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Research Article
Bisacurone Ameliorated Pressure Overload-Induced Cardiac Hypertrophy in Experimental Rats Through Inhibition of Oxidative Stress and Bax/Caspase-3 Pathway

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Abstract

Background and Objective: The sustained Left Ventricular Hypertrophy (LVH) causes cardiac function failure, resulting in Congestive Heart Failure (CHF) and mortality. Bisacurone, a bioactive terpenoid, has been reported for its antioxidant and anti-inflammatory potential. To determine the cardioprotective efficacy of bisacurone against pressure overload-induced cardiac hypertrophy in a rat model of Aortic Stenosis (AS). **Materials and Methods:** Constriction of abdominal aortic of Sprague-Dawley rats caused induction of pressure overload-induced cardiac hypertrophy. Then rats were treated with either vehicle (AS) group or lisinopril (15 mg kg^{-1}) or bisacurone (25, 50 and 100 μg kg^{-1}, p.o.) for 4 weeks. **Results:** AS control group showed a significant ($p<0.05$) in alteration hemodynamic and left ventricular function tests compared to sham control rats, however bisacurone (50 and 100 μg kg^{-1}) treatment markedly ($p<0.05$) inhibited these alterations. Bisacurone effectively ($p<0.05$) augmented pressure overload-induced diminished levels of cardiac SOD, GSH, Na-K-ATPase, Ca-ATPase and mRNA expressions of Bcl2. The up-regulated MDA, NO and mRNA expressions of ANP, BNP, cTn-I, Bax and Caspase-3were efficiently ($p<0.05$) were down-regulated by bisacurone. AS-induced histological alteration in cardiac tissue was reduced by bisacurone treatment. **Conclusion:** Bisacurone treatment inhibited pressure overload-induced progressive cardiac hypertrophy by inhibiting elevated oxidative stress (SOD, GSH, MDA and NO) and apoptosis (Bax, Bcl-2 and Caspase-3). Thus, bisacurone can be considered as an important therapeutic moiety in the management of congestive heart failure.

Key words: Aortic stenosis, apoptosis, atrial natriuretic peptide, bisacurone, cardiac hypertrophy, pressure overload

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Left Ventricular Hypertrophy (LVH) is an adaptive process in response to increased pressure overload, however, sustained LVH causes failure of cardiac functions results in Congestive Heart Failure (CHF) and mortality[1-4]. A study suggested that Aortic Stenosis (AS) is one of the most common reasons for CHF, affecting approximately 3% of individuals older than 65 years, with the overall survival rate in symptomatic patients is 2-3 years[5]. It has been estimated that more than 4 M Chinese individuals are affected by CHF every year, demonstrating an approximately 30% of mortality rate over a 3-year[6]. According to the China PEACE Retrospective Heart Failure Study, the combined in-hospital mortality and treatment withdrawal rate was 3.5%[6]. Aghamohammadi *et al.*[1] reported that one out of 33 people would be suffered from CHF by 2030[1]. Although CHF is a fast-growing cardiovascular disease, it is associated with a substantial economic burden contributing $126.819 per patient as a cost of treatment. It is expected to be triple by 2030[1]. Thus, there is a need for cost-effective therapy for the management of patients with CHF.

The myocardial remodelling induced by aortic stenosis, which causes pressure overload in cardiac tissue, represents a complex phenomenon that includes alteration in myocardial contractility and hemodynamics, activation of a neurohumoral system such as renin-angiotensin system, atrial natriuretic peptide and sympathetic nervous system, elevated expressions of cytokines and apoptotic markers[3,7-10]. These biochemical and structural perturbation leads of myocardial hypertrophy and fibrosis results in myocardial stiffness and alteration in diastolic function leading to death[11,12]. Furthermore, excessive production of Reactive Oxygen Species (ROS), releases of inflammatory mediators (such as cyclooxygenase-II (COX-II) and nitric oxide (NO)) and inflammatory cytokines (including tumour necrosis factor-α (TNF-α) and interleukins (ILs)), activation of G protein-coupled receptor (GPCR) signalling and endoplasmic reticulum stress has been implicated in the pathogenesis of cardiac hypertrophy[13-16]. Furthermore, researchers have well documented the importance of Angiotensin-Converting Enzyme (ACE) during the regulation of secondary hypertension induced by impaired renal and cardiac functions[17]. The activation of vasoconstricting octapeptide angiotensin I from decapeptide angiotensin I in the presence of renal ACE cause vasoconstriction and release of aldosterone that plays a significant role in the development of long-term hypertension[13,18,19]. Additionally, pressure overload-induced cardiac apoptosis followed by necrosis increases myocardial fibrosis and alters myocardial tissue contractility. Thus, researchers have targeted the inhibition of apoptosis to offer protection against myocardial remodeling[20,21].

