxy radicals and H_2O_2 damage. ISO also causes polyunsaturated lipid peroxidation and formed MDA which is a reactive aldehyde and considered as electrophile species that develops toxic stress to the cardiomyocytes (Panda and Naik, 2008). BVC treatment prevented myocardium from oxidative damage by improving the levels of GSH, SOD and CAT and also prevented formation of reactive aldehyde in myocardium via its anti-oxidant mechanism.

Administration of ISO induced histological changes like myocardial degeneration, lymphocytic infiltration, tissue fibrosis and necrosis. This damage was controlled by BVC treatments probably because of its membrane stabilizing and anti-inflammatory effect. This finding provides additional evidence of cardio protective effect of BVC Rasa.

6. CONCLUSION:

1. Bruhat Vata Chintamani Rasa (BVC) treatment prevented ISO induced changes in the electrocardiogram and haemodynamic parameters in animals.
2. BVC treatment reduced the levels of cardiac marker enzymes in serum which shows its preventive effect against ISO-induced myocardial damage.
3. Most importantly, BVC prevented the loss of anti-oxidant enzymes from the myocardium and also improved the serum levels of AMPK and SIRT1.
4. Histopathological findings provide evidence for protective effect of BVC treatment against myocardial fibrosis and necrosis.
5. Hence, it can be concluded that Bruhat Vata Chintamani Rasa offers cardioprotection in Isoprenaline or isoproterenol (ISO) induced cardio-toxicity / cardiac damage.

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