

# The Possible Cardio-Reno- Protective Effects of Vanillin on Isopretrenol- Induced Myocardial Infarction in Rats

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**Abstract** Myocardial infarction (MI) continues to be a major public health problem in the world. Vanillin is a natural phenolic compound that possesses significant anti-oxidant and anti-inflammatory activities. The present study aimed to investigate the effects of pretreatment with vanillin (150 mg/kg, p.o.) and vanillin (300 mg/kg, p.o.) on isoprenaline-induced MI in rats. Markers chosen to assess cardiac damage included serum activity of creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH), in addition to serum level of cardiac troponin-I (cTn-I), as well as antioxidant activity of cardiac catalase (CAT) as well as cardiac contents of RGH, lipid peroxides, and nitrite. Furthermore Electrocardiograph (ECG) monitoring and histological examinations of cardiac tissues were done. In addition markers chosen to assess renal impairment included serum and urine levels of creatinin, blood urea nitrogen (BUN), also creatinine clearance (Ccr), urea clearance (Ucr) and glomerular filtration rate (GFR) were assessed. In addition, antioxidant activity of renal CAT and renal contents of RGH, lipid peroxides, and nitrite. Furthermore, histological examinations of renal tissues were done. Isoprenaline increased serum CK-MB and LDH activity and cTn-I level, cardiac and renal oxidative stress biomarkers. In addition, it produced ST-segment elevation and degenerative changes in heart and renal tissues. Pretreatment with vanillin in previous doses significantly suppressed isoprenaline-induced pathological changes, as the elevated levels of cTn-I, LDH and CK-MB in serum coupled with reduction in cardiac and renal oxidative stress markers. Moreover marked improvement in ECG and histopathologic alterations in both cardiac and renal tissues were produced. In conclusion, vanillin can be regarded as a promising cardio- and reno- protective natural agent in MI.

**Keywords** Myocardial infarction, renal impairment, vanillin, oxidative stress.

## Introduction

Acute myocardial infarction (AMI) continues to be a health problem causing mortality and morbidity despite clinical care and public concern (Ruff and Braunwald, 2011). It is well known that MI is a common symptom of myocardial ischemia, and occurs when cardiac injury surpasses a critical threshold, resulting in mortal cardiac damage (Kim, 2012). In MI, reactive oxygen species (ROS) are the primary components of oxidative damage of cardiomyocytes (Dhalla et al., 2000). Renal dysfunction is a strong independent predictor of cardiovascular outcomes and mortality in the general population (Go et al., 2004) after MI (Lekston et al., 2009 and Amin et al., 2012).

To study possible protective effects of drugs on the myocardial injury from AMI, a widely used experimental model is the induction of MI by means of the receiving isoproterenol in rats, since this substance causes a myocardial damage similar to the one observed in AMI in humans (Ithayarasi and Devi, 1997). Several mechanisms proposed to explain the

isoproterenol-induced myocardial harm, one might say: an unbalance between oxygen supply to and demand from cardiomyocytes inwardly, which is related to myocardial hyperfunction due to increase both in chronotropism and inotropism as well as to hypotension in the coronary bed (Yeager and Whitehurst, 1982). Secondly, it is also claimed that there is an elevation of Ca<sup>++</sup> overcharge inside the cell (Bloom and Davis, 1972), in addition, that ion is related to the activation of the adenylate cyclase enzyme and the depletion of ATP levels on the course of the events (Bhagat et al., 1976). Eventually, there is an oxidative stress augmentation because of several metabolic products originated from isoproterenol (Singal et al., 1982). Compromised antioxidant defense leads to metabolic and functional impairment and membrane permeability changes consequent to lipid peroxidation and ultimately irreversible damage to the myocyte membrane (Zhang et al., 2014).

Myocardial infarction (MI) is a major form of ischemic heart disease, characterized by an imbalance of coronary blood supply and myocardial demand which results in ischemia and myocardial death. Experimental and clinical studies have shown that, during ischemic injury, produced oxidative stress plays a key role in the development of MI (Tullio et al., 2013). In ischemic tissues, the oxygen-free radicals have been implicated in oxidative chain reactions, which damage the cell membrane and subcellular structures containing phospholipids and proteins. These reactions further cause phospholipid peroxidation and subsequently lead to functional, structural, and metabolic alterations in the heart (Tappia et al., 2001). The central role of ROS in the pathophysiology of MI has been confirmed by the ability of antioxidants to reduce ischemic injury in the animal model of isoproterenol- induced MI (Ojha et al., 2012). A large number of epidemiological, clinical, and experimental studies have demonstrated that the use of antioxidants as a preventive approach may limit the infarct size and attenuate myocardial dysfunction as well as slowing down the progression and consequences of MI (Agrawal et al., 2014). Antioxidants not only suppress the formation of ROS and free radical generation and or augmentation of endogenous antioxidant enzymes but also modulate heart function (Ertracht et al., 2014).

Numerous previous studies have shown that antioxidants may inhibit the progression of cardiac ischemia (Visioli et al., 2000). Accordingly, many natural antioxidants are recognized to have potential as herbal medicines for reducing the occurrence of cardiovascular diseases (Ignarro et al., 2007). Vanillin, for example, is a chemical compound that confers the smell and flavor of vanilla, is used as a sleep prevention agent and an aphrodisiac (Bythrow, 2005). Functional uses of vanillin indicate that it exhibits chemopreventive effects in multiorgan carcinogenesis models in rats (Akagi et al., 1995) and prevents the invasion and migration of cancer cells (Cheng et al., 2008); it can also be used to treat sickle cell anemia (Zhang et al., 2004). Besides, vanillin has been shown to inhibit lipopolysaccharide-stimulated NF-KB activation and cyclooxygenase-2 gene expression in murine macrophages (Murakami et al., 2007). In addition it has anti-oxidant, anti-inflammatory and hepatoprotective effects against CCl<sub>4</sub>-induced acute liver injury in adult rats (Makni et al., 2011).

In the present study, we investigated the cardio- and reno- protective effect of vanillin against isoproterenol-induced myocardial injury, a clinically relevant animal model, by measuring the markers of myocyte injury, cardiac and renal biochemical markers, antioxidant defense system, and echo- cardiogram (ECG) parameters. Furthermore, to support our findings, we also examined the effects of vanillin on histopathological changes in the myocardium and kidney tissues.

## Materials and methods

### Drugs and chemicals

Chemicals: All chemicals used in this study were analytically pure and purchased from Sigma-Aldrich Chemical Co., (St Louis, MO, USA), isoprenaline

which was used for MI induction and vanillin which was used for protection were in powder form.

