VEDICINALS-9

Series of Organ Effects of Vedicinal-9: 1
Vedicinals -9 and Cardioprotection against Ischemia induced by Isoproterenol

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Abstract:

Given the exponential rise in the number of patients with myocarditis and ischemic heart disease in COVID-19, Long-COVID and post-vaccination states in the ongoing pandemic, there is an urgent need for safe and tolerable compounds that have undergone clinical trials and exhibit efficacy in the above patients. Biopsy findings reveal that the majority of the patients have myocarditis with inflammatory infiltrate that has been shown to cause myocardial damage and sudden cardiac deaths in many young individuals, particularly in males. A component of increased sympathetic nervous system activity is expected and has been shown in the above set of patients due to the stress that accompanies the illness and dysautonomia. We selected isoproterenol, a cardiac stimulant with beta-1 adrenoceptor agonist to mimic the above-described elevated sympathetic state in rats to induce myocardial damage and inflammatory injury with and without the formulation Vedicinals®-9. RecentlyVedicinals®-9 which is a combination of nine essential herbs and nutraceuticals has undergone human clinical trial and appears to be efficacious based on its results in COVID-19. Here we present data showing the effect of Vedinical-9 in isoproterenol-induced myocardial infarction and myocarditis in rats. Parameters such as CK-MB, TROPONIN, GPT: GOT ratio, and increase in heart weights indicated successful induction of the myocardial infarction model in rats. A significant decrease in CK-MB parameters in all the groups as compared with that of the isoproterenol-administered group. The change observed was dose-dependent as it was pronounced in the Vedicinal-9 group followed by high dose combinations, medium dose, and then low doses in both male and female rats. Similar dose-dependent changes were seen in the troponin parameter, although there was no statistical significance seen, values of troponin were markedly reduced compared with those of G2 group animals receiving only isoproterenol. Evaluation of interleukin parameters showed that isoproterenol increased the values of TNF-a and IL-6. In the case of males, there is a significant decrease in values of TNF-a in the majority of the treatment groups. Other parameters were evaluated that include mortality, clinical signs, body weight, and clinical chemistry, gross and histopathological examination, where Vedicinals®-9 was able to clear the inflammatory infiltrate in myocarditis. With the use of drug synergism as in Vedicinals®-9 has diverse molecules that have been proven previously without a shred of doubt to be virucidal, anti-inflammatory, antioxidant cytoprotective, and with a wide margin of safety, we may be able to attain similar results in large human clinical trials and reduce the myocardial damages causing mortalities and morbidities in COVID-19, post-COVID-19-vaccinated and Long-COVID patients

Keywords:

COVID-19, post-COVID-19-vaccinated cardiac adverse effects, Long-COVID, Vedicinals®-9, Sudden cardiac deaths, vaccine and heart.

A- Introduction:

Emerging studies are providing strong evidence regarding the damages inflicted by the Spike (S) protein in the end organs like the heart and brain in patients with COVID-19 and alarmingly in patients vaccinated for SARS-CoV-2 during the pandemic. Cases of myocarditis following the acute and possibly chronic phase of COVID-19 infection called Long-COVID are believed to be caused by the effects of S protein combined with the inflammatory reactions that accompany the viral infection of the myocardium (1). The fact that reports of myocarditis following mRNA vaccines (2) have evidenced the S protein to be the key player in the cases that have been reported. It is pertinent here to mention that myocardial damages caused by SARS-CoV-2 in the acute phase of COVID-19 also involved ischemic injury caused by coronary artery thrombosis and similar occurrences have been reported following COVID-19 vaccinations suggest the possible role of S protein as the etiological factor that either by endothelial damage or mechanisms independent to it (3) is capable of driving the activation of the coagulation pathways leading to the thrombotic occlusion of the coronary vessels and subsequent myocardial infarction and fibrosis. It is important to note that a complex cascade of events (Fig.1) resulting from acute phase COVID-19, Long-COVID and vaccine-adverse cardiovascular events in patients who continue to produce S protein following vaccination for COVID-19 should be anticipated and multitargeted anticoagulation therapy should be attempted by drug synergism if the incidences of the coronary thrombosis and consequent myocardial infraction is to be prevented in the above group of the patients which is a clear and present danger in the ongoing pandemic and era of COVID-19 vaccination. The current therapies to address the complex coronary and related vital organ thrombosis involve the use of anticoagulants, apheresis (4), and thrombi-dissolving drugs and molecules, which have remained partly beneficial or temporary therapies. Presently the approach remains limited to preventive and symptomatic supportive therapies, in an attempt to prevent further complications and organ failure. Several marketed drugs like Remdesivir, Liponavir, Ritonavir, and Chloroquine are being used but none of them have shown promising results in the acute phase of COVID-19. Since the advent of the SARS-CoV-2 coronavirus, researchers and clinical experts are being exposed to a constant onslaught of a variety of emerging clinical manifestations, research data, and even autopsy studies that all indicate that the COVID-19 disease which the novel coronavirus induces is not just a simple respiratory disease but one that is far more complex and is assaulting the human host via a variety of ways including disrupting and damaging the immune host immune system, triggering a variety of inflammation pathways, disrupting numerous human cellular pathways resulting in a variety of medical conditions arising. The long-term health implications for those that have survived the disease are far more alarming.

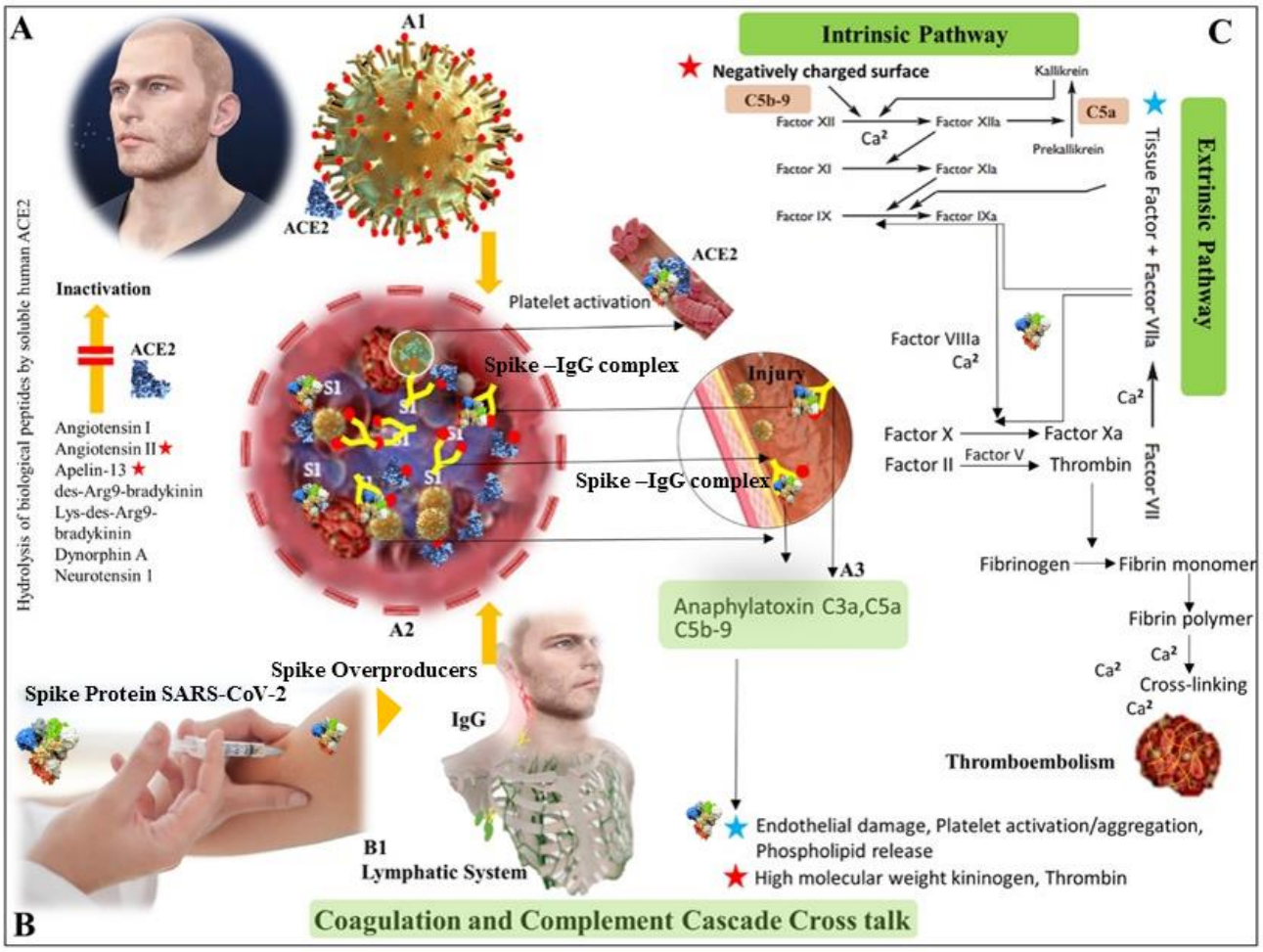


Figure-1 Complex cascade of coagulation leading to thrombosis and damage to end-organ injury

results from the COVID-19 S-protein mediated damage and the overproduced S-protein as seen in vaccine-related cardiovascular adverse events. Note that multiple stimuli like endothelial injury, activation of the complement cascade, and direct clotting activation by S-protein can form thrombi to cause myocardial infarction.

Despite the usage of certain existing drugs under EUA approvals and antibody-based treatment protocols, it has been shown that the SARS-CoV-2 coronavirus virus is fast mutating to develop drug-resistant variants. Even in conditions where such treatment protocols have not been used extensively, the novel coronavirus is still evolving cum mutating and giving rise to a variety of more potent and transmissible variants. While vaccination programs are underway to hopefully control this pandemic, a group of international researchers and scientists led by German biotech Health Shield with its collaborators in India have developed multiple molecular-based therapeutic suspension (5) that is not only able to effectively halt the SARS-Cov-2 replication through a variety of targets but can restore the affected lymphocytes to address the immune dysregulation actions of the SARS-CoV-2 coronavirus (6). The study team carefully selected a total of nine bioactive phytochemical molecules based on extensive silico computational docking studies, a meta-analysis of thousands of past published studies on these molecules and their mechanisms along with detailed studies of

conditions arising in COVID-19 patients. After going through extensive constituent blending studies, safety studies, and animal trials, the Vedicinals-9 team developed a potent therapeutic compound that has performed extremely well in a WHO-registered randomized clinical trial involving its use as an adjuvant along with a variety of existing standard treatment protocols. Informal observation studies involving the usage of the product as a stand-alone therapeutic have also shown extremely positive results.

The molecules Hesperidin, Baicalin, Curcumin, Rutin, Glycyrrhizin, Epigallocatechin Gallate, Piperine, and Quercetin can act as an antiviral (5, 6) and also address more than several hundred cellular pathways that are affected as a result of a SARS-CoV-2 virus infection. Plans are already underway for bigger clinical trials in a variety of countries and also more detailed studies to assess the therapeutic compound as potential prophylaxis for COVID-19 and also for the treatment of a variety of Long COVID-19 conditions.

B- MATERIAL AND METHOD:

1.1 Objectives

The objective of the study was to assess the effect of Vedinical-9 in isoproterenol-induced myocardial infarction in rats.

1.2 Study Guidelines

The design and scope of this study were based on consideration of the study objectives and the Sponsor's recommendations. The experimental procedures were performed as per the approved study plan.

1.3 Good Laboratory Practice

The test facility is certified by the National GLP Compliance Monitoring Authority (NGCMA) (Certificate Number: GLP/C-168/2021), Department of Science & Technology, Govt. of India, for compliance with OECD-GLP. This toxicity study was performed following OECD Principles on Good Laboratory Practice (7, 8, 9, 10) (revised 1997, issued January 1998) ENV/MC/CHEM (98) 17 Environment Directorate, Organization for Economic Co-operation and Development, Paris, 1998.

1.4 Study Period

Study Initiation Date	:	May 10, 2021
Experiment Start Date	:	May 10, 2021
Experiment Completion Date	:	Jul 15, 2021
Study Completion Date	:	Jul 31, 2021

1.0 DETAILS: MATERIALS AND METHODS

Details of the methods mentioned in the subsequent sections of the Study Plan are as per the appropriate Standard Operating Procedures (SOPs) at PRADO. Different formulations, ready to be administered, were provided by the sponsor.

2.1 Test Item Details

Name of Test Item	:	Vedicinals-9 and Vedicinals-9 Bio-enhanced
Appearance	:	xxx
Assay	:	xx%
Storage conditions	:	Ambient temperature
Handling Precautions	:	Standard laboratory precautions were followed

Note:

2.2 Details of Isoproterenol

Name of Compound	:	Isoproterenol
Appearance	:	xxx
Storage conditions	:	Ambient temperature
Handling Precautions	:	Standard laboratory precautions were followed

2.3 Details of the Test System

Species (Strain)	:	Rats (Sprague Dawley)
Sex	:	Female
Age	:	6-7 weeks
Body weight at initiation	:	200-250 gm
Source	:	National Institute of Biosciences, Pune.