The current treatment regimen of CHF management includes various pharmacological interventions such as angiotensin-II receptor blockers (ARBs), β-blockers, RAS inhibitors, ACE inhibitors, antifibrogenic agents and aldosterone antagonists[22]. However, these therapeutic options are associated with a high mortality rate (50-60%) over 5-year[3,23]. The non-pharmacological intervention includes surgical replacement of aortic valve, nevertheless, these surgical interventions are associated with a high operative mortality rate and significant treatment cost[24]. Thus, there is a scarcity of safe and effective therapeutic regimens that improve outcomes during CHF. Cardiac hypertrophy induced by aortic constriction is a widely used, well-established and highly reproducible experimental animal model employed by several researchers to determine the potential of various therapeutic interventions against LV remodeling[7,20,25-27].

Bisacurone, a bioactive terpenoid from *C. longa*, has been well documented for its antioxidant and anti-inflammatory properties, exerted via the inhibition of release of pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α)[28]. Furthermore, researchers have demonstrated the hepatoprotective property of bisacurone in preclinical[29] and clinical[30] settings. Recently, Cui *et al.*[31] reported the protective efficacy of bisacurone against diabetic cardiomyopathy via inhibition of elevated cardiac oxido-nitrosative (SOD, GSH, MDA and NO) and apoptosis (Bax, Caspase-3 and Caspase-9)[31]. Therefore, in light of existing evidence, it was hypothesized that bisacurone might protect against pressure overload-induced cardiac fibrosis. Thus, to validate this hypothesis, this study aimed to investigate the potential of bisacurone against pressure overload-induced cardiotoxicity in an experimental animal model of aortic stenosis.

MATERIALS AND METHODS

Study area: The experiment was performed in the Pharmacology laboratory of the Department of Cardiology, Pidu District People's Hospital Chengdu, China, from 5th April, to 16th July, 2021. All the experiments were carried out between 0800 and 1700 hrs in a quiet laboratory environment.

Animals: Adult male Sprague-Dawley rats (200-250 g, 7-8 weeks, n = 110) were obtained from the experimental animal centre of the Third Affiliated Hospital of Chengdu

Medical College. They were maintained at 24±1°C, with a 45-55% relative humidity and a 12:12 hrs dark/light cycle. The animals had free access to standard pellet chow and water throughout the experimental protocol. All experiments were carried out between 09:00 and 17:00 hrs. The Third Affiliated Hospital approved the experimental protocol number (No. CYFYEC-C-F0605) of Chengdu Medical College. In addition, all the experimental protocols involved in this experiment were carried out according to the National Institute of Health Guide for Care and Use of Laboratory Animals and were approved by the Animal Ethics and Use.

Chemicals: Total Ribonucleic Acid (RNA) Extraction kit and One-step qualitative reverse transcriptase-polymerase chain reaction (RT-PCR) kit were purchased from MP Biomedicals India Private Limited, India.

Surgical procedure for pressure overload-induced myocardial hypertrophy: The animals were anaesthetized with sodium thiopental (35 mg kg^{-1}) intraperitoneally (i.p.). A mid-abdominal incision was made to expose the abdominal aorta. The aorta above the left renal artery was dissected and constricted at the suprarenal level using a cannula of size 0.9 into 40 mm, which was ligated with the aorta and withdrawn afterwards. In an age and body-weight-matched sham-operated rat, the abdominal aorta was isolated and placed without ligation. After surgery, the rats were administered with penicillin (200 kU/kg/day) intramuscularly (im) for 1 week to prevent infection[32].

Experimental design: One week after surgery, the AS rats were randomly divided into the following experimental groups (n = 15):

- **Aortic stenosis (AS) group:** AS rats received saline for 4 weeks, p.o.
- **Lisinopril (15):** AS rats received lisinopril (15 mg kg^{-1}, p.o.) for 4 weeks
- **Bisacurone (25) group:** AS rats received Bisacurone (25 µg kg^{-1}, p.o.) for 4 weeks
- **Bisacurone (50) group:** AS rats received Bisacurone (50 µg kg^{-1}, p.o.) for 4 weeks
- **Bisacurone (100) group:** AS rats received Bisacurone (100 µg kg^{-1}, p.o.) for 4 weeks

Other groups of rats without aortic ligation were maintained separately into the following experimental groups (n = 15):