### Animals

Eighty four male Wister rats weighed 150-180g were obtained from the Holding Company for Biological Products & Vaccines VACCERA, Egypt. The normal chow was acquired from Meladco for Animal Food, Egypt. Pellets and tap water were provided *ad libitum*. Temperature was maintained at 25°C. A 12/12 h light/dark cycle was maintained. Rats were allowed at least 1 week to acclimatize to the lab conditions. All procedures were done according to guidelines of Ethical Committee, Faculty of Pharmacy, Al-Azhar University.

### Experimental design

Rats were divided into six equal groups, the first group acted as control (-ve control). The second was the MI group, received isoprenaline powder which was dissolved in normal saline in a dose of 85 mg/kg, subcutaneously (S.C.) for two consecutive days at 24 hrs interval. and used as +ve control (Ojha et al., 2008). The third group received vanillin powder that was prepared in a suspension form in distilled water, by using tween 80 as a suspending agent, in a dose of 150 mg/kg, orally by gavage (P.O.) for five days (Ket et al., 2010). The fourth group received vanillin in a dose of 150mg/kg, P.O. for five days followed by isoprenaline in a dose of 85 mg/kg, S.C. for two successive days. The fifth group received vanillin in a dose of 300 mg/kg, P.O. for five days (Ket et al., 2010), and the sixth group received vanillin in a dose of 300 mg/kg, P.O. for five days followed by isoprenaline in a dose of 85 mg/kg, S.C. for two successive days.

Twenty four hours after the last dose of isoprenaline, urine was collected in a metabolic cage for determination of Ccr, Ucr and Ucr, then the rats were anaesthetized by using urethane dissolved in saline (0.9% NaCl in a dose of 1.2g/kg, intraperitoneally (I.P.) (Karl et al., 1993) for the ECG estimation. Blood was collected from the retro-orbital venous plexus, and allowed to clot and serum was separated by centrifugation at 3000 g for 15 min for biochemical assessment. Rats were then anaesthetized and sacrificed by cervical dislocation (painless death ethics). The hearts and kidneys were dissected out. The heart was weighed and immersed in ice-cold saline. The hearts and kidneys from two rats were fixed in 10% buffered formalin for staining with haematoxylin and eosin and undergo histopathological examination (Banchroft et al., 1996). Heart and kidney tissues were homogenized in saline, centrifuged, and the supernatant fluid was used for assessment of oxidative stress biomarkers using Shimadzu Spectrophotometer UV-1201 (Japan).

### Hemodynamic assessments

Anesthetized rats were placed in the supine position on a board and ECG was recorded continuously with standard artifact free lead II (right forelimb to left hind limb). Needle electrodes were inserted subcutaneously into paw pads of each rat, and connected to Biocare ECG 101 (Shenzhen Biocare Electronics Co., Ltd., China). The ECG was measured to determine duration

and amplitude of the P wave, QRS complex, and ST segment alterations.

Heart index= (heart weight/body weight) ×100.

### Biochemical analysis

#### Cardio-toxicity markers

Serum CK-MB and LDH activities were determined according to standard methods using available commercial kits (Spectrum diagnostics, Cairo, Egypt), cTn-I, was determined using an enzyme-linked immunosorbent assay (ELISA) developed by Life Diagnostics Inc. West Chester PA, USA, according to the manufacturer's instructions.

#### Kidney function tests

Serum concentrations of BUN and creatinine were estimated. In addition concentrations of urea and creatinine in urine were measured, using standard diagnostic kits (Quimica Clinica Aplicada s.a., Amposta, Spain).

Determination of glomerular filtration rate (GFR): was calculated according to the following equation (Pestel *et al.*, 2007).

$$\text{GFR} = \sqrt{\text{Creatinine Clearance (Ccr)} \times \text{Urea Clearance (Ucr)}}$$

Determination of creatinine clearance (C<sub>Cr</sub>): was calculated on the basis of urinary Cr, serum Cr, urine volume body weight, using the equation shown below (Kim *et al.*, 2004).

$$\text{Ccr (ml/min/kg body weight)} = (\text{urinary Cr (mg/dl)} \times \text{urine volume (ml)/serum Cr (mg/dl)}) \times (1000/\text{body weight (g)} \times (1/1440 \text{ (min)}))$$

Determination of urea clearance (Ucr) = Urine urea (mg/dl) × V (ml/min) / Plasma urea (mg/dl) (Pestel *et al.*, 2007).

#### Assessment of cardiac and renal oxidative stress markers and antioxidant enzymes activities:

Catalase (CAT) activities were assessed according to the manufacturer's instructions of available kits (Trevigen, Inc., USA), (Aebi, 1984).

Determination of reduced glutathione (RGH) level: RGH level in cardiac and renal tissues homogenates was measured by the method of Ellman (1959). Trichloroacetic acid (5%) was added to adequate dilution of tissue homogenates (0.5 ml) to precipitate the protein content in the samples. Then, this mixture was centrifuged at 10,000 g, 5 min and the supernatant was recovered. Finally, 5, 5'-dithiobis (2-nitrobenzoic acid) solution was added to the reaction mixtures, and the absorbance was recorded at 412 nm using spectrophotometer.

Determination of lipid peroxide level: Lipid peroxidation was determined by estimating the level of thiobarbituric acid reactive substances measured as malondialdehyde (MDA), according to the method of Uchiyama and Mihara (1978). Briefly, the reaction mixture (0.5 ml homogenate + 2.5 ml 20% trichloroacetic acid + 1.0 ml 0.6% thiobarbituric acid) was heated for 20 min in a boiling water bath followed by cooling and addition of 4 ml n-butanol with shaking. The alcohol layer was separated by centrifugation at 2000 g for 10 min and absorbance was measured at 535 nm. The results were expressed as nmol MDA/g wet tissue using 1,1,3,3-tetraethoxypropane as standard.

Determination of Nitric oxide (NO) content: Using the Griess reaction (Griess, 1879), the accumulated nitrite was measured in the cardiac and renal tissue homogenate as an indicator of NO production. Briefly, 0.3 ml of colon homogenate was mixed with an equal volume of 1% (w/v) sulfanilamide [in 5% (v/v) phosphoric acid] and 0.1% (w/v) N-1-naphthylethylenediamine dihydrochloride, and incubated at room temperature for 10 min. The absorbance at 548 nm was measured. Sodium nitrite serial dilution standard curve was used to calculate concentrations in the samples. All samples were analyzed in triplicate.

#### Cardiac and renal histopathological analysis:

Specimens from cardiac and renal tissues were cleared in xylene and embedded in paraffin at 56 degrees in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness and stained by hematoxylin and eosin stains for histopathological examination under light microscope (Banchroft *et al.*, 1996).