2.4 Vehicle Details

The vehicle was selected based on a solubility and syringibility trial conducted at PRADO. 0.5% Carboxy Methyl Cellulose in deionized water was considered as Vehicle. Details of the vehicle. Details of the Carboxy Methyl Cellulose was used for this study mentioned below:

Name of Vehicle	:	Carboxy Methyl Cellulose
Batch Number	:	
Manufacturing Date	:	
Expiry / Retest Date	:	

2.5 Justification of Selection of Vehicle

The vehicle was selected based on the solubility trial.

2.6 Test System Details

Species (Strain)	:	Rat (Sprague Dawley)
Sex	:	Male and Female (nulliparous and nonpregnant)
Age (at the initiation of acclimation)	:	6-8 weeks
Body weight (at the initiation of dosing)	:	Male – 199.0-360.0g. Female – 170.0 to 271.0 g.
Source	:	National Institute of Biosciences, Pune, India. 1901/GO/Bt/S/07/CPCSEA

2.7 Justification for Selection of Test System

Rat is one of the recommended rodent species by regulatory guidelines for the conduct of safety and toxicity studies because of the availability of vast historical data.

2.0 EXPERIMENTAL PROCEDURES

3.1 Animal Welfare

All animal welfare procedures to be followed for this conduct of study were following the guidelines set by the Committee for Control and Supervision of Experiments on Animals (CPCSEA; Registration number: 1723/PO/RcBiBt/S/13/CPCSEA), Department of Animal Husbandry and Dairying, Ministry of Fisheries, Government of India for conducting experiments on small laboratory animals as published in The Gazette of India, December 15, 1998. Prior approval of the Institutional Animal Ethics Committee (IAEC) is in place (IAEC-21-027).

3.2 Husbandry

Location	:	ARF Room No. 06
Temperature	:	21.0 to 22.9 °C
Humidity	:	38 to 69 %
Lighting	:	The photoperiod was 12 hrs light and 12 hrs dark. Light hours are controlled by an automated system.
Air Changes	:	10 to 15 air changes per hour were maintained throughout the in-life phase of this study.
Cages	:	3 animals per sex per cage were housed together in clean, sterilized polycarbonate cages having provision for holding pelleted food and drinking water in bottles with stainless steel sipper tubes throughout the study period.
Cage Rotation Frequency	:	Cage Rotation was done weekly.
Cage Dimensions	:	Approximately 41.0 cm x 28.2 cm x 15.2 cm
Feed	:	A standard pelleted rodent diet was provided *ad libitum.*
Water	:	Reverse Osmosis water was provided *ad libitum* in autoclaved polypropylene bottles.
Analysis of feed, water, and Bedding Material	:	Contaminant and nutrient content analysis of feed and pesticide analysis of water samples is done routinely. Details are included in the raw data file.
Animal Identification	:	Animals were identified by tail marking. The group of animals per cage was identified by different colored cage cards.

3.3 Acclimatization

A total of 84 Sprague Dawley rats (42 males and 42 females) were received from the Animal Research facility and were allowed to acclimatize for 05 days before assignment to groups. During this period, animals were identified by tail marking. Animals were observed once daily for clinical signs and twice daily for morbidity and mortality.

3.4 Randomization

A total of 42 male and 42 female animals were randomly allocated to control and different treatment groups (03 males and 03 females per group). Animals were randomized based on their recent body weight and weight variation of the animals were not exceed ± 20 % of the mean body weight for each sex.

3.5 Preparation of the Dose Formulation

The required amount of test items was triturated by using mortar and pestle and transferred to a measuring cylinder containing carboxy methyl cellulose (CMC). The final volume was made up using 0.5% carboxy methyl cellulose (CMC). The exact quantity of test items and vehicles used was recorded in the raw data. All formulations were prepared fresh, on each day of test item administration. Details of the formulation preparation were maintained in raw data.

3.6 Experimental Design

Group No.	Group	Treatment	Dose (mg/kg /day)	Animal Numbers	
				Male	Female
G1	Control	Isoproterenol one single dose	90	01-03	04-06
G2	Medium	ECGC	138	07-09	10-12
G3		Biacalin	138	13-15	16-18
G4		Quercetin	138	19-21	22-24
G5		Mix	138	25-27	28-30
G6	Low	ECGC	55.2	31-33	34-36
G7		Biacalin	55.2	37-39	40-42
G8		Quercetin	55.2	43-45	46-48
G9		Mix	55.2	49-51	52-54
G10	High	ECGC	207	55-57	58-60
G11		Biacalin	207	61-63	64-66
G12		Quercetin	207	67-69	70-72
G13		Mix	207	73-75	76-78
G-14	Vedicinals-9	Vedicinals-9	464	79-81	82-84

3.7 Justification for Selection of Dose and Route of Administration

The oral route was selected based on the recommendation of the sponsor and it is the intended clinical route of administration.

3.8 Dose Administration

After injection of isoproterenol different treatment group animals received daily doses of test items by oral route daily for 7 days.

3.0 OBSERVATIONS

All the following observations were recorded from all the animals.

4.1 Mortality and Clinical Signs Observations

After dose administration, all the animals were observed once a day throughout the study period for clinical signs, and twice a day for morbidity and mortality.

4.2 Body Weights

Fasting body weights on the day of the necropsy were also recorded.

4.3 Clinical Pathology Observations

After completion of the dosing period on day 7, all the animals were fasted overnight (water allowed). The blood samples were withdrawn from retro-orbital sinuses under mild anesthesia, on day 9 for hematological and clinical chemistry analysis for CK-MB, LDH, GOT, GPT, GPT: GOT, and Creatinine.

4.3.1 Clinical Chemistry

Blood samples were collected in vials containing heparin (250 IU/ml) as an anticoagulant for plasma and without anticoagulant for serum. Plasma was separated by centrifugation at 3000 rpm for 10-15 minutes at room temperature. The plasma sample was processed for clinical chemistry analysis.

Plasma samples were processed using ERBA EM 200 Auto Analyzer. Serum samples were outsourced for the estimation of CK-MB, Troponin-I, and immunogenicity parameters.

Sr. No.	Parameter	Unit
1	Glutamate Oxaloacetate Transaminase (GOT or AST)	U/L
2	Glutamate Pyruvate Transaminase (GPT or ALT)	U/L
3	Creatinine (CREAT)	mg/dl
4	GPT : GOT	NA
5	Lactate dehydrogenase (LDH)	mg/dl
6	CK-MB	g/dl

4.4 Immunogenicity Evaluation

The IL-6 and TNF-α in the serum were determined in all animals by ELISA. Estimation of ELISA will be outsourced.

4.5 Necropsy and Gross Pathology

On day 9, all animals were humanely euthanized by using CO_2 asphyxiation. All the animals were subjected to detailed gross pathological examination, which include careful examination of the heart.

4.6 Heart Weight

After gross pathology examination, the hearts of all animals were trimmed off to remove any adherent tissue and were weighed wet. Relative organ weights were calculated for each animal.

The hearts of all animals were fixed in 10% Neutral Buffered Formalin for subsequent histopathological examination.

4.7 Histopathology

The heart from all control (G1) and high-dose group (G4) animals were processed routinely and embedded in paraffin. The sections of 3-5 µ thickness were cut and stained with hematoxylin-eosin stain.

4.8 Data Analysis

All the individual data were summarized in terms of groups and sex to obtain the mean and standard deviation. The clinical chemistry and absolute well as relative heart weight data were analyzed using way ANOVA test followed by Dunnett's test using Graph Pad Prism (Version 7.03). All analyses and comparisons were evaluated at the 5% ($P \leq 0.05$) level in comparison with control.

4.9 Archives

All original raw data, QAU audited draft study plan, signed study plan, QAU audited draft report, final report along with study specimen (wet tissue samples, tissue blocks, and slides) and electronic files of the study will be retained for 1 year from study completion date, at PRADO, Pune. Thereafter, the archived material will be disposed of or stored for an extended period according to the written instructions of the Sponsor.

SUMMARY

Study No.	PRADO/B-2102
Test Item	Vedicinals
Study Title	Cardioprotective Effects of Various formulations of Vedicinals-9 on Isoproterenol-Induced Myocardial Infarction in Sprague Dawley Rats
Route	Oral
Dose	
No. of Groups	14 (3 Animals/Sex/Group)

The objective of the study is to assess the effect of Vedinical-9 in isoproterenol-induced myocardial infarction in rats.

Parameters were evaluated including mortality, clinical signs, body weight, clinical chemistry, heart weights, and gross and histopathological examination.

The increased clinical chemistry parameters such as CK-MB, GPT: GOT ratio, and increase in heart weights indicated successful induction of the myocardial infarction model in rats. All animals survived till the scheduled necropsy, except one male and one female from G2 and G10 groups were found dead on days 3 and 4, respectively. No abnormal clinical signs were

observed in any animals throughout the experiment period. No test item-related adverse effects were observed in body weights during the experiment period.

The significant decrease in CK-MB parameter in all the groups as compared with that of the isoproterenol-administered group. This change was dose-dependent as it was pronounced in the Vedicinal-9 group followed by high dose combinations, medium dose, and then low doses for both males and females. Similar dose-dependent changes were seen in the troponin parameter, although there was no statistical significance seen, values of troponin were markedly reduced compared with those of G2 group animals receiving only isoproterenol.

Evaluation of interleukin parameters showed that isoproterenol increased the values of TNF-a and IL-6. In the case of males, there is a significant decrease in values of TNF-a in all the treatment groups except groups G6, G8, and G9. In the case of females except G3, G5, G7, G9, and G10 all the other groups have significantly reduced TNF-a values.

Values of IL-6 were reduced in all treatment groups as compared with those of isoproterenol-treated animals, however, there was no statistical significance seen in any group.

Gross examination of animals during the necropsy revealed that isoproterenol treatment caused congested blood vessels and a rounded heart apex. Treatment of test items in various combinations has reduced the thickness of the ventricles.

Microscopic examination of the heart of animals receiving a single dose of isoproterenol revealed myocardial degeneration minimal focal (1/3 each male and female) to mild (2/3 each male and female), MNC infiltration minimal focal(2/3 males and 1/3 female) to mild focal (2/3 females) and congested blood vessels minimal focal (1/3 female) to mild focal (3/3 males and 2/3 females).

The incidence and severity of histopathological observations were reversed due to different combinations of vedicinal-9 formulations. However, the reversal was more pronounced in the Vedicinal-9 group followed by high dose combinations, medium dose, and then low dose.

Absolute and relative heart weights did not show any statistically significant changes, however, isoproterenol treatment caused an increase in heart weight. Treatment of various test items alone and in combination with various doses reduced the heart weights compared with the G2 group both in males and females.

Based on the results obtained in the present study conditions, it can be concluded that the myocardial damage caused by a toxic dosage of isoproterenol was reversed by the administration of various combinations of Vedicinals-9 formulations. the reversal was more pronounced in the Vedicinal-9 group followed by high dose combinations, medium dose, and then low dose.

C- RESULTS

5.1 Development of Myocardial Infarction Model

The increased clinical chemistry parameters such as CK-MB, GPT:GOT ratio and increase in heart weights indicated successful induction of the myocardial infarction model in rats details the groups of rats tested and the dose range used are shown (Fig.1) (Also, supplementary material)

Tissue/ Findings/Sex				Females		
Dose Group	G1	G2	G3	G4	G5	G6
Dose (mg/kg)	Isoproterenol (Single Dose 60)	Vedicinals-9 (100)	Vedicinals-9 Bioenhanced (100)	Isoproterenol (Divided Dose 85)	Vedicinals-9 (100)	Vedicinals-9 Bioenhanced (100)
Number Examined	6	6	6	6	6	6
Heart						
Mean	0.75	0.76	0.71	0.72	0.77	0.78*

Figure 1. The experimental group receiving a Single dose of isoproterenol, a bio-enhanced formulation of Vedicinal-9 reduced the heart weights. Divided doses of isoproterenol, bio-enhanced Vedicinals-9 formulation significantly increased heart weights above that of normal Vedicinals-9 formulation or only isoproterenol.

5.2 Mortality and Clinical Signs Observations

All animals survived till the scheduled necropsy, except one male and one female from G2 and G10 groups were found dead on days 3 and 4, respectively. No abnormal clinical signs were observed in any animals throughout the experiment period. **(Supplementary Material Table 1; Appendix - I)**

5.3 Body Weights

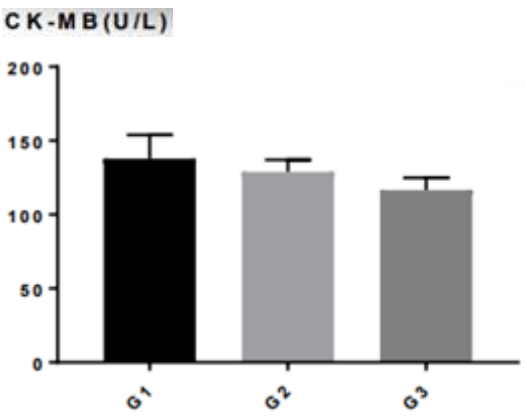
Body weights and body weight gains of all the treatment groups males and females were non significantly increased when compared with that of the control group males and females. **(Supplementary Material Table 2; Appendix - II)** (**Table 3** depicts the Mean and SD of clinical chemistry results.