- **Sham group:** Rats underwent surgery to isolate the abdominal aorta without its ligation and received saline for 4 weeks, p.o.
- **Per se group:** Rats did not undergo any surgery however, they received Bisacurone (100 µg kg^{-1}, p.o.) 4 weeks

The bisacurone was freshly prepared in three different dosages (25, 50 and100 µg kg^{-1})[33,34] and administered orally to all groups at a pre-fixed time once daily for 28 days. Animal care was taken following institutional guidelines. At the end of the treatment period, rats were anaesthetized with urethane injection (1.25 g kg^{-1}, i.p.) and blood was withdrawn by a retro-orbital puncture. Each blood sample was collected into separate vials for the determination of serum parameters. After blood collection and surgical procedure for the assessment of electrocardiographic, hemodynamic and Left Ventricle (LV) contractile function then animals were sacrificed by cervical dislocation, the heart was rapidly removed and stored at 80°C for biochemical (n = 6) and RT-PCR analysis (n = 6). Finally, the heart of three rats from each group was isolated and fixed for histopathological evaluation.

Serum biochemistry: Serum was separated by centrifugation using Eppendorf Microcentrifuge (model No. 5810, Germany), maintained at 4°C and run at a speed of 5488 g for 15 min. Serum Lactate Dehydrogenase (LDH), Creatine Kinase-MB (CK-MB) and alkaline phosphatase (ALP) were measured by spectrophotometer (UV/VIS spectrophotometer, Jasco V-530, Japan) using reagent kits according to the procedure provided by the manufacturer (Accurex Biomedical Pvt. Ltd., Mumbai, India).

Measurement of electrocardiographic, hemodynamic and left ventricular function: At the end of the study, animals were anaesthetized with urethane (1.25 g kg^{-1}, i.p.). Since urethane anaesthesia has minimal effects on the cardiovascular and respiratory systems and long-lasting anaesthesia with rapid onset following i.p., administration. The duration of anaesthesia is sufficient to carry out a surgical procedure and record hemodynamic and electrocardiographic parameters. A polyethylene cannula (PE 50) filled with heparinized saline (100 IU mL^{-1}) connected with a transducer bio amplifier (for signal amplification) was used to determined hemodynamic changes such as HR (Heart rate), SBP (Systolic Blood Pressure), DBP (Diastolic Blood Pressure), MABP (Mean arterial Blood Pressure) and left ventricular functions. In

Table 1: Primer sequence for cardiac ANP, BNP, cTn-I, Bax, Bcl-2, Caspase-3 and β-actin

	Sequence		
Genes	Forward primer	Reverse primer	Size (bp)
ANP	CTGCTAGACCACCTGGAGGA	AAGCTGTTGCAGCCTAGTCC	320
BNP	TGATTCTGCTCCTGCTTTTC	GTGGATTGTTCTGGAGACTG	91
cTn-I	ACTTCGCAGAGGCAGCAATCA	GGTTGCCTTGTTCTTCCTTCAG	267
Bax	GGGAATTCTGGAGCTGCAGAGGATGATT	GCGGATCCAAGTTGCCATCAGCAAACAT	96
Bcl-2	CTGTACGGCCCCAGCATGGCG	GCTTTGTTTCATGGTACATC	231
Caspase-3	CTCGGTCTGGTACAGATGTCGATG	GGTTAACCCGGGTAAGAATGTGCA	238
β-actin	GTCACCCACACTGTGCCCATCT	ACAGAGTACTTGCGCTCAGGAG	764

addition, Millar Mikro-tip transducer catheter (Model SRP-320, Millar instrument, INC 320-7051, Houston, TX) was utilized to assess left ventricular systolic pressure (LVESP) whereas left ventricular end-diastolic pressure (LVEDP), dp/dt_{max} and dp/dt_{min} estimated by using AD Instrument data acquisition system (LabChart 7.3, AD Instrument Pvt. Ltd., Australia).

Biochemical estimation

Tissue homogenate preparation: All animals were sacrificed at the end of the study, i.e., on the 29th day, the heart was immediately isolated. Tissue homogenates were prepared with 0.1 M Tris-HCl buffer (pH 7.4) and supernatant of homogenates was employed to estimate superoxide dismutase (SOD), reduced glutathione (GSH), lipid peroxidation (MDA content), nitric oxide (NO content), Na-K-ATPase and Ca-ATPase as described previously[32].

Determination of cardiac ANP, BNP, cTn-I, Bax, Bcl-2 and Caspase-3mRNAexpressionsby RT-PCR: The levels of cardiac atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), cardiac troponin (cTn-I), B-cell lymphoma 2 (Bcl-2), Bcl-2-associated X protein (Bax) and Caspase-3 messenger ribonucleic acid (mRNA) were analyzed using qualitative RT-PCR as described previously[32]. The primer sequence was provided in Table 1.