#### Statistical analysis

Results were expressed as Mean ± Standard Error of the Mean (SEM) of six animals and the different groups were compared using one-way analysis of variance followed by Tukey-Kramer test for multiple comparisons. Prism (version5) was used.

#### Results

#### Protective effects of vanillin on electro-cardiographic changes in isopretrenol- induced myocardial infarction in rats

Figure (1) showing lead II ECG trace pattern of: (A) normal rat showing regular ECG pattern with defined P, QRS, and T waves, (B) isoprenaline treated rat showing positive T wave, ST segment elevation, and decreased R wave amplitude, (C) vanillin (150mg/kg) treated rat showing regular ECG pattern, (D) MI + vanillin (150mg/kg) pretreated rat showing a marked decrease in ST segment elevation and an increase in R wave amplitude; (E) vanillin (300mg/kg) treated rat showing regular ECG pattern and (F) MI+ Vanillin (300mg/kg) pretreated rat showing a decrease in ST segment elevation and an increase in R wave amplitude.

#### Protective effects of vanillin on heart index in isopretrenol- induced myocardial infarction in rats:

Our findings in table (3) indicated that in MI group heart index was increased by 43.29% compared with the control group, (the ratio of heart/body weight is an index of cardiac hypertrophy) while pretreatment of MI with vanillin in both doses (150mg/kg and 300mg/kg) significantly reduced cardiac hypertrophy as evidenced by reduction of heart/body weight by 26.20 % and 13.10% respectively compared with MI group.

#### Protective effects of vanillin on serum cardiac biomarkers in isopretrenol- induced myocardial infarction in rats

Our findings in table (1) indicated that in MI group all cardiac biomarkers cTn-I, CK-MB and LDH were increased by 51.4%, 135.5% and 187.5%, respectively compared with control group. While pretreatment of MI with vanillin in both doses (150mg/kg and

300mg/kg), significantly reduced cardiac biomarkers cTn-I by 47.70% and 74.43%, respectively, CK-MB by 188.82% and 140.74%, respectively and LDH by 43.93% and 48.52%, respectively, compared with MI group. In addition pretreatment of MI with vanillin in a dose of 300mg/kg, significantly reduced cTn-I and CK-MB by 51.11% and 25.46%, respectively compared with MI group pretreated with vanillin in a dose of 150mg/kg.

#### **Protective effects of vanillin on kidney function tests in isopretrenol- induced myocardial infarction in rats**

Our findings in table (2) indicated that in MI group serum renal biomarkers BUN and serum creatinine were increased by 67.92%, 61.76% respectively compared with control group. While pretreatment of MI with vanillin in both doses (150mg/kg and 300mg/kg), significantly reduced BUN by 30.22% and 34.49%, respectively and serum creatinine by 62.98% and 63.55%, respectively, compared with MI group.

In addition to that, our findings in table (2) indicated that MI induced significant decrease in urine volume, but significantly increased urinary creatinine and urinary urea by 65.1%, 76.43% and 64.72%, respectively compared with control group. While pretreatment of MI with vanillin in both doses (150mg/kg and 300mg/kg), significantly increased urine volume by 32.09% and 44.85%, respectively and significantly decreased urinary creatinine by 33.62% and 38.00% respectively. Also they induced significant decrease in urinary urea by 26.54% and 37.69% respectively, compared with MI group.

#### **Protective effects of vanillin on glomerular filtration rate (GFR) and renal clearance of creatinine (Ccr) and urea (Ucr) in isopretrenol-induced myocardial infarction in rats**

Our findings in figure (2) indicated that MI induced significant decrease in GFR, Ccr and Ucr by 33.25%, 33.93% and 36.21%, respectively compared with control group. While pretreatment of MI with vanillin in both doses (150mg/kg and 300mg/kg), significantly increased GFR by 42.12% and 38.98%, respectively, Ccr by 49.33% and 49.61%, respectively and Ucr by 42.31% and 38.28%, respectively compared with MI group.

#### **Protective effects of vanillin on cardiac and renal catalase (CAT) activity in isopretrenol-induced myocardial infarction in rats**

Our findings in table (3 and 4) indicated that in MI group cardiac and renal CAT was decreased by 36.20% and 34.88%, respectively compared with control group. While pretreatment of MI with vanillin in a dose of (150mg/kg), significantly increased cardiac CAT by 56.97% and renal CAT by 46.17%, respectively compared with MI group. While pretreatment of MI with vanillin in a dose of 300mg/kg, didn't produce any significant variation in cardiac or renal CAT compared with MI group. In addition pretreatment of MI with vanillin in a dose of 150mg/kg, significantly increase cardiac CAT by 54.21% and renal CAT by 53.21%, respectively compared with MI group pretreated with vanillin in a dose of 300mg/kg.

#### **Protective effects of vanillin on cardiac and renal reduced glutathione (RGH) content in isopretrenol-induced myocardial infarction in rats**

Our findings in table (3 and 4) indicated that in MI group cardiac and renal RGH were decreased by 36.25% and 16.89% respectively compared with control group. While pretreatment of MI with vanillin in both doses (150mg/kg and 300mg/kg), significantly increased cardiac RGH by 56.88% and 17.56%, respectively and renal RGH by 26.36% and 24.50%, respectively, and compared with MI group. In addition pretreatment of MI with vanillin in a dose of 150mg/kg, significantly increase renal RGH by 33.44% compared with MI group pretreated with vanillin in a dose of 300mg/kg.

#### **Protective effects of vanillin on cardiac and renal malondialdehyde (MDA) content in isopretrenol-induced myocardial infarction in rats**

Our findings in table (3 and 4) revealed that in MI group cardiac and renal MDA were increased by 67.68%, 63.42% respectively compared with control group. While pretreatment of MI with vanillin in both doses (150mg/kg and 300mg/kg), significantly decreased cardiac MDA by 35.18% and 29.84%, respectively and renal MDA by 56.83% and 28.90%, respectively, and compared with MI group.

#### **Protective effects of vanillin on cardiac and renal nitric oxide (NO) content in isopretrenol-induced myocardial infarction in rats**

Our findings in table (3 and 4) revealed that in MI group cardiac and renal NO were increased by 109.67%, 51.7% respectively compared with control group. While pretreatment of MI with vanillin in both doses (150mg/kg and 300mg/kg), significantly decreased cardiac NO by 29.40% and 51.58%, respectively and renal NO by 34.46% and 37.40%, respectively, compared with MI group.