5.4 Clinical Chemistry

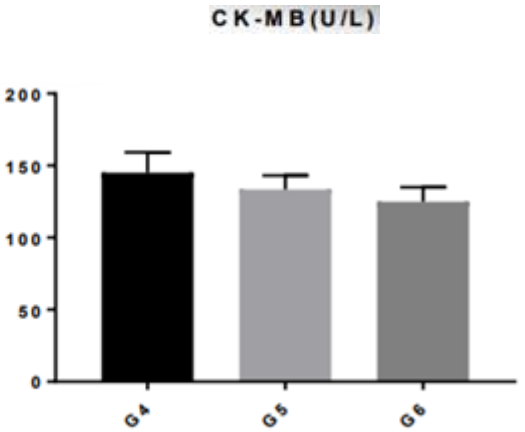
In the case of males, there is a significant decrease in CK-MB parameters in all the groups as compared with that of the isoproterenol-administered group. This change was dose-dependent

as it was pronounced in the Vedicinal-9 group followed by high dose combinations, medium dose, and then low dose. A similar trend was observed in females.

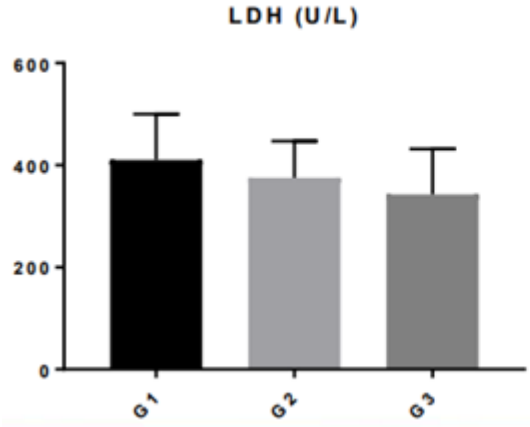
Similar dose-dependent changes were seen in the troponin parameter, although there was no statistical significance seen, values of troponin were markedly reduced compared with those of G2 group animals receiving only isoproterenol. **(Fig-2 and Supplementary Material Table 3; Appendix - III).**

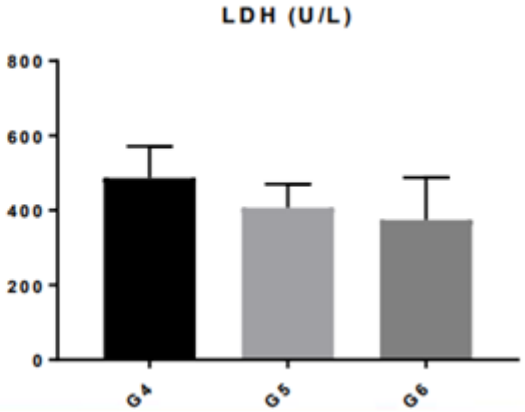


Single Dose of Isoproterenol



Divided Dose of Isoproterenol





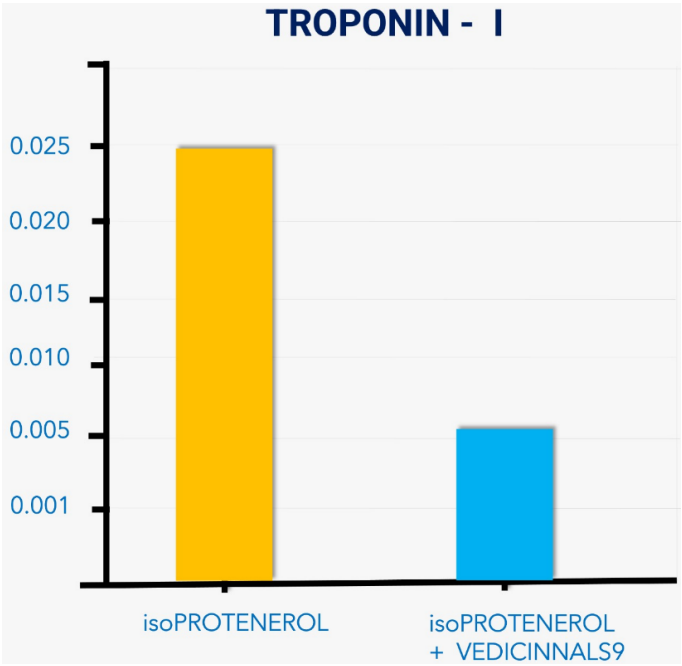


Figure -2 Shows biomarkers for cardiac muscle cell damage and the effects of vedicinal-9 on the biomarker profile with or without Isoproterenol.

5.5 Immunogenicity Evaluation

Evaluation of interleukin parameters showed that isoproterenol increased the values of TNF- α and IL-6 (Fig-4). In the case of males, there is a significant decrease in values of TNF-α in all the treatment groups except groups G6, G8, and G9. In the case of females except G3, G5, G7, G9, and G10 all the other groups have significantly reduced TNF-α values.

Values of IL-6 were reduced in all treatment groups as compared with those of isoproterenol-treated animals, however, there was no statistical significance seen in any group. **(Supplementary Material Table 4; Appendix - IV).**

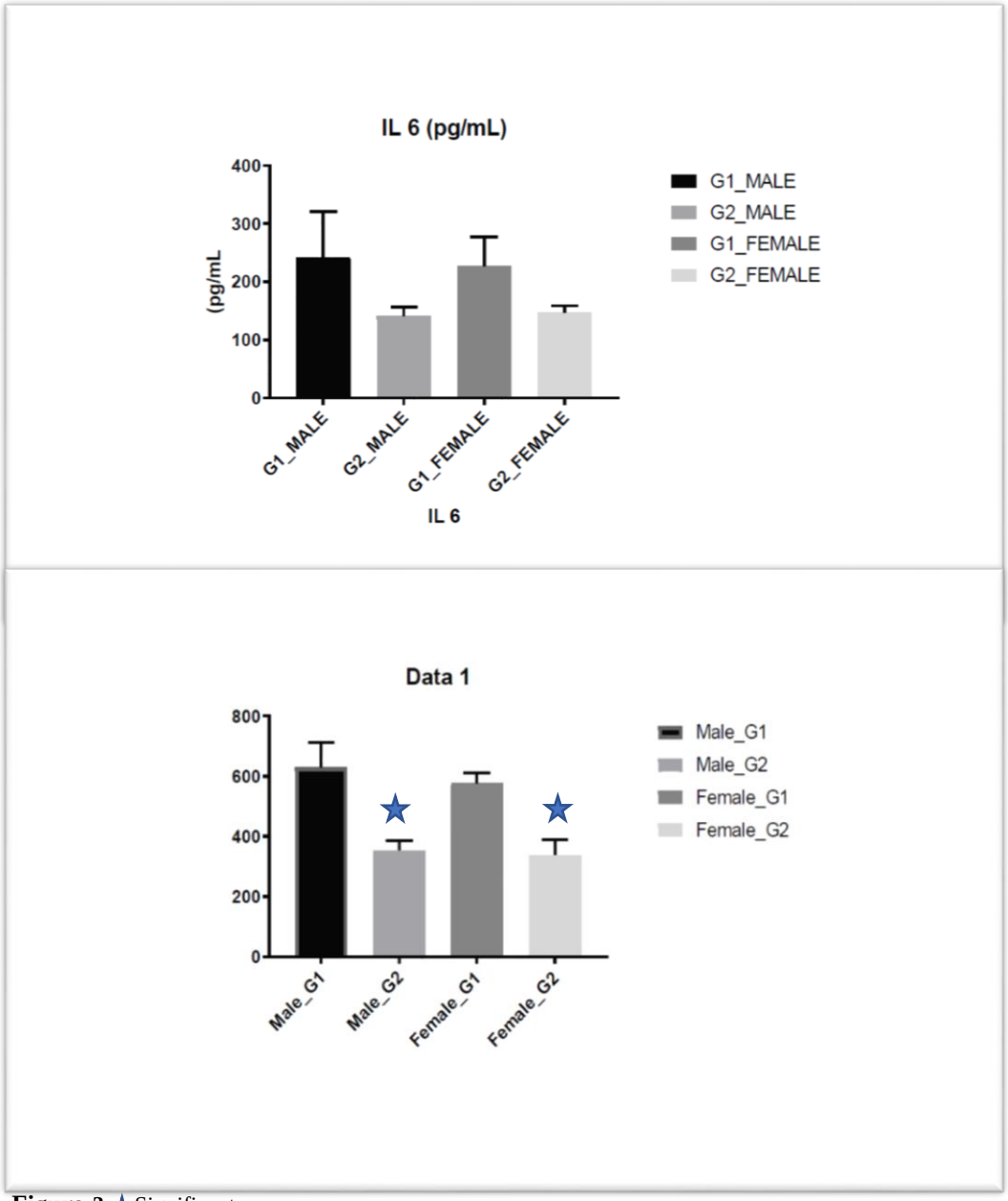


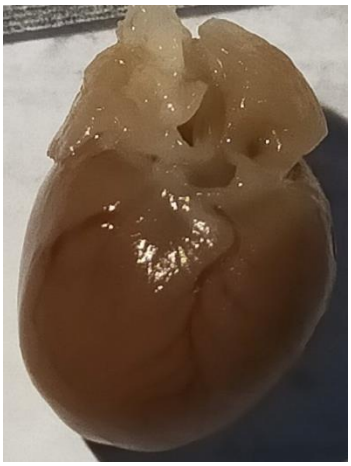
Figure-3 ★Significant

5.6 Gross Pathology

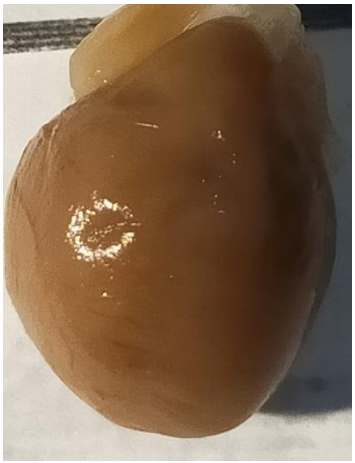
Gross examination of animals during the necropsy revealed that isoproterenol treatment caused congested blood vessels and a rounded heart apex. Treatment of test items in various combinations has reduced the thickness of the ventricles. **Fig-4 (Table 5; Appendix - V).**

5.7 Heart Weights

Absolute and relative heart weights did not show any statistically significant changes, however, isoproterenol treatment caused an increase in heart weight. Treatment of various test items alone and in combination with various doses reduced the heart weights compared with the G2 group both in males and females. **(Table 2; Appendix - II).**



G13: Isoproterenol + Mix (90 + 207 mg/kg): Minimal congestion of blood vessels



G14: Isoproterenol + Vedicinals-9 (90 + 464 mg/kg): No abnormality detected

Figure-4 Effects of Isoproterenol on myocardium (A) alone, and Isoproterenol+Vedicinals-9 (B). Note the Vedicinals-9 treated heart showed no congestion, hyperemia, and normal subpericardial coronary arteries (B) in contrast to dilated coronaries with dark-colored myocardium (A).

5.8 Histopathology

Microscopic examination of the heart of animals receiving a single dose of isoproterenol revealed myocardial degeneration minimal focal (1/3 each male and female) to mild (2/3 each male and female), MNC infiltration minimal focal(2/3 males and 1/3 female) to mild focal (2/3 females) and congested blood vessels minimal focal (1/3 female) to mild focal (3/3 males and 2/3 females).