Histopathological evaluation: The isolated tissue was trimmed into small pieces and preserved in 10% formalin for 24 hrs. Specimens were cut in sections of 3-5 m in thickness by microtome and stained by hematoxylin-eosin. The samples were mounted by Disterene Phthalate Xylene (DPX). For myocardial fibres staining, Yuccafine™ Masson's trichrome staining kit (Yucca Diagnostics, India) was used. Each tissue section's photomicrographs were observed using Cell Imaging software for Life Science microscopy (Olympus Soft Imaging Solution GmbH, Munster, Germany). Microscopic scoring was performed by an experienced histologist, unaware of the treatment groups as described previously[20,35].

Statistical analysis: Data were expressed as Mean±Standard Error Mean (SEM). Data analysis was performed using Graph Pad Prism 5.0 software (Graph Pad, San Diego, CA, USA). Data were analyzed by one-way analysis of variance (ANOVA) and Tukey's multiple range tests were applied for *post hoc* analysis. Data of the percentage of survival was analyzed by log-rank tests. The Pearson Correlation test estimated a correlation between the two groups. A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of bisacurone on relative heart weight and percent survival of rats: There was no significant change in the bodyweight of sham (230.20±2.69 g) as control (229.30±1.94 g), Lisinopril (15 mg kg^{-1}) treated (229.00±1.83 g) and bisacurone (25, 50 and 100 µg kg^{-1}, 226.20±2.09, 234.30±2.69 and 226.80±2.98 g, respectively) treated and per se treated (229.70±2.54 g) rats. However, absolute and relative heart weight was increased effectively ($p<0.05$) in AS control rats (0.79±0.03 g and $3.44\pm0.17\times10^{-3}$) compared to sham-treated rats (0.46±0.02 g and $2.00\pm0.08\times10^{-3}$). Administration of lisinopril noticeably suppressed ($p<0.05$) pressure overload-induced elevated absolute (0.56±0.03 g) and relative heart weight ($2.44\pm0.15\times10^{-3}$) compared to AS control rats. Administration of bisacurone (50 and 100 µg kg^{-1}) also strikingly repressed ($p<0.05$) absolute (0.65±0.03 and 0.59±0.03 g) and relative heart weight (2.78 ± 0.12 and $2.60\pm0.17\times10^{-3}$) compared to AS control rats. However, lisinopril showed more effective ($p<0.05$) attenuation pressure overload-induced elevated absolute and relative heart weight than bisacurone treated rats in Table 2.

Aortic stenosis induces significant ($p<0.05$, 40%) mortality in AS control rats as compared to the sham group (0%). However, when compared with AS control rats, administration of Lisinopril (15 mg kg^{-1}) and bisacurone (50 and 100 µg kg^{-1})

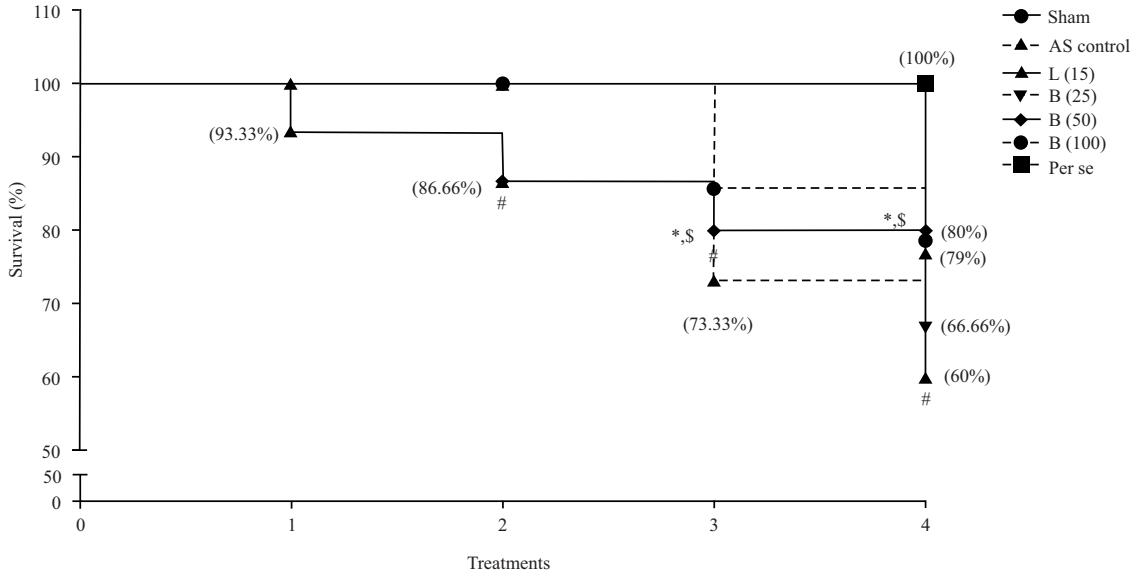

Fig. 1: Effect of bisacurone on pressure overload-induced alterations in percent survival of rats