#### **Protective effects of vanillin on cardiac histopathological changes in isopretrenol-induced myocardial infarction in rats**

Figure (3) Photomicrograph of longitudinal section from heart of rat in (A) control group showing no abnormal histopathological findings in myocardial muscle bundles, (B) MI group showing focal inflammatory cells infiltration detected in the degenerated (*myd*) and hyalinized (*myh*) myocardial bundles, while (C) vanillin (150 mg/kg) group showing no abnormal histopathological alterations in myocardial muscle bundles, while (D) MI + vanillin (150 mg/kg) group showing focal inflammatory cells infiltration in the degenerated myocardium (*myd*). In addition (E) vanillin (300 mg/kg) group, the myocardial bundles showed mild congestion in the blood vessels (v), and (F) MI + vanillin (300 mg/kg) Oedema (o) with inflammatory cells (m) infiltration were detected in the subendocardium tissue.

### Protective effects of vanillin on renal histopathological changes in isopretrenol-induced myocardial infarction in rats

Figure (4) Photomicrograph of longitudinal section from kidney of rat in (A) control group showing no abnormal histopathological findings observed in the glomeruli and tubules at the cortex as well as in the tubules at the corticomedullary junction, (B) MI group showing swelling and vacuolization in the lining endothelium of the glomerular tufts (g) associated with degenerative change in the lining epithelium of the tubules at the cortex (d), while (C) vanillin (150 mg/kg) group showing no abnormal histopathological alteration, while (D) MI + vanillin (150 mg/kg) group showing perivascular inflammatory cells aggregations surrounding the congested blood vessels (v). In addition (E) in vanillin (300 mg/kg) group, mild congestion was detected in the tufts of the glomeruli (g), finally (F) MI + vanillin (300 mg/kg) no abnormal histopathological alterations were recorded.

**Table (1): Protective effects of vanillin on serum cardiac biomarkers in isopretrenol- induced myocardial infarction in rats**

Groups Parameter (Mean±SEM)	Control	MI	Vanillin (150mg/kg)	MI+Van (150mg/kg)	Vanillin (300mg/kg)	MI+Van (300mg/kg)
cTn-I (ng/dl)	0.776 ± 0.042	8.357 ± 0.261 <sup>a</sup>	1.168 ± 0.0149 <sup>b</sup>	4.367 ± 0.272 <sup>a,b,c</sup>	1.550 ± 0.028 <sup>a,b,d</sup>	2.135 ± 0.122 <sup>a,b,c,d</sup>
Serum CK-MB (ng/dl)	2.045 ± 0.198	16.97 ± 0.713 <sup>a</sup>	4.815 ± 0.192 <sup>a,b</sup>	9.092 ± 0.324 <sup>a,b,c</sup>	6.592 ± 0.143 <sup>a,b,c,d</sup>	6.777 ± 0.2552 <sup>a,b,c,d</sup>
Serum LDH (U/L)	99.70 ± 1.762	287.5 ± 12.46 <sup>a</sup>	102.6 ± 6.008 <sup>b</sup>	161.2 ± 14.81 <sup>a,b,c</sup>	105.3 ± 3.790 <sup>b,d</sup>	148.0 ± 14.8 <sup>a,b,c,e</sup>

Data are expressed as mean ± SEM (n=6), a, b, c, d or e: indicates significantly different from the control, myocardial infarction, vanillin (150mg/kg), MI+ vanillin (150mg/kg) and vanillin (300mg/kg) group, respectively at P<0.05 using one way ANOVA followed by Tukey–Kramer as a post hoc test.

**Table (2): Protective effects of vanillin on kidney function tests in isopretrenol- induced myocardial infarction in rats**

Groups Parameter (Mean±SEM)	Control	MI	Vanillin (150mg/kg)	MI+Van (150mg/kg)	Vanillin (300mg/kg)	MI+Van (300mg/kg)
BUN (mg/dl)	20.39 ± 1.097	34.24 ± 2.291 <sup>a</sup>	22.26 ± 1.961 <sup>b</sup>	23.89 ± 2.105 <sup>b</sup>	18.70 ± 1.238 <sup>b</sup>	22.36 ± 1.800 <sup>b</sup>
Serum creatinine (mg/dl)	1.849 ± 0.08	2.991 ± 0.08 <sup>a</sup>	1.856 ± 0.09 <sup>b</sup>	1.884 ± 0.06 <sup>b</sup>	1.832 ± 0.11 <sup>b</sup>	1.901 ± 1.10 <sup>b</sup>
Urine volume (ml)	8.217 ± 0.18	5.350 ± 0.24 <sup>a</sup>	8.417 ± 0.27 <sup>b</sup>	7.067 ± 0.20 <sup>b</sup>	8.533 ± 0.31 <sup>b,d</sup>	7.750 ± 0.40 <sup>b</sup>
Urinary creatinine (mg/dl)	112.00 ± 3.88	197.61 ± 12.22 <sup>a</sup>	110.30 ± 7.65 <sup>b</sup>	131.17 ± 9.29 <sup>b</sup>	110.74 ± 5.86 <sup>b</sup>	122.5 ± 6.95 <sup>b</sup>
Urinary urea (g/dl)	3.43 ± 0.26	5.65 ± 0.44 <sup>a</sup>	3.55 ± 0.31 <sup>b</sup>	4.15 ± 0.40 <sup>b</sup>	3.25 ± 0.26 <sup>b</sup>	3.52 ± 0.34 <sup>b</sup>

Data are expressed as mean ± SEM (n=6), a or b: indicates significantly different from the control and myocardial infarction group, respectively at P<0.05 using one way ANOVA followed by Tukey–Kramer as a post hoc test.

### Figure (2): Protective effects of vanillin on renal function tests in isopretrenol- induced myocardial infarction in rats

- Protective effects of vanillin on GFR in isopretrenol- induced myocardial infarction in rats
- Protective effects of vanillin on Ccr in isopretrenol- induced myocardial infarction in rats
- Protective effects of vanillin on Ucr in isopretrenol- induced myocardial infarction in rats

Data are expressed as mean ± SEM (n=6).

a, b, c, d or e: indicates significantly different from the control, myocardial infarction, vanillin (150mg/kg), MI+ vanillin (150mg/kg) and vanillin (300mg/kg) group, respectively at P<0.05 using one way ANOVA followed by Tukey–Kramer as a post hoc test.