The incidence and severity of histopathological observations were reversed due to different combinations of vedicinal-9 formulations. However, the reversal was more pronounced in the Vedicinal-9 group followed by high dose combinations, medium dose, and then low dose Fig-5. **(Table 6; Appendix - VI).**

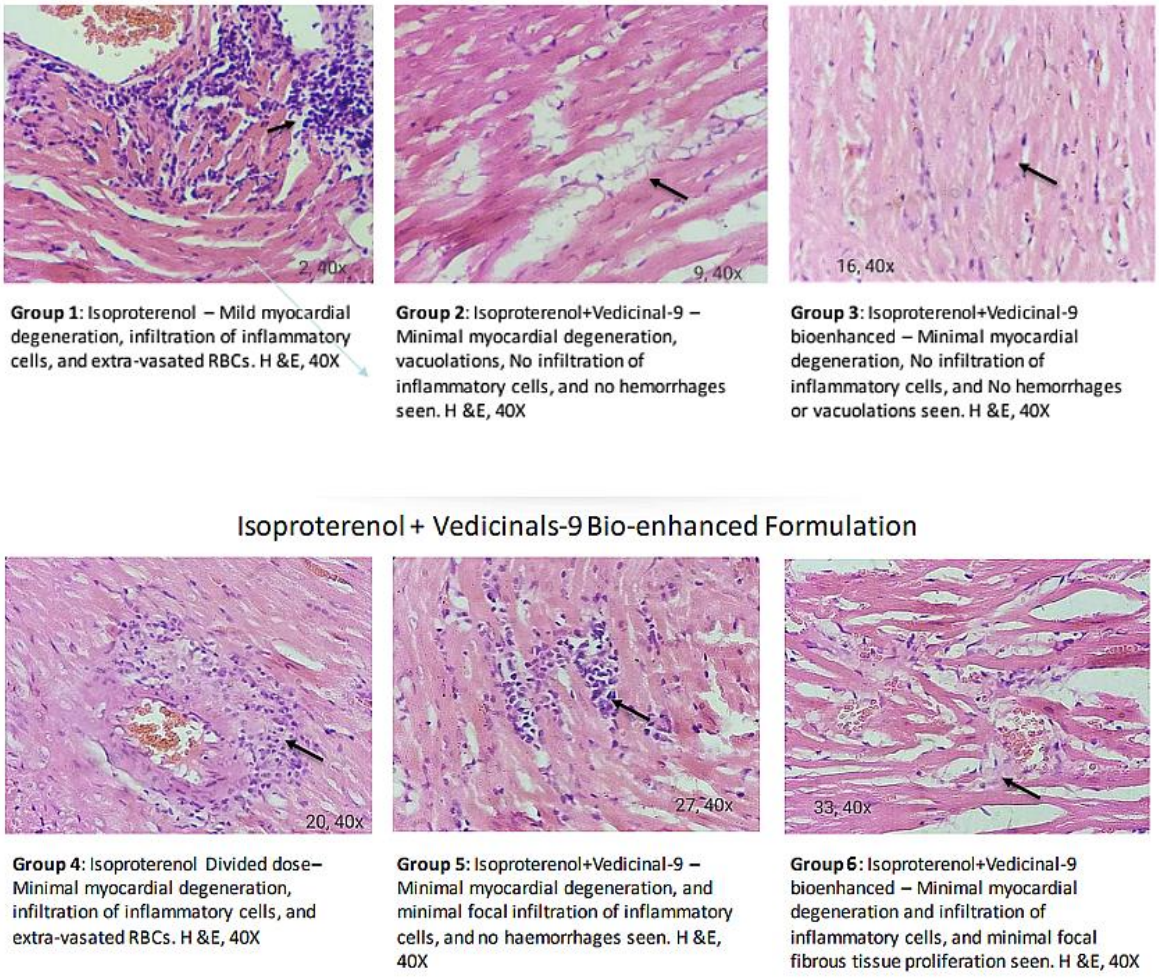


Figure- 5a: Isoproterenol with and without Vedicinals-9 in myocardial tissue lymphocytic infiltrate. Note the effects of Vedicinals-9 in the prevention and clearance of inflammatory infiltrates (top-row). The Vedicinals-9 minimized the myocardial inflammation and degeneration limiting the fibrosis. (bottom-row).

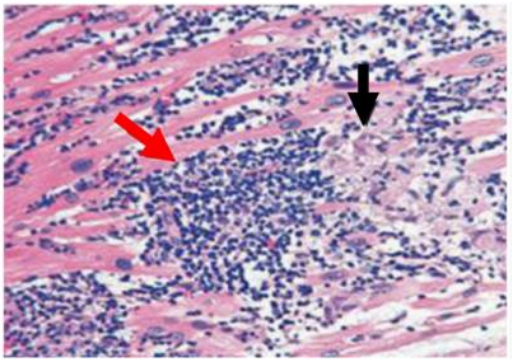


Figure 1.Active myocarditis suggestive of lymphocytic and histiocytic infiltrate and higher T lymphocytes in heart-tissue sections. Hematoxylin and eosin immunohistochemistry of heart tissue samples depicts characteristic lesions of acute myocarditis with widespread lymphocytic and histiocytic infiltrate (red arrow) and associated myocyte damage (black arrow).

Nappi F, Avtaar Singh SS. SARS-CoV-2-Induced Myocarditis: A State-of-the-Art Review. Viruses. 2023; 15(4):916. https://doi.org/10.3390/v15040916

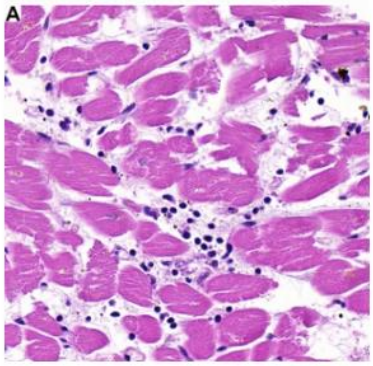


Fig.1 A; Lymphocytic aggregates in the interventricular septum of a case associated myocardiocyte destruction
https://link.springer.com/article/10.1007/s00392-022-02129-5

Figure 5b: For comparison with 6a, the known myocarditis dominated by lymphocytic infiltrates is a feature of acute phase COVID-19 (left) and following vaccine-associated myocarditis (right).

D- DISCUSSION AND CONCLUSION

This study was performed to observe the clinical efficacy of the formulation vedicinal-9 on myocardial damage and inflammation caused by isoproterenol and compare the effects of V9 on myocardial cells with the spike protein injury caused in COVID-19 infection and in the cardiovascular adverse events reported with mRNA vaccines. It has been demonstrated that isoproterenol is capable of inducing myocardial infarction in animal models resulting in inflammatory infiltration in the zones of cell injury. Isoproterenol is a beta-adrenergic receptor agonist that has been widely used to induce experimental animal models of myocardial damage, resulting in morphological and biochemical changes in the heart similar to the change in humans. It has been shown that isoproterenol can increase myocardial contraction and oxygen demand, resulting in electron leakage in the respiratory chain. These free electrons interact with molecular oxygen to create reactive oxygen species (ROS), further oxidizing elements of the cells, including proteins, DNA, and membrane lipids, directly damaging the myocardium. The mechanism of myocardial damage caused by spike protein is been studied in extensive detail and ROS-mediated myocardial injury coupled with coronary thrombosis has been reported. Common to both the damages is the secondary inflammatory infiltrate, lymphocytes in particular (Figure 6a-6b), which further aggravates the cell damage by cytokine-mediated injuries. Our study showed that vedicinal-9 was capable of clearing the inflammatory infiltrate and preventing myocardial cell damage. The restoration of the cardiac enzymes with vedicinal-9 was a particularly significant finding in our study. Based on the results obtained in the present study conditions, it can be concluded that the myocardial damage caused by a toxic dosage of isoproterenol was reversed by the administration of various combinations of Vedicinals-9 formulations. the reversal was more pronounced in the Vedicinal-9 group followed by high dose combinations, medium dose, and then low dose. The results show the potential of vedicinal-9 if used in COVID-19-affected patients with or without myocardial infraction, as prevention of further damage in myocardial ischemia before the ST –segment changes do develop signifying an infraction appears to be a potential efficacious effect of vedincinal-9. The same appears to hold for spike protein-mediated damage now been reported as a vaccine-adverse event in a large group of individuals. With the current evidence of vedicinal-9 use in both of these groups of people prevention of serious myocardial damage needs to be evaluated in a large cohort for which clinical trials are underway.

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Supporting Data and Supplementary Material

TABLE 1: Summary: Clinical Signs, Morbidity and Mortality Observations - Males

Group	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14
	Isoproterenol 90 mg/kg													
	Control	EGCG	Biacalin	Quercetin	**Mix Medium**	EGCG	Biacalin	Quercetin	**Mix Low**	EGCG	Biacalin	Quercetin	**Mix High**	Vedicinals -9
Dose (mg/kg)	90	138	138	138	138	55.2	55.2	55.2	55.2	207	207	207	207	464
No of Animals	3	2	3	3	3	3	3	3	3	3	3	3	3	3
Mortality	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Signs	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

TABLE 1 Summary (Contd.): Clinical Signs, Morbidity and Mortality Observations - Females

Group	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14
	Isoproterenol 90 mg/kg													
	Control	EGCG	Biacalin	Quercetin	**Mix Medium**	EGCG	Biacalin	Quercetin	**Mix Low**	EGCG	Biacalin	Quercetin	**Mix High**	Vedicinals -9
Dose (mg/kg)	90	138	138	138	138	55.2	55.2	55.2	55.2	207	207	207	207	464
No of Animals	3	3	3	3	3	3	3	3	3	2	3	3	3	3
Mortality	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Signs	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

TABLE 2 : Summary: Body Weights and Absolute and Relative Heart Weight (gm)- Male and Female

Mean/ SD/N	Males				Females			
	BW1	**BW8**	**AHW**	**RHW**	**BW1**	**BW8**	**AHW**	**RHW**
G1 Isoproterenol								**Dose: 90 mg/kg**
Mean	214.67	236.67	1.30	0.54	223.00	216.25	1.10	0.47
SD	26.27	34.78	0.50	0.13	41.57	6.01	0.27	0.07
N	3	3	3	3	3	3	3	0
G2 (Isoproterenol + EGCG)								**Dose: 90+138 mg/kg**
Mean	241.17	256.25	0.97	0.38	213.00	222.83	1.01	0.45
SD	30.40	6.72	0.18	0.08	50.09	20.55	0.09	0.01
N	3	3	3	3	3	2	2	0
G3 (Isoproterenol + Biacalin)								**Dose: 90+138 mg/kg**
Mean	238.33	262.33	1.31	0.50	213.67	230.67	1.01	0.44
SD	28.82	27.89	0.12	0.07	40.29	40.44	0.13	0.06
N	3	3	3	3	3	3	3	3
G4 (Isoproterenol + Quercetin)								**Dose: 90+138 mg/kg**
Mean	268.00	294.17	1.41	0.49	212.00	236.67	0.93	0.40
SD	79.90	85.13	0.30	0.07	30.94	47.93	0.14	0.04
N	3	3	3	3	3	3	3	3
G5 (Isoproterenol + Mix)								**Dose: 90+ 138 mg/kg**
Mean	262.67	289.17	1.14	0.41	212.00	234.17	0.97	0.42
SD	84.00	0.14	0.07	0.00	30.94	35.65	0.12	0.02
N	3	3	3	3	3	3	3	3
G6 (Isoproterenol + EGCG)								**Dose: 90+55.2 mg/kg**
Mean	261.33	289.50	1.07	0.38	214.17	232.50	0.93	0.41
SD	65.96	66.22	0.14	0.04	27.31	39.77	0.01	0.07
N	3	3	3	3	3	3	3	3
G7 (Isoproterenol + Biacalin)								**Dose: 90+55.2 mg/kg**
Mean	258.33	292.33	1.07	0.37	215.83	243.33	1.01	0.42
SD	62.18	51.69	0.10	0.05	24.87	31.87	0.07	0.03
N	3	3	3	3	3	3	3	3

TABLE 2 (Contd.): Summary: Body Weights and Absolute and Relative Heart Weight (gm)- Male and Female

Mean/ SD/N	Males				Females			
	BW1	BW8	AHW	RHW	BW1	BW8	AHW	RHW
G8 (Isoproterenol + Quercetin)						**Dose: 90 + 55.2mg/kg**		
Mean	254.17	287.67	0.81	0.27	216.17	241.17	0.88	0.37
SD	55.89	57.76	0.47	0.10	23.91	25.93	0.07	0.02
N	3	3	3	3	3	3	3	3
G9 (Isoproterenol +Mix)						**Dose: 90+ 55.2 mg/kg**		
Mean	253.50	277.33	0.78	0.28	216.17	236.33	0.92	0.40
SD	54.77	63.09	0.18	0.02	22.91	28.11	0.06	0.07
N	3	3	3	3	3	3	3	3
G10 (Isoproterenol + EGCG)						**Dose: 90+207 mg/kg**		
Mean	252.83	274.83	0.79	0.30	216.33	219.75	0.70	0.31
SD	55.26	52.49	0.11	0.09	21.03	16.62	0.15	0.04
N	3	3	3	3	3	2	2	2
G11 (Isoproterenol + Biacalin)						**Dose: 90+207 mg/kg**		
Mean	258.33	285.67	1.01	0.36	220.50	238.00	0.97	0.41
SD	52.52	48.22	0.03	0.07	21.27	23.39	0.04	0.06
N	3	3	3	3	3	3	3	3
G12 (Isoproterenol + Quercetin)						**Dose: 90+207 mg/kg**		
Mean	250.67	277.67	1.16	0.42	213.67	242.00	1.03	0.43
SD	61.78	62.08	0.21	0.05	29.19	30.00	0.04	0.04
N	3	3	3	3	3	3	3	3
G13 (Isoproterenol + Mix)						**Dose: 90+207 mg/kg**		
Mean	243.17	268.67	0.96	0.37	216.67	234.17	1.02	0.43
SD	66.29	60.80	0.11	0.10	20.13	26.60	0.13	0.03
N	3	3	3	3	3	3	3	3
G14 (Isoproterenol + Vedicinals-9)						**Dose: 90+ 464 mg/kg**		
Mean	220.83	248.17	1.00	0.40	225.50	242.00	0.93	0.39
SD	14.27	21.78	0.10	0.06	29.01	29.75	0.04	0.06
N	3	3	3	3	3	3	3	3