Data are expressed as Mean±SEM. (n = 6) and analyzed by log-rank tests. *p<0.05 as compared to the aortic stenosis (AS)-control group, #p<0.05 as compared to the sham group, $p<0.05 as compared to one another (lisinopril and bisacurone). AS: Aortic stenosis control rats, L (15): Lisinopril (15 mg kg^{-1}, p.o.) treated rats, B (25): Bisacurone (25 μg kg^{-1}, p.o.) treated rats, B (50): Bisacurone (50 μg kg^{-1}, p.o.) treated rats and B (100): Bisacurone (100 μg kg^{-1}, p.o.) treated rats

also markedly decreased (p<0.05) AS-induced mortality (20%). Bisacurone (25 μg kg^{-1}) failed to show any significant protection against pressure overload-induced mortality (33%) in Fig. 1.

Effect of bisacurone on serum CK-MB, LDH and ALP levels in rats: The serum CK-MB, LDH and ALP levels were elevated (p<0.05) in AS control (2181.00±45.68, 2705.00±38.59 IU L^{-1} and 367.70±11.94 mg (%), respectively) rats compared to sham-treated rats (1052.00±58.67, 1213.00±49.65 IU L^{-1} and 107.80±11.05 mg (%), respectively). However, lisinopril administration notably reduces serum CK-MB (1331.00± 68.11 IU L^{-1}), LDH (1535.00±69.42 IU L^{-1}) and ALP levels (142.70±10.37 mg) (%) (p<0.05) compared to AS control rats. Bisacurone (50 and 100 μg kg^{-1}) treatment also effectively (p<0.05) inhibited pressure overload-induced elevated serum CK-MB (1663.00±54.27 and 1331.00± 63.35 IU L^{-1}), LDH (1988.00±46.17 and 1802.00±47.99 IU L^{-1}) and ALP (246.60±14.03 and 150.60±14.21 mg (%)) levels compared to AS control rats. However, when compared with bisacurone treated rats, lisinopril treatment markedly (p<0.05) educe serum CK-MB, LDH and ALP levels in Table 2.

Effect of bisacurone on electrocardiographic, hemodynamic and left ventricular function tests in rats: In AS control rats, pressure overload-induced myocardial hypertrophy showed noticeably (p<0.05) variations in heart rate (267.70±7.97 BPM), QRS interval (30.33±0.67 ms), QT interval (84.50±2.63 ms), QTc interval (171.00±5.59 ms), RR interval (208.20±6.66 ms), SBP (161.00±4.28 mmHg), DBP (115.5±4.19 mmHg), LVEDP (11.83±0.70 mmHg), $Max_{dp/dt}$ (2099.00±162.60), $Min_{dp/dt}$ (-2085.00±62.40), pressure time index (23.83±0.95), contractility index (29.17±1.97) and Tau (10.33±0.67 ms) compared to sham-treated rats (heart rate (385.70±6.30 BPM), QRS interval (13.17±0.60 ms), QT interval (48.17±3.73 ms), QTc interval (129.80±6.54 ms), RR interval (151.30±5.04 ms), SBP (104.30±4.34 mmHg), DBP (83.83±3.50 mmHg), LVEDP (4.50±0.56 mmHg), $Max_{dp/dt}$ (4081.00±112.90), $Min_{dp/dt}$ (-2634.00±83.49), pressure time index (17.83±0.40), contractility index (54.67±1.12) and Tau (5.33±0.56 ms). Administration of lisinopril efficiently (p<0.05) attenuated pressure overload-induced modification in heart rate (346.70±10.72 BPM), QRS interval (17.83±0.54 ms), QT interval (63.17±2.21 ms), QTc interval (134.20±5.69 ms), RR interval (171.00±4.97 ms), SBP (118.80±4.38 mmHg), DBP (91.50±2.60 mmHg), LVEDP (7.17±0.54 mmHg), $Max_{dp/dt}$ (3670.00±75.56), $Min_{dp/dt}$ (-2403.00±60.48), pressure time index (19.17±0.87), contractility index (48.50±1.48) and Tau (6.00±0.77 ms) compared to AS control rats. Bisacurone (50 and 100 μg kg^{-1}) treatment noticeably (p<0.05) restored heart rate (325.20±5.94 and 353.20±8.33 BPM), QRS interval (24.00±0.77 and 18.83±0.40 ms), QT interval (69.17±2.75 and 62.83±3.20 ms), QTc interval (154.00±4.75 and 142.80±5.33 ms), RR interval (176.50±6.98 and