**Table (3): Protective effects of vanillin on heart index and cardiac antioxidant parameters in isopretrenol-induced myocardial infarction in rats**

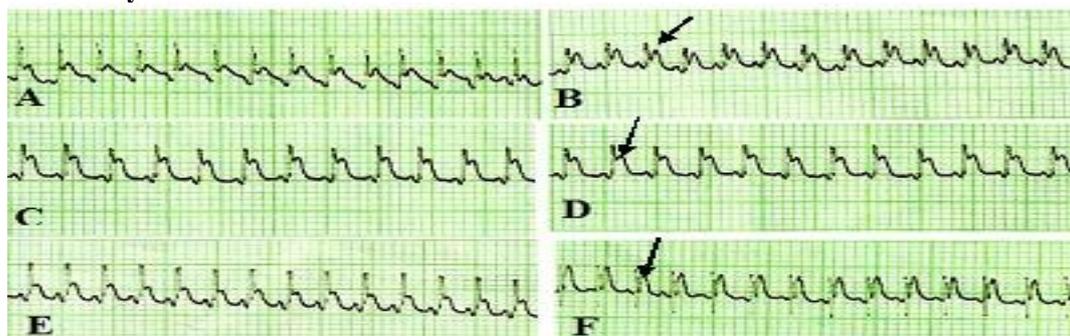
Parameter (Mean±SEM)	Control	MI	Vanillin (150mg/kg)	MI+Van (150mg/kg)	Vanillin (300mg/kg)	MI+Van (300mg/kg)
Heart Index (g)	0.261 ± 0.004	0.374 ± 0.004 <sup>a</sup>	0.277 ± 0.006 <sup>b</sup>	0.276 ± 0.008 <sup>b</sup>	0.272 ± 0.002 <sup>b</sup>	0.325 ± 0.007 <sup>a,b,c,d,e</sup>
CAT (U/g tissue)	4.408 ± 0.338	2.812 ± 0.206 <sup>a</sup>	4.361 ± 0.243 <sup>b</sup>	4.409 ± 0.334 <sup>b</sup>	4.182 ± 0.466 <sup>b</sup>	2.859 ± 0.088 <sup>a,c,d,e</sup>
RGH (nmol/ g tissue)	7.86 ± 0.184	5.01 ± 0.176 <sup>a</sup>	10.92 ± 0.088 <sup>a,b</sup>	7.86 ± 0.317 <sup>b,c</sup>	7.48 ± 0.414 <sup>b,c</sup>	5.89 ± 0.134 <sup>a,c,d,e</sup>
MDA ( nmol/g tissue)	42.42 ± 3.78	71.13 ± 6.24 <sup>a</sup>	39.03 ± 3.14 <sup>b</sup>	46.10 ± 1.80 <sup>b</sup>	43.02 ± 4.27 <sup>b</sup>	49.90 ± 4.02 <sup>b</sup>
NO (µM)	21.70 ± 1.35	45.50 ± 4.54 <sup>a</sup>	21.17 ± 1.19 <sup>b</sup>	32.12 ± 2.56 <sup>b</sup>	21.83 ± 2.10 <sup>b</sup>	22.03 ± 2.17 <sup>b</sup>

Data are expressed as mean ± SEM (n=6), a, b, c, d or e: indicates significantly different from the control, myocardial infarction, vanillin (150mg/kg), MI+ vanillin (150mg/kg) and vanillin (300mg/kg) group, respectively at P<0.05 using one way ANOVA followed by Tukey–Kramer as a post hoc test.

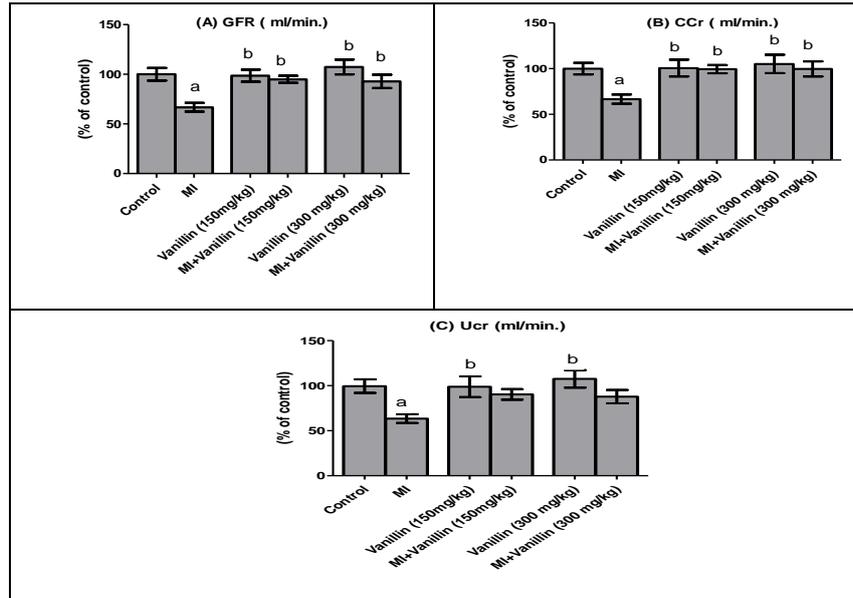
**Table (4): Protective effects of vanillin on renal antioxidant parameters in isopretrenol-induced myocardial infarction in rats**

Parameter (Mean±SEM)	Control	MI	Vanillin (150mg/kg)	MI+Van (150mg/kg)	Vanillin (300mg/kg)	MI+Van (300mg/kg)
CAT (U/g tissue)	6.286 ± 0.17	4.093 ± 0.09 <sup>a</sup>	6.520 ± 0.31 <sup>b</sup>	5.983 ± 0.25 <sup>b</sup>	6.146 ± 0.21 <sup>b</sup>	3.905 ± 0.14 <sup>a,c,d,e</sup>
RGH (nmol/ g tissue)	19.35 ± 0.34	16.08 ± 0.28 <sup>a</sup>	22.84 ± 0.26 <sup>a,b</sup>	20.32 ± 0.36 <sup>b,c</sup>	21.32 ± 0.79 <sup>b</sup>	20.02 ± 0.33 <sup>b,c</sup>
MDA (nmol/g tissue)	43.80 ± 4.09	71.58 ± 4.69 <sup>a</sup>	35.73 ± 3.37 <sup>b</sup>	30.90 ± 2.95 <sup>b</sup>	30.60 ± 1.76 <sup>b</sup>	51.47 ± 4.30 <sup>b,e</sup>
NO (µM)	85.80 ± 4.76	130.21 ± 11.56 <sup>a</sup>	83.87 ± 2.15 <sup>b</sup>	85.33 ± 3.41 <sup>b</sup>	75.43 ± 4.50 <sup>b</sup>	81.50 ± 6.62 <sup>b</sup>