TABLE 3: Summary: Clinical Chemistry Analysis- Male

Mean/ SD/N	GPT	GOT	CK-MB	LDH	CREAT	Troponin-I	GPT:GOT
	U/L	U/L	U/L	U/L	mg/dl	ng/mL	mg/dl
G1 Isoproterenol							**Dose: 90 mg/kg**
Mean	92.83	109.17	19.35	188.33	0.46	0.025	0.85
SD	11.46	7.23	1.87	121.04	0.05	0.01	0.05
N	3	3	3	3	3	3	3
G2 (Isoproterenol + EGCG)							**Dose: 90+138 mg/kg**
Mean	75.30	139.15	9.61*	138.67	0.46	0.005	0.55*
SD	13.45	39.53	0.27	26.50	0.04	0.001	0.02
N	3	3	3	3	3	2	3
G3 (Isoproterenol + Biacalin)							**Dose: 90+138 mg/kg**
Mean	79.57	110.37	12.27*	138.67	0.42	0.005	0.72*
SD	9.71	9.27	0.52	15.18	0.05	0.001	0.04
N	3	3	3	3	3	3	3
G4 (Isoproterenol + Quercetin)							**Dose: 90+138 mg/kg**
Mean	90.47	103.33	9.10*	121.00	0.46	0.006	0.88
SD	7.32	3.66	2.35	11.27	0.06	0.002	0.07
N	3	3	3	3	3	3	3
G5 (Isoproterenol + Mix Medium (E+B+Q))							**Dose: 90+ (91.4+36.3+10.3) mg/kg**
Mean	74.17	97.43	9.78*	119.67	0.44	0.006	0.76
SD	10.20	15.21	0.33	9.71	0.05	0.002	0.02
N	3	3	3	3	3	3	3
G6 (Isoproterenol + EGCG)							**Dose: 90+55.2 mg/kg**
Mean	79.90	108.80	11.96*	230.67	0.42	0.007	0.75
SD	5.01	21.36	2.49	180.07	0.02	0.002	0.12
N	3	3	3	3	3	3	3
G7 (Isoproterenol + Biacalin)							**Dose: 90+55.2 mg/kg**
Mean	75.90	98.83	14.14*	117.67	0.41	0.005	0.77
SD	9.83	7.16	0.69	29.37	0.02	0.001	0.12
N	3	3	3	3	3	3	3

TABLE 3 (Contd.): Summary: Clinical Chemistry Analysis- Male

Mean/ SD/N	GPT	GOT	CK-MB	LDH	CREAT	Troponin-I	GPT:GOT
	U/L	U/L	U/L	U/L	mg/dl	ng/mL	mg/dl
G8 (Isoproterenol + Quercetin)							**Dose: 90+55.2 mg/kg**
Mean	79.17	113.37	11.59*	161.33	0.42	0.007	0.70*
SD	6.56	8.95	2.55	27.79	0.05	0.003	0.07
N	3	3	3	3	3	3	3
G9 (Isoproterenol + Mix Low (E+B+Q))							**Dose: 90+ (36.6+ 14.5+ 4.1) mg/kg**
Mean	79.90	108.80	11.96*	121.67	0.43	0.007	0.75*
SD	12.41	17.30	1.49	16.44	0.09	0.002	0.03
N	3	3	3	3	3	3	3
G10 (Isoproterenol + EGCG)							**Dose: 90+207 mg/kg**
Mean	66.50	122.50	9.39*	104.67	0.38	0.005	0.54*
SD	13.97	22.42	1.57	10.02	0.02	0.002	0.02
N	3	3	3	3	3	3	3
G11 (Isoproterenol + Biacalin)							**Dose: 90+207 mg/kg**
Mean	75.00	101.63	10.03*	117.67	0.37	0.003	0.74
SD	3.53	4.84	0.46	4.73	0.03	0.002	0.06
N	3	3	3	3	3	3	3
G12 (Isoproterenol + Quercetin)							**Dose: 90+207 mg/kg**
Mean	92.83	99.87	8.43*	138.33	0.41	0.006	0.72
SD	11.46	13.24	0.82	18.50	0.06	0.002	0.14
N	3	3	3	3	3	3	3
G13 (Isoproterenol + Mix High)							**Dose: (137.1+ 54.5 +15.45) mg/kg**
Mean	78.37	109.90	9.93*	141.67	0.42	0.004	0.71
SD	11.82	12.30	1.71	32.15	0.02	0.002	0.03
N	3	3	3	3	3	3	3
G14 (Isoproterenol + Vedicinals-9)							**Dose: 90+ 902 mg/kg**
Mean	79.53	94.47	8.14*	133.67	0.42	0.005	0.85
SD	4.42	7.93	0.60	48.85	0.03	0.003	0.09
N	3	3	3	3	3	3	3

TABLE 3 (Contd.): Summary: Clinical Chemistry Analysis- Female

Mean/ SD/N	GPT	GOT	CK-MB	LDH	CREAT	Troponin-I	GPT:GOT
	U/L	U/L	U/L	U/L	mg/dl	ng/mL	mg/dl
G1 Isoproterenol							**Dose: 90 mg/kg**
Mean	63.57	80.35	18.46	100.00	0.39	0.024	0.74
SD	15.71	22.70	1.92	22.07	0.06	0.015	0.04
N	3	3	3	3	3	3	3
G2 (Isoproterenol + EGCG)							**Dose: 90+138 mg/kg**
Mean	58.87	85.60	10.15*	94.00	0.38	0.006	0.70
SD	6.73	20.28	0.40	1.41	0.04	0.001	0.08
N	3	3	3	3	3	3	3
G3 (Isoproterenol + Biacalin)							**Dose: 90+138 mg/kg**
Mean	69.20	94.87	10.54*	111.67	0.38	0.005	0.73
SD	4.14	9.36	1.65	11.72	0.04	0.001	0.08
N	3	3	3	3	3	3	3
G4 (Isoproterenol + Quercetin)							**Dose: 90+138 mg/kg**
Mean	78.93	107.10	9.34*	130.33	0.37	0.006	0.73
SD	18.71	15.54	1.09	16.20	0.04	0.002	0.07
N	3	3	3	3	3	3	3
G5 (Isoproterenol + Mix Medium (E+B+Q))							**Dose: 90+ (91.4+36.3+10.3) mg/kg**
Mean	76.87	103.67	10.17*	114.00	0.37	0.004	0.74
SD	2.48	1.25	0.43	18.25	0.04	0.001	0.03
N	3	3	3	3	3	3	3
G6 (Isoproterenol + EGCG)							**Dose: 90+55.2 mg/kg**
Mean	56.37	93.33	12.20*	134.00	0.35	0.008	0.60
SD	1.29	5.07	1.95	22.52	0.01	0.003	0.02
N	3	3	3	3	3	3	3
G7 (Isoproterenol + Biacalin)							**Dose: 90+55.2 mg/kg**
Mean	65.75	89.33	13.74	106.00	0.37	0.007	0.52
SD	11.24	17.45	3.20	8.72	0.02	0.002	0.45
N	3	3	3	3	3	3	3

TABLE 3 (Contd.): Summary: Clinical Chemistry Analysis- Female

Mean/ SD/N	GPT U/L	GOT U/L	CK-MB U/L	LDH U/L	CREAT mg/dl	Troponin-I ng/mL	GPT:GOT mg/dl
G8 (Isoproterenol + Quercetin)							**Dose: 90+55.2 mg/kg**
Mean	62.40	95.27	11.96*	132.67	0.39	0.009	0.66
SD	6.12	12.34	1.22	47.25	0.02	0.005	0.02
N	3	3	3	3	3	3	3
G9 (Isoproterenol + Mix Low (E+B+Q))							**Dose: 90+ (36.3+ 14.5+ 4.1) mg/kg**
Mean	67.80	94.10	11.28*	122.67	0.39	0.009	0.73
SD	16.24	29.77	0.79	21.13	0.03	0.002	0.12
N	3	3	3	3	3	3	3
G10 (Isoproterenol + EGCG)							**Dose: 90+207 mg/kg**
Mean	63.95	103.25	8.86*	183.00	0.39	0.006	0.62
SD	0.78	7.71	0.35	18.38	0.01	0.001	0.04
N	2	2	2	2	2	2	2
G11 (Isoproterenol + Biacalin)							**Dose: 90+207 mg/kg**
Mean	49.23	80.67	10.48*	82.33	0.34	0.005	0.61
SD	13.64	2.10	1.03	16.26	0.05	0.001	0.17
N	3	3	3	3	3	3	3
G12 (Isoproterenol + Quercetin							**Dose: 90+207 mg/kg**
Mean	59.43	99.30	9.06*	94.67	0.36	0.004	0.60
SD	3.31	8.14	1.14	29.14	0.01	0.001	0.08
N	3	3	3	3	3	3	3
G13 (Isoproterenol + Mix High)							**Dose: (137.1+ 54.5 +15.45) mg/kg**
Mean	57.80	92.50	9.21*	94.67	0.37	0.004	0.65
SD	19.62	10.79	0.29	8.02	0.05	0.002	0.27
N	3	3	3	3	3	3	3
G14 (Isoproterenol + Vedicinals-9)							**Dose: 90+464 mg/kg**
Mean	48.17	73.37	8.52*	82.67	0.48	0.006	0.69
SD	2.51	23.07	0.83	41.04	0.21	0.001	0.16
N	3	3	3	3	3	3	3

TABLE 4: Summary: Immunogenicity Parameters - Male and Female

Mean/ SD/N	Males		Females	
	IL 6 (pg/mL)	TNF α	IL 6 (pg/mL)	TNF α
G1 Isoproterenol				**Dose: 90 mg/kg**
Mean	242.23	630.57	226.92	577.56
SD	78.45	81.88	50.21	35.47
N	3	3	3	3
G2 (Isoproterenol + EGCG)				**Dose: 90+138 mg/kg**
Mean	157.92	447.66*	167.191	408.29*
SD	39.13	22.99	52.430	44.97
N	2	2	3	3
G3 (Isoproterenol + Biacalin)				**Dose: 90+138 mg/kg**
Mean	228.54	376.64*	144.48	438.58
SD	10.12	22.10	7.68	97.48
N	3	3	3	3
G4 (Isoproterenol + Quercetin)				**Dose: 90+138 mg/kg**
Mean	189.09	414.59*	185.65	411.70*
SD	68.18	59.43	47.95	16.07
N	3	3	3	3
G5 (Isoproterenol + Mix Medium (E+B+Q))				**Dose: 90+ (91.4+36.3+10.3) mg/kg**
Mean	201.65	379.23*	182.28	400.47
SD	51.53	40.86	41.60	152.27
N	3	3	3	3
G6 (Isoproterenol + EGCG)				**Dose: 90+55.2 mg/kg**
Mean	165.52	509.45	195.81	453.20
SD	54.59	128.21	67.24	115.98
N	3	3	3	3
G7 (Isoproterenol + Biacalin)				**Dose: 90+55.2 mg/kg**
Mean	226.90	449.30*	173.83	440.62
SD	41.65	33.62	13.87	109.11
N	3	3	3	3

TABLE 4 (Contd.): Summary: Immunogenicity Parameters - Male and Female

Mean/ SD/N	Males		Females	
	IL 6 (pg/mL)	TNF α	IL 6 (pg/mL)	TNF α
G8 (Isoproterenol + Quercetin)				**Dose: 90 + 55.2mg/kg**
Mean	226.02	502.75	202.42	431.08*
SD	24.14	66.69	13.91	59.45
N	3	3	3	3
G9 (Isoproterenol + Mix Low (E+B+Q))				**Dose: 90+ (36.3+ 14.5+ 4.1) mg/kg**
Mean	214.23	509.45	210.01	441.65
SD	31.61	125.29	28.72	104.52
N	3	3	3	3
G10 (Isoproterenol + EGCG)				**Dose: 90+207 mg/kg**
Mean	142.81	342.89*	151.62	338.40
SD	16.47	56.54	33.78	110.36
N	3	3	2	3
G11 (Isoproterenol + Biacalin)				**Dose: 90+207 mg/kg**
Mean	163.79	383.56*	146.99	340.51*
SD	32.87	78.25	9.52	80.95
N	3	3	3	3
G12 (Isoproterenol + Quercetin)				**Dose: 90+207 mg/kg**
Mean	157.05	379.04*	165.46	360.01*
SD	11.34	87.72	29.66	65.50
N	3	3	3	3
G13 (Isoproterenol + (E+B+Q))				**Dose: 90+ (137.1+ 54.5 +15.45) mg/kg**
Mean	144.48	356.99*	162.93	351.08*
SD	16.16	63.68	21.50	40.36
N	3	3	3	3
G14 (Isoproterenol + Vedicinals-9)				**Dose: 90+ 902 mg/kg**
Mean	141.97	353.82*	147.41	339.28*
SD	15.14	33.38	11.40	50.61
N	3	3	3	3

TABLE 5: Summary: Gross Pathological Observations - Males

Tissue/ Findings/	Isoproterenol +												
Dose mg/kg/day	C	EGCG		Mix	Biacalin			Mix	Quecetin			Mix	V-9
	90	90+138	90+138	90+138	90+5.2	90+5.2	90+5.2	90+5.2	90+207	90+207	90+207	90+207	90+464
Dose Group	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14
Number Examined	3	2	3	3	3	3	3	3	3	3	3	3	3	3
Round Heart	1	1	2	0	0	0	0	0	0	0	0	0	0	0
Minimal congestion of blood vessels	2	1	3	1	1	0	0	0	0	1	1	2	0	0
NAD	1	2	0	2	2	3	3	3	3	2	2	1	3	3