Table 2: Effect of bisacurone on pressure overload-induced alterations in absolute heart weight, relative heart weight, serum CK-MB, LDH and ALP in rats

Parameters	Sham	AS control	L (15)	B (25)	B (50)	B (100)	Per se
Body weight (g)	230.20±2.69	229.30±1.94	229.00±1.83	226.20±2.09	234.30±2.69	226.80±2.98	229.70±2.54
Heart weight (g)	0.46±0.02	0.79±0.03$^{\#}$	0.56±0.03$^{*,\$}$	0.79±0.02	0.65±0.03$^{*,\$}$	0.59±0.03$^{*,\$}$	0.48±0.02
Heart weigh/body weight ($\times 10^{-3}$)	2.00±0.08	3.44±0.17$^{\#}$	2.44±0.15$^{*,\$}$	3.50±0.11	2.78±0.12$^{*,\$}$	2.60±0.17$^{*,\$}$	2.11±0.11
Serum CK-MB (IU L^{-1})	1052.00±58.67	2181.00±45.68$^{\#}$	1331.00±68.11$^{*,\$}$	2161.00±20.22	1663.00±54.27$^{*,\$}$	1331.00±63.35$^{*,\$}$	956.50±32.88
Serum LDH (IU L^{-1})	1213.00±49.65	2705.00±38.59$^{\#}$	1535.00±69.42$^{*,\$}$	2641.00±59.43	1988.00±46.17$^{*,\$}$	1802.00±47.99$^{*,\$}$	1101.00±56.01
ALP (mg %)	107.80±11.05	367.70±11.94$^{\#}$	142.70±10.37$^{*,\$}$	335.00±11.9	246.60±14.03$^{*,\$}$	150.60±14.21$^{*,\$}$	125.00±12.17

Data are expressed as Mean±SEM, (n = 6) and analyzed by one-way ANOVA followed by Tukey's multiple range tests. *p<0.05 as compared to the aortic stenosis (AS)-control group, $^{\#}$p<0.05 as compared to the sham group, $^{\$}$p<0.05 as compared to one another (lisinopril and bisacurone). AS: Aortic stenosis control rats, L (15): Lisinopril (15 mg kg^{-1}, p.o.) treated rats, B (25): Bisacurone (25 µg kg^{-1}, p.o.) treated rats, B (50): Bisacurone (50 µg kg^{-1}, p.o.) treated rats, B (100): Bisacurone (100 µg kg^{-1}, p.o.) treated rats. ALP: Alkaline phosphatase, CK-MB: Creatine kinase-MB and LDH: Lactate dehydrogenase

Table 3: Effect of bisacurone on pressure overload-induced alterations electrocardiographic, hemodynamic and left ventricular function tests changes in rats