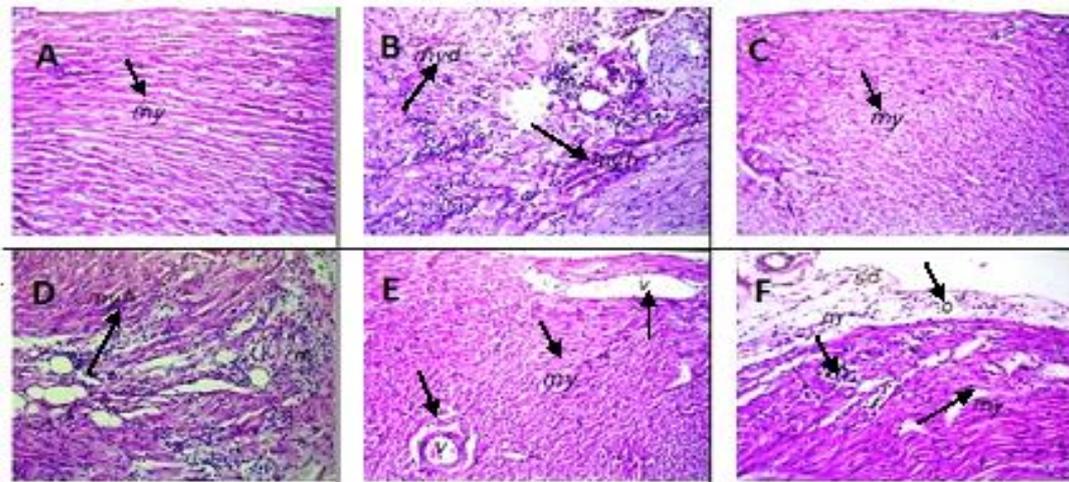
Data are expressed as mean ± SEM (n=6), a, b, c, d or e: indicates significantly different from the control, myocardial infarction, vanillin (150mg/kg), MI+ vanillin (150mg/kg) and vanillin (300mg/kg) group, respectively at P<0.05 using one way ANOVA followed by Tukey–Kramer as a post hoc test.

**Figure (1): Protective effects of vanillin on electro-cardio graphic changes in isopretrenol-induced myocardial infarction in rats**

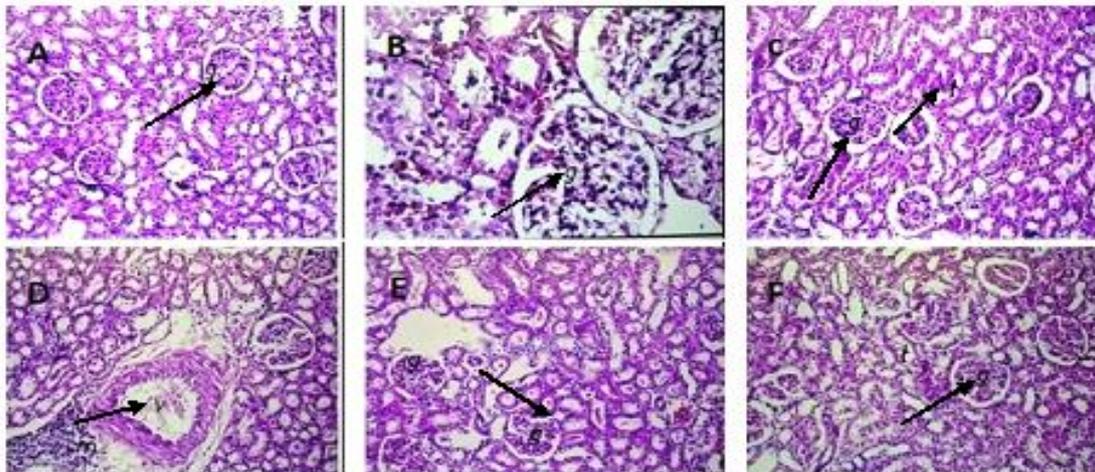
**Figure (2): Protective effects of vanillin on renal function tests in isopretrenol- induced myocardial infarction in rats**



**Figure (3): Protective effects of vanillin on cardiac histopathological changes in isopretrenol- induced myocardial infarction in rats**



**Figure (4): Protective effects of vanillin on renal histopathological changes in isopretrenol- induced myocardial infarction in rats**



## Discussion

The main criteria generally used for the definite diagnosis of MI are the evolving pattern of Electrocardiograph (ECG) abnormalities. Several studies have demonstrated that isoprenaline administration produce marked hemodynamic alterations manifested in the form of systolic or diastolic dysfunction and increased heart rate (Goyal et al., 2010). The adrenergic receptors stimulation due to overproduction of catecholamines is known to be a major cause of stress-induced cardiac dysfunction (Shao et al., 2013).

Beta-blocker therapy plays a major role in the pretreatment of cardiovascular diseases. They are used for their anti-ischemic, anti-arrhythmic and antihypertensive properties (Davel et al., 2014). Anti-ischemic action of beta-blockers decreases myocardial oxygen demand by reducing heart rate, cardiac contractility, and systolic blood pressure.

During the acute phase of MI, beta-blockers limit infarct size, reduce life-threatening arrhythmias, relieve pain and reduce mortality including sudden cardiac death (Srivastava et al., 2007). Vanidilol, newly synthesized from vanillin, is a vanilloid-type beta-adrenoceptor blocker. It inhibits the tachycardiac effects induced by isoproterenol (Wu et al., 1994). Thus, vanillin can be used as a protective agent of MI as an adjuvant. In the present study, we noted a significant increase in heart rate and elevation of ST-segments in isoprenaline-induced rats. The observed ST-segment elevation might be due to myocardial necrosis accelerated by isoprenaline, which is consistent with the observations of earlier reports (Patel et al., 2010).

Pretreatment with vanillin markedly inhibited isoproterenol-induced tachycardia and ST-segment elevation, suggestive of its cell membrane protecting effects. In addition, ECG monitoring of isoprenaline-treated rats showed positive T-wave and ST segment elevation coupled with marked decrease in R wave amplitude that reflect isoprenaline-induced myocardial ischemia and infarction. ECG pattern alterations by isoprenaline were previously demonstrated by previous investigators (Prince and Sathya, 2010). Pretreatment with vanillin produced a marked protection against isoprenaline-induced myocardial damage. Indeed, the suppression of MI by vanillin was reflected in the current investigation through the decrease in heart rate, suppression of ST-segment elevation and the marked increase in R wave amplitude upon ECG monitoring as compared with the isoprenaline group.

Rats treated with isoprenaline have been reported to undergo increase in heart weight. Hypertrophy of hearts as well as cardiomyocytes was also observed in our rat model. One postulate about increased heart wet weight is due to increase in water content and development of oedema in intramuscular spaces culminating in extensive necrotic changes and invasion of inflammatory cells (Patel et al., 2010). Also, Cardiac hypertrophy was shown to be induced by

isoprenaline via  $\beta_1$ -adrenergic signaling (Patterson et al., 2004), but the pretreatment of MI group with vanillin improved these changes in heart index.