TABLE 5: Summary (Contd.): Gross Pathological Observations - Females

Tissue/ Findings/	Isoproterenol +												
Dose mg/kg/day	C	EGCG		Mix	Biacalin			Mix	Quercetin			Mix	V-9
	90	90+138	90+138	90+138	90+5.2	90+5.2	90+5.2	90+5.2	90+207	90+207	90+207	90+207	90+464
Dose Group	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14
Number Examined	3	3	3	3	3	3	3	3	3	2	3	3	3	3
Round Heart	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Minimal congestion of blood vessels	0	1	0	0	1	3	1	2	0	0	1	0	2	0
NAD	3	2	3	3	2	0	2	1	3	3	2	3	1	3

TABLE 6: Summary: Histopathology Observations - Males

Tissue/ Findings/Sex	Males													
Dose mg/kg/day	Isoproterenol	EGCG	Biacalin	Quercetin	**Mix Medium**	EGCG	Biacalin	Quercetin	**Mix Low**	EGCG	Biacalin	Quercetin	**Mix High**	Vedicinals -9
Group	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**	**G9**	**G10**	**G11**	**G12**	**G13**	**G14**
G15Number Examined	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Heart														
Myocardial degeneration														
Minimal Focal	1	2	2	2	1	2	2	1	2	2	2	2	1	1
Mild Focal	2	1	1	1	1	1	1	1	0	0	0	0	1	0
NAD	0	0	0	0		0	0	1	1	1	1	1	0	2
MNCs infiltration														
Minimal Focal	1	0	2	2	3	2	1	2	3	2	3	2	2	2
Mild Focal	2	1	0	0	0	1	1	1	0	0	0	0	0	0
NAD	0	2	1	1	0	0	1	0	0	1	0	1	1	1
Congested blood vessels														
Minimal Focal	0	2	3	3	2	1	2	3	3	2	2	3	2	2
Mild Focal	3	1	0	0	0	2	0	0	0	0	0	0	0	0
NAD	0	0	0	0	1	0	1	0	0	1	1	0	1	1

TABLE 6 (Contd.): Summary: Histopathology Observations – Females

Tissue/ Findings/Sex	**Females**													
Dose mg/kg/day	Isoproterenol	EGCG	Biacalin	Quercetin	**Mix Medium**	EGCG	Biacalin	Quercetin	**Mix Low**	EGCG	Biacalin	Quercetin	**Mix High**	Vedicinals -9
Group	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**	**G9**	**G10**	**G11**	**G12**	**G13**	**G14**
G15Number Examined	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Heart														
Myocardial degeneration														
Minimal Focal	1	0	1	2	1	1	2	1	2	0	2	3	2	2
Mild Focal	2	2	1	0	1	1	0	0	1	0	0	0	0	0
MNCs infiltration														
Minimal Focal	1	1	2	3	2	1	2	1	3	0	2	2	1	1
Mild Focal	2	1	1	0	0	2	0	0	0	0	2	0	0	0
Congested blood vessels														
Minimal Focal	1	1	1	2	2	3	2	3	2	1	2	1	1	1
Mild Focal	2	1	0	0	0	0	0	0	1	0	0	0	0	0

APPENDIX- 1: Clinical Signs, Morbidity and Mortality Observations – Males and Females

Animal No.	Treatment Day							Animal No.	Treatment Day						
	1	2	3	4	5	6	7		1	2	3	4	5	6	7
G1 Isoproterenol (Dose: 90 mg/kg)								**G5 (Isoproterenol + Mix Medium (E+B+Q)) Dose: 90+ (91.4+36.3+10.3) mg/kg**							
01	N	N	N	N	N	N	N	25	N	N	N	N	N	N	N
02	N	N	N	N	N	N	N	26	N	N	N	N	N	N	N
03	N	N	N	N	N	N	N	27	N	N	N	N	N	N	N
04	N	N	N	N	N	N	N	28	N	N	N	N	N	N	N
05	N	N	N	N	N	N	N	29	N	N	N	N	N	N	N
06	N	N	N	N	N	N	N	30	N	N	N	N	N	N	N
G2 (Isoproterenol + EGCG) (Dose: 90+138 mg/kg)								**G6 (Isoproterenol + EGCG) Dose: 90+55.2 mg/kg**							
07	N	N	N	N	N	N	N	31	N	N	N	N	N	N	N
08	N	N	N	N	N	N	N	32	N	N	N	N	N	N	N
09	N	N	N	-	-	-	-	33	N	N	N	N	N	N	N
10	N	N	N	N	N	N	N	34	N	N	N	N	N	N	N
11	N	N	N	N	N	N	N	35	N	N	N	N	N	N	N
12	N	N	N	N	N	N	N	36	N	N	N	N	N	N	N
G3 (Isoproterenol + Biacalin) (Dose: 90+138 mg/kg)								**G7 (Isoproterenol + Biacalin) Dose: 90+55.2 mg/kg**							
13	N	N	N	N	N	N	N	37	N	N	N	N	N	N	N
14	N	N	N	N	N	N	N	38	N	N	N	N	N	N	N
15	N	N	N	N	N	N	N	39	N	N	N	N	N	N	N
16	N	N	N	N	N	N	N	40	N	N	N	N	N	N	N
17	N	N	N	N	N	N	N	41	N	N	N	N	N	N	N
18	N	N	N	N	N	N	N	42	N	N	N	N	N	N	N
G4 (Isoproterenol + Quercetin) (Dose: 90+138 mg/kg)								**G8 (Isoproterenol + Quercetin) Dose: 90 + 55.2mg/kg**							
19	N	N	N	N	N	N	N	43	N	N	N	N	N	N	N
20	N	N	N	N	N	N	N	44	N	N	N	N	N	N	N
21	N	N	N	N	N	N	N	45	N	N	N	N	N	N	N
22	N	N	N	N	N	N	N	46	N	N	N	N	N	N	N
23	N	N	N	N	N	N	N	47	N	N	N	N	N	N	N
24	N	N	N	N	N	N	N	48	N	N	N	N	N	N	N

APPENDIX- 1 (Contd.): Clinical Signs, Morbidity and Mortality Observations – Males and Females

Animal No.	Treatment Day							Animal No.	Treatment Day						
	1	2	3	4	5	6	7		1	2	3	4	5	6	7
G9 (Isoproterenol + Mix Low (E+B+Q)) Dose: 90+ (36.3+ 14.5+ 4.1) mg/kg								**G13 (Isoproterenol + (E+B+Q)) Dose: 90+ (137.1+ 54.5 +15.45) mg/kg**							
49	N	N	N	N	N	N	N	73	N	N	N	N	N	N	N
50	N	N	N	N	N	N	N	74	N	N	N	N	N	N	N
51	N	N	N	N	N	N	N	75	N	N	N	N	N	N	N
52	N	N	N	N	N	N	N	76	N	N	N	N	N	N	N
53	N	N	N	N	N	N	N	77	N	N	N	N	N	N	N
54	N	N	N	N	N	N	N	78	N	N	N	N	N	N	N
G10 (Isoproterenol + EGCG) Dose: 90+207 mg/kg								**G14 (Isoproterenol + Vedicinals-9) Dose: 90+ 902 mg/kg**							
55	N	N	N	N	N	N	N	79	N	N	N	N	N	N	N
56	N	N	N	N	N	N	N	80	N	N	N	N	N	N	N
57	N	N	N	N	N	N	N	81	N	N	N	N	N	N	N
58	N	N	N	N	N	N	N	82	N	N	N	N	N	N	N
59	N	N	N	N	N	N	N	83	N	N	N	N	N	N	N
60	N	N	N	N	-	-	-	84	N	N	N	N	N	N	N
G11 (Isoproterenol + Biacalin) Dose: 90+207 mg/kg															
61	N	N	N	N	N	N	N	-	-	-	-	-	-	-	-
62	N	N	N	N	N	N	N	-	-	-	-	-	-	-	-
63	N	N	N	N	N	N	N	-	-	-	-	-	-	-	-
64	N	N	N	N	N	N	N	-	-	-	-	-	-	-	-
65	N	N	N	N	N	N	N	-	-	-	-	-	-	-	-
66	N	N	N	N	N	N	N	-	-	-	-	-	-	-	-
G12 (Isoproterenol + Quercetin) Dose: 90+207 mg/kg															
67	N	N	N	N	N	N	N	-	-	-	-	-	-	-	-
68	N	N	N	N	N	N	N	-	-	-	-	-	-	-	-
69	N	N	N	N	N	N	N	-	-	-	-	-	-	-	-
70	N	N	N	N	N	N	N	-	-	-	-	-	-	-	-
71	N	N	N	N	N	N	N	-	-	-	-	-	-	-	-
72	N	N	N	N	N	N	N	-	-	-	-	-	-	-	-

APPENDIX- II: Individual Animal Absolute and Relative Heart Weight (gm)- Male and Female

Animal No.	Heart Weight (Male)		Animal No.	Heart Weight (Female)	
	Absolute	Relative		Absolute	Relative
G1 Isoproterenol					**Dose: 90 mg/kg**
01	0.84	0.40	4	0.83	0.39
02	1.23	0.55	5	1.10	0.50
03	1.84	0.67	6	1.36	0.53
G2 (Isoproterenol + EGCG)					**Dose: 90+138 mg/kg**
07	0.84	0.32	10	0.93	0.46
08	1.10	0.44	11	0.99	0.44
09	1.87	0.62	12	1.11	0.46
G3 (Isoproterenol + Biacalin)					**Dose: 90+138 mg/kg**
13	1.23	0.52	16	0.86	0.43
14	1.45	0.56	17	1.10	0.51
15	1.24	0.42	18	1.07	0.39
G4 (Isoproterenol + Quercetin)					**Dose: 90+138 mg/kg**
19	1.09	0.46	22	0.8	0.38
20	1.45	0.57	23	0.92	0.44
21	1.69	0.43	24	1.07	0.37
G5 (Isoproterenol + Mix)					**Dose: 90+ 138 mg/kg**
25	1.02	0.40	28	0.89	0.44
26	1.10	0.49	29	0.92	0.40
27	1.30	0.34	30	1.11	0.41
G6 (Isoproterenol + EGCG)					**Dose: 90+55.2 mg/kg**
31	0.93	0.40	34	0.92	0.48
32	1.08	0.40	35	0.94	0.40
33	1.20	0.33	36	0.93	0.34
G7 (Isoproterenol + Biacalin)					**Dose: 90+55.2 mg/kg**
37	0.96	0.37	40	0.93	0.44
38	1.12	0.42	41	1.05	0.43
39	1.13	0.32	42	1.05	0.38

APPENDIX- II(Contd.): Individual Animal Absolute and Relative Heart Weight (gm)- Male and Female

Animal No.	Heart Weight (Male)		Animal No.	Heart Weight (Female)	
	Absolute	Relative		Absolute	Relative
G8 (Isoproterenol + Quercetin)					**Dose: 90 + 55.2 mg/kg**
43	0.46	0.19	46	0.81	0.38
44	0.62	0.23	47	0.94	0.37
45	1.34	0.38	48	0.90	0.35
G9 (Isoproterenol +Mix)					**Dose: 90+ 55.2 mg/kg**
49	0.66	0.30	52	0.99	0.48
50	0.69	0.26	53	0.87	0.36
51	0.99	0.29	54	0.91	0.35
G10 (Isoproterenol + EGCG)					**Dose: 90+207 mg/kg**
55	0.91	0.40	58	0.59	0.28
56	0.69	0.26	59	0.80	0.35
57	0.76	0.23	60	1.00	0.38
G11 (Isoproterenol + Biacalin)					**Dose: 90+55.2 mg/kg**
61	1.03	0.44	64	1.01	0.48
62	1.03	0.35	65	0.94	0.37
63	0.97	0.29	66	0.96	0.38
G12 (Isoproterenol + Quercetin)					**Dose: 90 + 55.2mg/kg**
67	0.93	0.44	70	1.00	0.47
68	1.34	0.46	71	1.07	0.39
69	1.20	0.36	72	1.02	0.42
G13 (Isoproterenol + Mix)					**Dose: 90+207 mg/kg**
73	0.85	0.29	76	0.95	0.45
74	0.97	0.49	77	0.93	0.40
75	1.07	0.34	78	1.17	0.45
G14 (Isoproterenol + Vedicinals-9)					**Dose: 90+ 464 mg/kg**
79	1.09	0.47	82	0.93	0.44
80	0.90	0.37	83	0.97	0.40
81	1.00	0.37	84	0.89	0.33