Parameters	Sham	AS control	L (15)	B (25)	B (50)	B (100)	Per se
Heart rate (BPM)	385.70±6.30	267.70±7.97$^{\#}$	346.70±10.72$^{*,\$}$	270.00±10.34	325.20±5.94$^{*,\$}$	353.20±8.33$^{*,\$}$	360.30±7.05
QRS interval (ms)	13.17±0.60	30.33±0.67$^{\#}$	17.83±0.54$^{*,\$}$	28.17±0.60	24.00±0.77$^{*,\$}$	18.83±0.40$^{*,\$}$	16.00±0.68
QT interval (ms)	48.17±3.73	84.50±2.63$^{\#}$	63.17±2.21$^{*,\$}$	83.00±2.25	69.17±2.75$^{*,\$}$	62.83±3.20$^{*,\$}$	49.67±3.19
QTc interval (ms)	129.80±6.54	171.00±5.59$^{\#}$	134.20±5.69$^{*,\$}$	160.20±5.19	154.00±4.75$^{*,\$}$	142.80±5.33$^{*,\$}$	125.80±6.14
RR interval (ms)	151.30±5.04	208.20±6.66$^{\#}$	171.00±4.97$^{*,\$}$	202.80±5.94	176.50±6.98$^{*,\$}$	175.50±5.12$^{*,\$}$	148.50±5.51
SBP (mm Hg)	104.30±4.34	161.00±4.28$^{\#}$	118.80±4.38$^{*,\$}$	152.70±4.41	132.00±4.74$^{*,\$}$	122.30±2.03$^{*,\$}$	112.30±4.49
DBP (mm Hg)	83.83±3.50	115.50±4.19$^{\#}$	91.50±2.60$^{*,\$}$	114.70±4.29	101.50±4.77$^{*,\$}$	91.17±3.26$^{*,\$}$	82.33±1.98
LVEDP (mm Hg)	4.50±0.56	11.83±0.70$^{\#}$	7.17±0.54$^{*,\$}$	11.50±0.62	9.17±0.48$^{*,\$}$	8.33±0.42$^{*,\$}$	5.83±0.60
$Max_{dp/dt}$	4081.00±112.90	2099.00±162.60$^{\#}$	3670.00±75.56$^{*,\$}$	2279.00±111.80	2851.00±125.20$^{*,\$}$	3689.00±130.20$^{*,\$}$	3976.00±65.59
$Min_{dp/dt}$	-2634.00±83.49	-2085.00±62.40$^{\#}$	-2403.00±60.48$^{*,\$}$	-1954.00±48.5	-2138.00±76.94$^{*,\$}$	-2576.00±74.70$^{*,\$}$	-2644.00±95.01
Pressure time index	17.83±0.40	23.83±0.95$^{\#}$	19.17±0.87$^{*,\$}$	24.00±0.86	21.33±1.05$^{*,\$}$	19.50±0.62$^{*,\$}$	17.50±0.56
Contractility index	54.67±1.12	29.17±1.97$^{\#}$	48.50±1.48$^{*,\$}$	32.67±1.48	39.67±1.76$^{*,\$}$	44.17±1.25$^{*,\$}$	53.17±2.02
Tau (ms)	5.33±0.56	10.33±0.67$^{\#}$	6.00±0.77$^{*,\$}$	10.17±0.48	8.67±0.80$^{*,\$}$	7.00±0.73$^{*,\$}$	5.00±0.63

Data are expressed as Mean±SEM, (n = 6) and analyzed by one-way ANOVA followed by Tukey's multiple range tests. *p<0.05 as compared to the aortic stenosis (AS)-control group, $^{\#}$p<0.05 as compared to the sham group, $^{\$}$p<0.05 as compared to one another (lisinopril and bisacurone). AS: Aortic stenosis control rats, L (15): Lisinopril (15 mg kg^{-1}, p.o.) treated rats, B (25): Bisacurone (25 µg kg^{-1}, p.o.) treated rats, B (50): Bisacurone (50 µg kg^{-1}, p.o.) treated rats, B (100): Bisacurone (100 µg kg^{-1}, p.o.) treated rats. SBP: Systolic blood pressure, DBP: Diastolic blood pressure and LVEDP: Left ventricular end-diastolic pressure

Table 4: Effect of bisacurone on pressure overload-induced alterations in cardiac oxido-nitrosative stress, Na-K-ATPase and Ca-ATPase in rats

Parameters	Sham	AS control	L (15)	B (25)	B (50)	B (100)	Per se
SOD (U mg^{-1} of protein)	10.41±0.42	3.96±0.49$^{\#}$	9.14±0.28$^{*,\$}$	4.37±0.27	5.63±0.39$^{*,\$}$	7.16±0.34$^{*,\$}$	10.11±0.48
GSH (µg mg^{-1} protein)	32.12±0.93	16.17±0.68$^{\#}$	28.09±1.01$^{*,\$}$	17.56±1.12	21.37±1.15$^{*,\$}$	26.03±1.03$^{*,\$}$	30.26±0.92
MDA (nmol $L^{-1}mg^{-1}$ of protein)	2.45±0.30	5.41±0.18$^{\#}$	3.56±0.24$^{*,\$}$	5.58±0.25	4.66±0.28$^{*,\$}$	3.65±0.19$^{*,\$}$	2.83±0.16
NO (µg mg^{-1} of protein)	221.50±26.40	717.20±27.02$^{\#}$	327.80±26.47$^{*,\$}$	645.30±26.47	530.30±25.05$^{*,\$}$	386.90±21.08$^{*,\$}$	204.10±25.99
Na-K-ATPase (µmol mg^{-1} of protein)	5.40±0.29	2.65±0.27$^{\#}$	5.13±0.34$^{*,\$}$	3.14±0.25	4.05±0.34$^{*,\$}$	5.05±0.24$^{*,\$}$	5.22±0.31
Ca-ATPase (µmol mg^{-1} of protein)	3.82±0.28	1.98±0.26$^{\#}$	3.35±0.21$^{*,\$}$	1.75±0.15	2.32±0.35$^{*,\$}$	3.01±0.16$^{*,\$}$	3.76±0.25