Myocardial cells contain several enzymes and macromolecules which on metabolic damage are released in the extracellular fluid and serve as diagnostic markers of myocardial injury (Ojha et al., 2008). The release of these enzymes reflects an alteration in the plasma membrane integrity and permeability in response to  $\beta$ -adrenergic stimulation (Goyal et al., 2010). Measuring of cTn-I level, CK-MB, and LDH activities are necessary to ascertain extent of myocardial injury. In the present study, isoprenaline administration caused a rise in the level of these cardiac diagnostic marker enzymes, due to leakage from the tissues to blood serum as a result of damaged or destroyed cardiomyocytes, as well as, the cells damaged because of insufficient supply of oxygen and oxidative damage of myocardium which render the cell membrane fragile, porous, or ruptured. The increased levels of these enzymes are indicative of severity of cell necrosis and isoprenaline mediated peroxidative myocyte injury (Heraldo et al., 2011). These observations are in accordance with previous studies performed on rats treated with isoproterenol (Wang et al., 2009).

Pretreatment of MI group with vanillin in both doses reduced the serum levels of cTn-I, CK-MB, and LDH activities compared to the histopathological changes in isoprenaline administered rats. The histopathological preservation and inhibition of lipid peroxidation due to antioxidant properties of vanillin (Makni et al., 2011), could be correlated with the reduced leakage of myocardial enzymes in serum. It can be inferred that vanillin might have preserved cell integrity and stabilized the myocardial membrane which restricts the leakage of these marker enzymes from the heart into blood.

Several studies have disclosed a complex relationship between cardiovascular and renal disease. Impaired renal function is detrimental for the heart (renocardiac interaction) both in clinical (Anavekar et al., 2004) and in experimental (Amann et al., 2003) settings. Impaired cardiac function is detrimental to the kidney (cardiorenal interaction), which is less well characterized in experimental settings.

Coexistence of renal and cardiac dysfunction, known as cardiorenal syndrome, has an adverse impact on clinical outcomes following AMI. Approximately one third of hospitalized AMI patients present with coexisting kidney dysfunction (Anavekar et al., 2004) and one fifth develop worsening renal function during hospitalization (Parikh et al., 2008). Lekawanvijit et al. (2012) demonstrated an experimental model of MI that worsening renal function which occur early post-MI, may be transient and is strongly related to activation of renal inflammatory-fibrosis pathways which leads to nonreversible functional impairment. Yujung et al. (2012) stated that vanillin has an anti-inflammatory effect, which can interpret the protective effect of

vanillin in kidney diseases resulted after MI. In addition the protective effects of vanillin were evaluated against carbon tetrachloride (CCl<sub>4</sub>)-induced kidney damages in rats, as evidenced by increased plasma creatinine, urea and uric acid levels, increased lipid peroxidation (MDA). While pretreatment of rats with vanillin (150 mg/kg/day, i.p.), for 3 consecutive days before CCl<sub>4</sub> injection, protected the kidneys against this damage (Makni et al., 2012).

The animal model of isoprenaline-induced myocardial injury recapitulates major metabolic and morphological changes similar to those occurring in human MI (Rona, 1985). Auto-oxidation of isoprenaline, which was used for induction of MI in our study, produces quinones, which react with oxygen to produce superoxide anions and hydrogen peroxide, leading to oxidative stress and depletion of the endogenous antioxidant system. Free radical scavenging enzymes, such as super oxide dismutase (SOD), CAT and RGH are the first line of cellular defense against oxidative injury, decomposing O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> before their interaction to form the more reactive hydroxyl radical (Wattanapitayakul and Bauer, 2001). The equilibrium between the enzymatic antioxidants and free radicals is an important process for the effective removal of oxidative stress in intracellular organelles (Senthila et al., 2004). However, in pathological conditions like MI, the generation of ROS can dramatically upset this balance with increased demand on the antioxidant defense system (Wu et al., 2009).

Vanillin(4-hydroxy-3-methoxybenzaldehyde), a compound isolated from the bean and pod of tropical vanilla orchid is widely used in the food and beverage industry and is responsible for the characteristic vanilla flavor (Oliveira et al., 2014). Besides its industrial and food application this compound has been the subject of several scientific investigations in the past few years, for the identification of antioxidant properties (Tai et al., 2011). Part of these biological properties can be attributed to the fact that vanillin is a phenolic compound. The antioxidant activity of phenols is attributed to their ability to scavenge free radicals (Surjadinata and Cisneros-Zevallos, 2012).

Myocardial infarction (MI) is characterized by cardiac dysfunction, lipid peroxidation, altered activities of cardiac injury markers, and depletion of endogenous antioxidants (Ojha et al., 2012). Furthermore, it has also been documented that the heart is highly susceptible to oxidative stress compared with other tissues due to lower activity of antioxidant defense in the heart tissues (Hrelia et al., 2004). The endogenous antioxidant defense network constitutes enzymatic (SOD and CAT) and non-enzymatic (RGH) molecules to neutralize the ROS mediated tissue injury in oxidative stress (Oyanagui, 1984). SOD catalyzes the dismutation of superoxide anions to oxygen and H<sub>2</sub>O<sub>2</sub>, which is further detoxified by CAT to water. Among several mechanisms proposed for isoprenaline-induced MI, production of highly cytotoxic-free radicals through autooxidation and disturbed

physiological balance between production of free radicals and antioxidative defense is widely accepted (Rathore et al., 1998). The decrease in activities of SOD and CAT following isoprenaline administration demonstrates overwhelming increase of free radicals, superoxide, and hydrogen peroxide which causes cellular injury. Vanillin pretreatment prevented decline of the myocardial CAT activities in isoprenaline administered rats.

Reduced glutathione (RGH) is a tripeptide which has a direct antioxidant function by reacting with superoxide radicals, peroxy radicals and singlet oxygen followed by the formation of oxidized RGH and other disulfides (Rathore et al., 1998). Thus, reduction in cellular RGH content could impair recovery after a short period of ischemia. In this study, isoprenaline administration was found to reduce the levels of RGH in cardiac tissue and the observation concurs with several earlier findings (Patel et al., 2010). Pretreatment with vanillin increased the level of RGH in the heart of isoprenaline-induced cardiotoxic rats when compared with individual treatment groups.