APPENDIX-III: Individual Animal Clinical Chemistry Observations- Males

Animal No.	LDH	GPT	GOT	CREAT	Troponin-I	GPT:GOT	CK-MB
	U/L	U/L	U/L	mg/dl	ng/mL	mg/dl	U/L
G1 Isoproterenol							**Dose: 90 mg/kg**
01	123.00	79.70	101.40	0.42	0.021	0.79	21.41
02	328.00	98.00	110.40	0.46	0.016	0.89	17.76
03	114.00	100.80	115.70	0.51	0.038	0.87	18.87
G2 (Isoproterenol + EGCG)							**Dose: 90+138 mg/kg**
07	165.00	89.90	167.10	0.49	0.006	0.54	9.42
08	139.00	63.40	111.20	0.42	0.004	0.57	9.80
09	112.00		-	0.47	-		-
G3 (Isoproterenol + Biacalin)							**Dose: 90+138 mg/kg**
13	155.00	75.80	102.80	0.46	0.004	0.74	12.02
14	125.00	90.60	120.70	0.43	0.006	0.75	11.92
15	136.00	72.30	107.60	0.37	0.005	0.67	12.86
G4 (Isoproterenol + Quercetin)							**Dose: 90+138 mg/kg**
19	114.00	92.00	99.50	0.49	0.004	0.92	10.48
20	115.00	96.90	106.80	0.39	0.006	0.91	6.39
21	134.00	82.50	103.70	0.51	0.007	0.80	10.44
G5 (Isoproterenol + Mix)							**Dose: 90+ 138 mg/kg**
25	128.00	79.70	105.40	0.49	0.004	0.76	9.91
26	109.00	62.4	79.90	0.39	0.007	0.78	9.40
27	122.00	80.40	107.00	0.43	0.006	0.75	10.02
G6 (Isoproterenol + EGCG)							**Dose: 90+55.2 mg/kg**
31	88.00	74.40	86.10	0.44	0.009	0.86	9.43
32	171.00	84.2	111.80	0.40	0.006	0.75	12.05
33	433.00	81.10	128.50	0.41	0.006	0.63	14.40
G7 (Isoproterenol + Biacalin)							**Dose: 90+55.2 mg/kg**
37	84.00	71.90	91.90	0.41	0.006	0.78	14.41
38	138.00	87.1	98.40	0.42	0.005	0.89	13.36
39	131.00	68.70	106.20	0.39	0.005	0.65	14.66

APPENDIX- 1II (Contd.): Individual Animal Clinical Chemistry Observations- Males

Animal No.	LDH	GPT	GOT	CREAT	Troponin-I	GPT:GOT	CK-MB
	U/L	U/L	U/L	mg/dl	ng/mL	mg/dl	U/L
G8 (Isoproterenol + Quercetin)							**Dose: 90 + 55.2mg/kg**
43	130.00	86.70	116.00	0.47	0.005	0.75	10.73
44	183.00	74.70	120.70	0.40	0.005	0.62	9.58
45	171.00	76.10	103.40	0.38	0.010	0.74	14.45
G9 (Isoproterenol +Mix)							**Dose: 90+ 55.2 mg/kg**
49	134.00	76.10	133.00	0.44	0.005	0.57	11.68
50	128.00	51.50	98.90	0.34	0.006	0.52	13.42
51	103.00	66.60	121.00	0.51	0.008	0.55	14.64
G10 (Isoproterenol + EGCG)							**Dose: 90+207 mg/kg**
55	104.00	82.50	147.80	0.38	0.004	0.56	9.26
56	115.00	56.70	105.10	0.40	0.007	0.54	11.02
57	95.00	60.30	114.60	0.37	0.005	0.53	7.88
G11 (Isoproterenol + Biacalin)							**Dose: 90+55.2 mg/kg**
61	114.00	79.00	97.20	0.37	0.005	0.81	9.56
62	123.00	73.70	106.80	0.40	0.002	0.69	10.47
63	116.00	72.30	100.90	0.34	0.002	0.72	10.06
G12 (Isoproterenol + Quercetin)							**Dose: 90 + 55.2mg/kg**
67	148.00	63.10	85.00	0.35	0.005	0.74	9.15
68	150.00	58.90	104.20	0.40	0.008	0.57	8.61
69	117.00	93.80	110.40	0.47	0.004	0.85	7.54
G13 (Isoproterenol + Mix)							**Dose: 90+207 mg/kg**
73	155.00	90.60	123.00	0.45	0.002	0.74	8.70
74	105.00	77.50	108.10	0.41	0.006	0.72	11.89
75	165.00	67.00	98.60	0.41	0.005	0.68	9.21
G14 (Isoproterenol + Vedicinals-9)							**Dose: 90+ 464 mg/kg**
79	190.00	76.50	103.10	0.39	0.003	0.74	8.29
80	103.00	77.50	87.50	0.44	0.005	0.89	7.48
81	108.00	84.60	92.80	0.43	0.008	0.91	8.66

APPENDIX- 1II (Contd.): Individual Animal Clinical Chemistry Observations- Females

Animal No.	LDH U/L	GPT U/L	GOT U/L	CREAT mg/dl	Troponin-I ng/mL	GPT:GOT mg/dl	CK-MB U/L
G1 Isoproterenol							**Dose: 90 mg/kg**
04	123.00	71.20	125.00	0.45	0.041		18.99
05	114.00	94.00	96.40	0.40	0.017	0.98	16.34
06	144.00	115.50	104.30	0.53	0.014	1.11	20.06
G2 (Isoproterenol + EGCG)							**Dose: 90+138 mg/kg**
10		66.30	108.70	0.40	0.007	0.61	9.89
11	95.00	57.10	77.40	0.40	0.006	0.74	10.61
12	93.00	53.20	70.70	0.33	0.006	0.75	9.94
G3 (Isoproterenol + Biacalin)							**Dose: 90+138 mg/kg**
16	107.00	70.80	105.40	0.40	0.005	0.67	11.14
17	103.00	64.50	91.70	0.41	0.005	0.70	11.81
18	125.00	72.30	87.50	0.34	0.004	0.83	8.67
G4 (Isoproterenol + Quercetin)							**Dose: 90+138 mg/kg**
22	149.00	98.00	121.30	0.42	0.005	0.81	10.06
23	120.00	78.20	109.50	0.36	0.008	0.71	9.88
24	122.00	60.60	90.50	0.34	0.004	0.67	8.08
G5 (Isoproterenol + Mix)							**Dose: 90+ 138 mg/kg**
28	93	75.8	102.80	0.40	0.003	0.74	10.48
29	126	75.10	105.10	0.39	0.003	0.71	10.34
30	123	79.70	103.10	0.32	0.005	0.77	9.68
G6 (Isoproterenol + EGCG)							**Dose: 90+55.2 mg/kg**
34	147.00	57.80	98.10	0.35	0.008	0.59	12.34
35	147.00	55.30	88.00	0.34	0.005	0.63	10.19
36	108.00	56.00	93.90	0.35	0.010	0.60	14.08
G7 (Isoproterenol + Biacalin)							**Dose: 90+55.2 mg/kg**
40	110.00	57.80	69.30	0.35	0.008	0.83	11.49
41	112.00	73.70	101.20	0.38	0.005	0.73	12.32
42	96.00	35.60	97.50	0.56	0.007	0.37	17.40

APPENDIX- 1II (Contd.): Individual Animal Clinical Chemistry Observations- Females

Animal No.	LDH	GPT	GOT	CREAT	Troponin-I	GPT:GOT	CK-MB
	U/L	U/L	U/L	mg/dl	ng/mL	mg/dl	U/L
G8 (Isoproterenol + Quercetin)							**Dose: 90 + 55.2 mg/kg**
46	91.00	55.70	81.60	0.37	0.005	0.68	10.66
47	184.00	67.70	105.60	0.39	0.014	0.64	13.09
48	123.00	63.80	98.60	0.41	0.008	0.65	12.12
G9 (Isoproterenol +Mix)							**Dose: 90+ 55.2 mg/kg**
52	147.00	81.10	127.70	0.42	0.010	0.64	12.17
53	112.00	72.60	83.60	0.39	0.010	0.87	10.66
54	109.00	49.70	71.00	0.37	0.006	0.70	11.02
G10 (Isoproterenol + EGCG)							**Dose: 90+207 mg/kg**
58	170.00	64.50	108.70	0.38	0.005	0.59	9.11
59	196.00	63.40	97.80	0.40	0.007	0.65	8.61
60	109.00	55.70	103.40	0.32	-	0.54	-
G11 (Isoproterenol + Biacalin)							**Dose: 90+55.2 mg/kg**
64	88.00	56.40	78.50	0.36	0.005	0.72	10.37
65	64.00	57.80	82.70	0.38	0.004	0.70	11.56
66	95.00	33.50	80.80	0.29	0.005	0.41	9.50
G12 (Isoproterenol + Quercetin)							**Dose: 90 + 55.2mg/kg**
70	64.00	60.60	94.50	0.37	0.004	0.64	7.75
71	122.00	62.00	94.70	0.36	0.004	0.65	9.88
72	98.00	55.70	108.70	0.35	0.005	0.51	9.54
G13 (Isoproterenol + Mix)							**Dose: 90+207 mg/kg**
76	103.00	70.50	83.60	0.42	0.004	0.84	8.95
77	94.00	67.70	89.40	0.36	0.003	0.76	9.52
78	87.00	35.20	104.50	0.33	0.006	0.34	9.16
G14 (Isoproterenol + Vedicinals-9)							**Dose: 90+ 464 mg/kg**
82	57.00	47.90	59.50	0.40	0.005	0.81	7.97
83	61.00	45.80	60.60	0.33	0.006	0.76	9.48
84	130.00	50.80	100.00	0.72	0.006	0.51	8.12

APPENDIX- IV: Individual Animal Immunogenicity Parameters - Males and Females

Animal ID	Males		Animal ID	Females	
	IL 6 (pg/mL)	TNF α		IL 6 (pg/mL)	TNF α
G1 Isoproterenol					**Dose: 90 mg/kg**
01	332.8	575.6	04	266.6	611.8
02	198.2	591.5	05	170.5	579.8
03	195.7	724.7	06	243.7	541.0
G2 (Isoproterenol + EGCG)					**Dose: 90+138 mg/kg**
07	185.6	463.9	10	110.2	415.3
08	130.2	431.4	11	213.4	449.4
09	-	-	12	178.0	360.2
G3 (Isoproterenol + Biacalin)					**Dose: 90+138 mg/kg**
13	218.4	363.0	16	152.9	339.5
14	238.7	364.7	17	142.8	441.8
15	228.5	402.1	18	137.8	534.4
G4 (Isoproterenol + Quercetin)					**Dose: 90+138 mg/kg**
19	122.7	419.3	22	238.7	429.7
20	185.6	471.5	23	173.0	406.7
21	259.0	352.9	24	145.3	398.7
G5 (Isoproterenol + Mix Medium (E+B+Q))					**Dose: 90+ (91.4+36.3+10.3) mg/kg**
25	238.7	340.1	28	140.3	273.9
26	223.5	421.6	29	223.5	358.0
27	142.8	376.0	30	183.1	569.5
G6 (Isoproterenol + EGCG)					**Dose: 90+55.2 mg/kg**
31	228.5	657.5	34	132.8	376.0
32	132.8	437.8	35	266.6	586.6
33	135.3	433.1	36	188.1	397.0
G7 (Isoproterenol + Biacalin)					**Dose: 90+55.2 mg/kg**
37	274.2	480.3	40	173.0	554.3
38	195.7	413.6	41	188.1	430.8
39	210.8	454.0	42	160.4	336.7

APPENDIX- IV (Contd.):Individual Animal Immunogenicity Parameters - Males and Females

Animal ID	Males		Animal ID	Females	
	IL 6 (pg/mL)	TNF α		IL 6 (pg/mL)	TNF α
G8 (Isoproterenol + Quercetin)					**Dose: 90 + 55.2mg/kg**
43	203.3	442.4	46	188.1	422.2
44	251.3	491.5	47	215.9	376.6
45	223.5	574.3	48	203.3	494.5
G9 (Isoproterenol + Mix Low (E+B+Q))					**Dose: 90+ (36.3+ 14.5+ 4.1) mg/kg**
49	213.4	590.3	52	178.0	321.2
50	246.3	345.1	53	233.6	495.7
51	183.1	422.8	54	218.4	508.1
G10 (Isoproterenol + EGCG)					**Dose: 90+207 mg/kg**
55	145.3	348.5	58	175.5	260.4
56	125.2	283.8	59	127.7	416.4
57	157.9	396.4	60	-	-
G11 (Isoproterenol + Biacalin)					**Dose: 90+207 mg/kg**
61	137.8	351.8	64	140.3	411.3
62	152.9	326.2	65	157.9	252.2
63	200.7	472.7	66	142.8	358.0
G12 (Isoproterenol + Quercetin)					**Dose: 90+207 mg/kg**
67	167.9	315.6	70	173.0	433.7
68	157.9	479.2	71	190.6	308.5
69	145.3	342.3	72	132.8	337.8
G13 (Isoproterenol + (E+B+Q))					**Dose: 90+ (137.1+ 54.5 +15.45) mg/kg**
73	162.9	289.8	76	140.3	353.5
74	132.8	416.4	77	183.1	309.6
75	137.8	364.7	78	165.4	390.2
G14 (Isoproterenol + Vedicinals-9)					**Dose: 90+ 902 mg/kg**
79	127.7	340.1	82	135.3	332.3
80	140.3	391.9	83	149.1	292.5
81	157.9	329.5	84	157.9	393.0

APPENDIX- V: Individual Animal Gross Pathology Findings - Male

Animal No.	Organ	Observations
G1 (Isoproterenol)		**Dose: 90 mg/kg**
01	Heart	NAD
02		Round heart and minimal congestion of Blood vessels
03		Minimal congestion of Blood vessels
G2 (Isoproterenol + EGCG)		**Dose: 90+138 mg/kg**
07	Heart	NAD
08		Round heart and minimal congestion of Blood vessels
G3(Isoproterenol + Biacalin)		**Dose: 90+138 mg/kg**
13	Heart	Round heart and Minimal congestion of Blood vessels
14		Minimal congestion of Blood vessels
15		Minimal congestion of Blood vessels and Round heart
G4(Isoprotereno+Quercetin)		**Dose:90+138 mg/kg**
19	Heart	NAD
20		NAD
21		Minimal congestion of Blood vessels
G5 (Isoproterenol + Mix)		**Dose: 90+ 138 mg/kg**
25	Heart	Minimal congestion of Blood vessels
26		NAD
27		NAD
G6 (Isoproterenol + EGCG)		**Dose: 90+55.2 mg/kg**
31	Heart	NAD
32		NAD
33		NAD
G7 (Isoproterenol + Biacalin)		**Dose: 90+55.2 mg/kg**
37	Heart	NAD
38		NAD
39		NAD

Key: NAD = No Abnormality Detected

APPENDIX-V (Contd.): Individual Animal Gross Pathology Findings - Male

Animal No.	Organ	Observation
G8(Isoproterenol + Quercetin)		**Dose: 90 + 55.2mg/kg**
43	Heart	NAD
44		NAD
45		NAD
G9 (Isoproterenol +Mix)		**Dose: 90+ 55.2 mg/kg**
49	Heart	NAD
50		NAD
51		NAD
G10 (Isoproterenol + EGCG)		**Dose: 90+207 mg/kg**
55	Heart	NAD
56		NAD
57		Minimal congestion of Blood vessels
G11(Isoproterenol + Biacalin)		**Dose: 90+207 mg/kg**
61	Heart	NAD
62		NAD
63		Minimal congestion of Blood vessels
G12(Isoproterenol + Quercetin)		**Dose: 90+207 mg/kg**
67	Heart	Minimal congestion of Blood vessels
68		Minimal congestion of Blood vessels
69		NAD
G13 (Isoproterenol + Mix)		**Dose: 90+207 mg/kg**
73	Heart	NAD
74		NAD
75		NAD
G14(Isoproterenol+Vedicinals-9)		**Dose: 90+464mg/kg**
79	Heart	NAD
80		NAD
81		NAD

Key: NAD = No Abnormality Detected

APPENDIX- V (Contd.): Individual Animal Gross Pathology Findings - Female

Animal No.	Organ	Observations
G1 Isoproterenol		**Dose: 90 mg/kg**
04	Heart	NAD
05		NAD
06		NAD
G2(Isoproterenol + EGCG)		**Dose: 90+138 mg/kg**
10	Heart	Minimal congestion of Blood vessels
11		NAD
12		NAD
G3(Isoproterenol + Biacalin)		**Dose: 90+138 mg/kg**
16	Heart	NAD
17		NAD
18		NAD
G4(Isoprotereno+Quercetin)		**Dose:90+138 mg/kg**
22	Heart	NAD
23		NAD
24		NAD
G5 (Isoproterenol + Mix)		**Dose: 90+ 138 mg/kg**
28	Heart	Minimal congestion of Blood vessels
29		NAD
30		NAD
G6 (Isoproterenol + EGCG)		**Dose: 90+55.2 mg/kg**
34	Heart	Minimal congestion of Blood vessels
35		Minimal congestion of Blood vessels
36		Minimal congestion of Blood vessels
G7 (Isoproterenol + Biacalin)		**Dose: 90+55.2 mg/kg**
40	Heart	NAD
41		Minimal congestion of Blood vessels
42		NAD

Key: NAD = No Abnormality Detected

APPENDIX- V (Contd.): Individual Animal Gross Pathology Findings - Female

Animal No.	Organ	Observation
G8(Isoproterenol + Quercetin)		**Dose: 90 + 55.2mg/kg**
46	Heart	NAD
47		Minimal congestion of Blood vessels
48		Minimal congestion of Blood vessels
G9 (Isoproterenol +Mix)		**Dose: 90+ 55.2 mg/kg**
52	Heart	NAD
53		NAD
54		NAD
G10 (Isoproterenol + EGCG)		**Dose: 90+207 mg/kg**
58	Heart	NAD
59		NAD
60		NAD
G11(Isoproterenol + Biacalin)		**Dose: 90+207 mg/kg**
64	Heart	Minimal congestion of Blood vessels
65		NAD
66		NAD
G12(Isoproterenol + Quercetin)		**Dose: 90+207 mg/kg**
70	Heart	NAD
71		NAD
72		NAD
G13 (Isoproterenol + Mix)		**Dose: 90+207 mg/kg**
76	Heart	Minimal congestion of Blood vessels
77		Minimal congestion of Blood vessels
78		NAD
G14(Isoproterenol+Vedicinals-9)		**Dose: 90+464mg/kg**
82	Heart	NAD
83		NAD
84		NAD

Key: NAD = No Abnormality Detected

APPENDIX- VI: Individual Animal Histopathology Observations- Males and Females

G 1 (Isoproterenol) **Dose: 90mg/kg**

Organ/Microscopic Finding (Heart)	Animal number					
	Males			Females		
	01	02	03	04	05	06
Myocardial degeneration	<2>	<1>	<2>	<1>	<2>	<2>
MNCs infiltration	<1>	<1>	NAD	<1>	<2>	<1>
Congested blood vessels	<2>	<2>	<2>	<1>	<2>	<2>

G 2 (Isoproterenol+EGCG) **Dose: 90+138 mg/kg**

Organ/Microscopic Finding (Heart)	Animal number				
	Males			Females	
	07	08	10	11	12
Myocardial degeneration	<2>	<1>	<1>	<2>	<2>
MNCs infiltration	<2>	NAD	NAD	<2>	<1>
Congested blood vessels	<1>	<2>	<1>	<2>	<1>

G 3 (Isoproterenol+Biacalin) **Dose: 90+138 mg/kg**

Organ/Microscopic Finding (Heart)	Animal number					
	Males			Females		
	13	14	15	16	17	18
Myocardial degeneration	<1>	<2>	<1>	NAD	<1>	<2>
MNCs infiltration	NAD	<1>	<1>	<2>	<1>	<1>
Congested blood vessels	<1>	<1>	<1>	NAD	<1>	NAD

G 4 (Isoproterenol+Quercetin) **Dose: 90+138 mg/kg**

Organ/Microscopic Finding (Heart)	Animal number					
	Males			Females		
	19	20	21	22	23	24
Myocardial degeneration	<2>	<1>	<1>	NAD	<1>	<1>
MNCs infiltration	<1>	<1>	NAD	<1>	<1>	<1>
Congested blood vessels	<1>	<1>	<1>	NAD	<1>	<1>

G 5 (Isoproterenol + Mix Medium (E+B+Q)) **Dose: 90+ (91.4+36.3+10.3) mg/kg**

Organ/Microscopic Finding (Heart)	Animal number					
	Males			Females		
	25	26	27	28	29	30
Myocardial degeneration	NAD	<1>	<2>	<1>	<2>	NAD
MNCs infiltration	<1>	<1>	<1>	NAD	<1>	<1>
Congested blood vessels	NAD	<1>	<1>	<1>	NAD	<1>

Key: NAD = No Abnormality Detected; 1= Minimal, 2= Mild, 3= Moderate < > = Focal, () = multifocal [] = diffuse

APPENDIX- VI (Contd.) : Individual Animal Histopathology Observations- Males and Females

G6 (Isoproterenol + EGCG) **Dose: 90+55.2 mg/kg**

Organ/Microscopic Finding (Heart)	Animal number					
	Males			Females		
	31	32	33	34	35	36
Myocardial degeneration	<2>	<1>	<1>	<2>	<1>	NAD
MNCs infiltration	<1>	<2>	<1>	<2>	<1>	<2>
Congested blood vessels	<2>	<1>	<2>	<1>	<1>	<1>

G7 (Isoproterenol + Biacalin) **Dose: 90+55.2 mg/kg**

Organ/Microscopic Finding (Heart)	Animal number					
	Males			Females		
	37	38	39	40	41	42
Myocardial degeneration	<1>	<1>	<2>	<1>	NAD	<1>
MNCs infiltration	<2>	<1>	NAD	<1>	<1>	NAD
Congested blood vessels	<1>	NAD	<1>	NAD	<1>	<1>

G8 (Isoproterenol + Quercetin) **Dose: 90 + 55.2mg/kg**

Organ/Microscopic Finding (Heart)	Animal number					
	Males			Females		
	43	44	45	46	47	48
Myocardial degeneration	NAD	<1>	<2>	<1>	NAD	NAD
MNCs infiltration	<2>	<1>	<1>	<1>	NAD	NAD
Congested blood vessels	<1>	<1>	<1>	<1>	<1>	<1>

G9 (Isoproterenol + Mix Low (E+B+Q)) **Dose: 90+ (36.3+ 14.5+ 4.1) mg/kg**

Organ/Microscopic Finding (Heart)	Animal number					
	Males			Females		
	49	50	51	52	53	54
Myocardial degeneration	<1>	NAD	<1>	<1>	<2>	<1>
MNCs infiltration	<1>	<1>	<1>	<1>	<1>	<1>
Congested blood vessels	<1>	<1>	<1>	<2>	<1>	<1>

G10 (Isoproterenol + EGCG) **Dose: 90+207 mg/kg**

Organ/Microscopic Finding (Heart)	Animal number				
	Males			Females	
	55	56	57	58	59
Myocardial degeneration	NAD	<1>	<1>	NAD	NAD
MNCs infiltration	<1>	NAD	<1>	NAD	NAD
Congested blood vessels	<1>	<1>	NAD	NAD	<1>

Key: NAD = No Abnormality Detected; 1= Minimal, 2= Mild, 3= Moderate < > = Focal, () = multifocal [] = diffuse

APPENDIX- VI (Contd.): Individual Animal Histopathology Observations- Males and Females

G11 (Isoproterenol + Biacalin) Dose: 90+207 mg/kg

Organ/Microscopic Finding (Heart)	Animal number					
	Males			Females		
	61	62	63	64	65	66
Myocardial degeneration	NAD	<1>	<1>	<1>	<1>	NAD
MNCs infiltration	<1>	<1>	<1>	<1>	<1>	NAD
Congested blood vessels	<1>	<1>	NAD	<1>	NAD	<1>

G12 (Isoproterenol + Quercetin) Dose: 90+207 mg/kg

Organ/Microscopic Finding (Heart)	Animal number					
	Males			Females		
	67	68	69	70	71	72
Myocardial degeneration	<1>	NAD	<1>	<1>	<1>	<1>
MNCs infiltration	<1>	<1>	NAD	<1>	<1>	NAD
Congested blood vessels	<1>	<1>	<1>	NAD	<1>	NAD

G13 (Isoproterenol + (E+B+Q)) Dose: 90+ (137.1+ 54.5 +15.45) mg/kg

Organ/Microscopic Finding (Heart)	Animal number					
	Males			Females		
	73	74	75	76	77	78
Myocardial degeneration	<2>	<1>	<1>	<1>	<1>	NAD
MNCs infiltration	<1>	<1>	NAD	NAD	NAD	<1>
Congested blood vessels	<1>	<1>	NAD	NAD	<1>	NAD

G14 (Isoproterenol + Vedicinals-9) Dose: 90+ 902 mg/kg

Organ/Microscopic Finding (Heart)	Animal number					
	Males			Females		
	79	80	81	82	83	84
Myocardial degeneration	<1>	NAD	NAD	<1>	NAD	<1>
MNCs infiltration	<1>	<1>	NAD	NAD	NAD	<1>
Congested blood vessels	<1>	<1>	NAD	NAD	NAD	<1>

Key: NAD = No Abnormality Detected; 1= Minimal, 2= Mild, 3= Moderate < > = Focal, () = multifocal [] = diffuse

LIST OF ABBREVIATIONS

^{0}C	Degree Celsius
%	Percentage
µL	Micro liter
ALB	Albumin
ALP	Alkaline Phosphatase
ARF	Animal Research Facility
BILT	Total Bilirubin
BUL	Blood urea
CBUN	Calculated Blood Urea Nitrogen
CHOLE	Cholesterol
CMC	Carboxyl Methyl Cellulose
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
CREAT	Creatinine
E	Edema
FD	Found Dead
f/L	Femtoliter
g/dL	Gram Per Deciliter
GLU	Glucose
gm	Gram
GOT	Glutamate oxaloacetate transaminase
GPT	Glutamate pyruvate transaminase
HCT	Haematocrit
HGB	Hemoglobin Concentration
Hrs	Hours
IAEC	Institutional Animal Ethics Committee
Kg	Kilogram
Ltd	Limited
mg/dl	Milligram Per Deciliter
MCV	Mean Corpuscular Volume
MCH	Mean Hemoglobin Concentration
MCHC	Mean Corpuscular Hemoglobin Concentration
mg	Milligram
ml	Milliliter
N	Number of Animals

NAD	No Abnormalities Detected
NOAEL	No Observed Adverse Effect Levels
OECD	The Organization for Economic Co-operation and Development
PAR	Protein Albumin Ratio
Pg	Picogram
PLT	Platelet
PRO	Protein
Pvt	Private
RBC	Red blood cells
SD	Standard Deviation
SOP	Standard Operating Procedures
TRGL	Triglycerides
U/L	Unit Per Liter
UV	Ultra Violet
WBC	White blood cells
w/v	Weight by volume