Lipid peroxidation has been defined as the oxidative deterioration of polyunsaturated lipid. It occurs constantly at a low level in most cellular biological systems. Oxygen-derived free radicals can react with lipids, if not blocked by sufficient antioxidant molecules, to form lipid peroxides which cause extensive damage (Tappel and Dillard, 1981). Since the major constituents of biological membranes are lipids, their peroxidation can lead to cell damage and death. A significant increase in the levels of lipid peroxidation products in isoprenaline -induced rats appear to be the initial stage to the tissue making it more susceptible to oxidative damage. Increased production of free radicals may be responsible for the observed membrane damage as evidenced by the elevated lipid peroxidation in terms of TBARS in the present study. The decrease in MDA level following pretreatment with vanillin can be ascribed to the enhanced activities of antioxidant status in myocardium. The ability of vanillin as a potent free radical scavenger and explain antioxidant may affect cardiac function and explicate its potential as a cardioprotective agent. The improved myocardial antioxidant status following vanillin treatment may presume to translate into the recovery of cardiac functions altered during isoprenaline -induced MI.

Nitric oxide (NO) level was also found to be increased in isoprenaline treated rats. It has been reported that inducible nitric oxide synthase (iNOS) expression and NO production increase in the myocardial infarcted heart (Pinto et al., 2007).  $\beta$ -Adrenergic stimulation also upregulated iNOS and significantly increases production of NO (Li et al., 2006). Increased NO concentration creates a nitrosative stress in presence of other reactive oxygen species (ROS) such as superoxides and generates the powerful oxidant molecule peroxynitrite (ONOO<sup>-</sup>). In our study, vanillin pretreatment in both doses prevented the rise of NO level in isoprenaline treated rats. Antioxidants

constitute the defense mechanism that limits the free radicals which initiate damage in tissues. A possible explanation for the decrease in NO production by vanillin may be mediated by its ability to inhibit iNOS expression through inhibition of nuclear factor-kappa B pathway, which further accounts for its anti-inflammatory potential (Makni et al., 2011).

Histopathologic examination of isoprenaline-treated rats revealed edema, infiltration of inflammatory cells along with myocyte degeneration and renal impairment. These findings are in accordance with previous studies (Kannan and Quine, 2013). However, vanillin pretreatment to isoprenaline-challenged rats has shown resistance towards necrosis, edema, and inflammation. It also protected cardiomyocytes and renal cells from the deleterious effects of isoprenaline. Rats which received vanillin pretreatment exhibited a normal myocardial and renal histology, which is suggestive of the fact that vanillin at this dose does not produce any significant adverse effects on both myocardium and kidney tissues.

Regarding mortality, the resulting data from our study showed a mortality of 25% in the MI group. This value is consistent with data found in literature, pointing to the isoproterenol-induced MI as a cause of mortality in this experimental model. Acikel et al. (2005) reported a mortality rate of 33.33% in rats after receiving isoproterenol, while pretreatment of MI group with both doses of vanillin decreased rate of mortality.

## Conclusion

In summary, the present study strongly demonstrates that a diversity of mechanisms may be responsible for the cardio- and reno- protective effects of vanillin. These beneficial potentials may be translated into improvement in heart weight/body weight ratio and reduced serum levels of myocyte marker enzymes along with restoration of antioxidants with concomitant reduction in reactive oxygen species in both cardiac and renal tissues. In addition to the improvement in renal biochemical parameters, vanillin significantly preserved the myocardial and renal histoarchitecture. In conclusion, the present study provides a scientific rationale of the utility value of vanillin. However, further well-controlled prospective clinical studies need to be performed to ascertain whether the present findings can be applied in the protection of human susceptible to ischemic heart diseases.

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### الملخص العربي

## التأثيرات المحتملة الواقية للقلب والكلية لمادة الفانيلين على إحتشاء عضلة القلب باستخدام الأيزوبروترنول في الجرذان

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يستمر احتشاء عضلة القلب هو سبب وجود مشكلة صحية عامة رئيسية في العالم. ويعد الفانيلين هو مركب الفينول الطبيعي الذي يمتلك فعالية هامة في مضادة الأكسدة ومضادة الإلتهابات. هذه الدراسة تهدف إلى تحقيق آثار وقائية للفانيلين (١٥٠ مجم / كجم)، والفانيلين (٣٠٠ مجم / كجم) على أعراض إحتشاء عضلة القلب والتي يسببها الأيزوبرينالين في الجرذان. تضمنت الدراسة اختياراتعلامات تقييم الضرر المحدث بالقلب لنشاط المصل من الكرياتين كيناز واللاكتات ديهيدروجيناز، بالإضافة إلى مستوى مصل القلب تروبونين-I. وكذلك النشاط المضاد للأكسدة للكاتالاز، وكذلك محتويات القلب من البيروكسيدات الدهنية والجلوتاثيون المختزل والنيترت في القلب. وعلاوة على ذلك تم القيام بقياس تخطيط القلب عن طريق جهاز رسم القلب وكذلك إجراء الفحوص النسيجية لأنسجة القلب. وبالإضافة إلى ذلك تضمنت الدراسة اختيار علامات تقييم القصور الكلوي عن طريق قياس مستويات الكرياتينين واليوريا في الدم والبول، وأيضا معدل إزالة الكرياتينين ومعدل إزالة اليوريا ومعدل الترشيح الكبيبي. وبالإضافة إلى ذلك تم قياس النشاط المضادة للأكسدة للكاتالاز بالكلية ومحتويات الكلية من البيروكسيدات الدهنية والجلوتاثيون المختزل والنيترت. وعلاوة على ذلك، تم القيام بإجراء الفحوص النسيجية للكلية. وقد تسبب الأيزوبرينالين في زيادة النشاط المصلي من الكرياتين كيناز واللاكتات ديهيدروجيناز بالإضافة إلى مستوى مصل القلب تروبونين-I، وزيادة المؤشرات الحيوية لحدوث أكسدة في القلب والكلية. وبالإضافة إلى ذلك، فإنه أدى لحدوث إرتفاع في شريحة ال س- ت والتغيرات التنكسية في القلب وأنسجة الكلية. وقد أدت المعالجة بالفانيلين بالجرعات السابقة بإحداث تغييرات بشكل كبير في فعل الأيزوبرينالين في مستويات النشاط المصلي من الكرياتين كيناز واللاكتات ديهيدروجيناز، بالإضافة إلى مستوى مصل القلب تروبونين-I. إلى جانب الحد من علامات الإجهاد التأكسدي القلب والكلية. وعلاوة على ذلك نتج تحسنا ملحوظا في رسم القلب والتعديلات النسيجية في أنسجة كل من القلب والكلية. في الختام يمكن إستنتاج انه يمكن اعتبار الفانيلين كمنتج من الطبيعة أن له تأثير وقائي على القلب والكلية من إحتشاء عضلة القلب.