Data are expressed as Mean±SEM, (n = 6) and analyzed by one-way ANOVA followed by Tukey's multiple range tests. *p<0.05 as compared to the aortic stenosis (AS)-control group, $^{\#}$p<0.05 as compared to the sham group, $^{\$}$p<0.05 as compared to one another (lisinopril and bisacurone). AS: Aortic stenosis control rats, L (15): Lisinopril (15 mg kg^{-1}, p.o.) treated rats, B (25): Bisacurone (25 µg kg^{-1}, p.o.) treated rats, B (50): Bisacurone (50 µg kg^{-1}, p.o.) treated rats, B (100): Bisacurone (100 µg kg^{-1}, p.o.) treated rats. SOD: Superoxide dismutase, GSH: Glutathione peroxidase, MDA: Malondialdehyde and NO: Nitric oxide

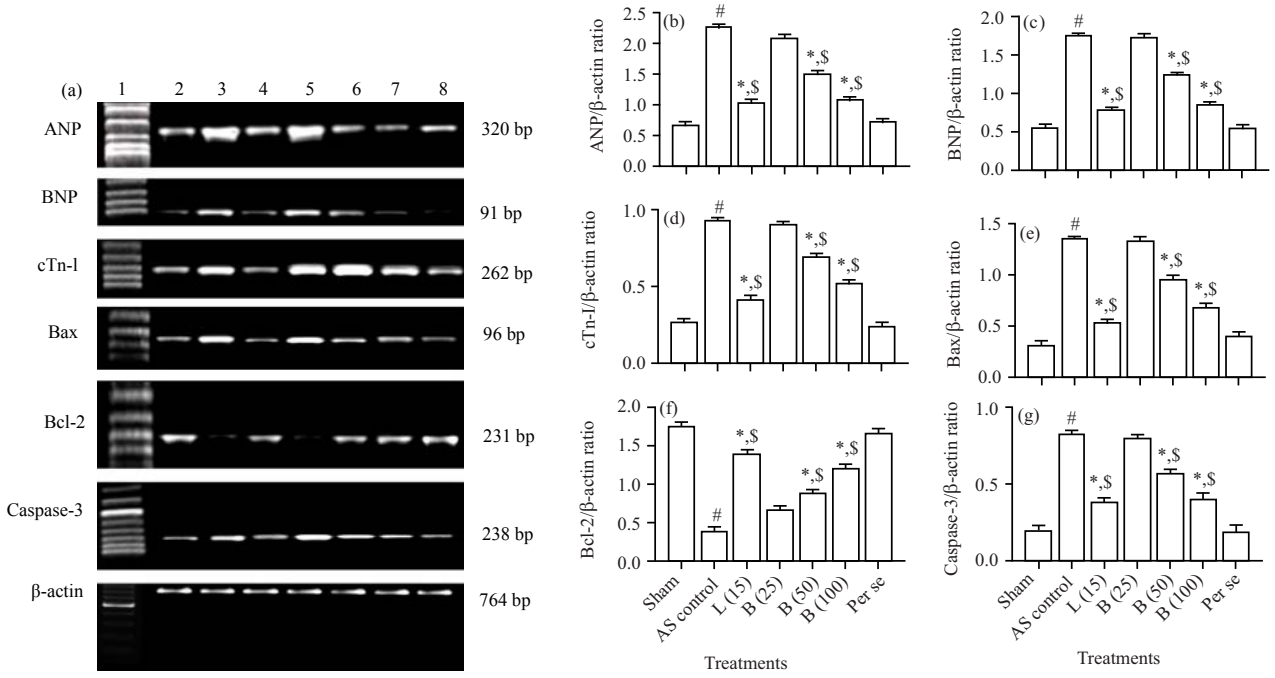

Fig. 2(a-g): Effect of bisacurone on pressure overload-induced alterations in cardiac ANP, BNP, cTn-I, Bax, Bcl-2, and Caspase-3 mRNA expressions determined by relative quantification, (a) Reverse transcriptase-polymerase chain reaction analysis, (b) Quantitative representation of mRNA expression of ANP, (c) Quantitative representation of mRNA expression of BNP, (d) Quantitative representation of mRNA expression of cTn-I, (e) Quantitative representation of mRNA expression of Bax, (f) Quantitative representation of mRNA expression of Bcl-2 and (g) Quantitative representation of mRNA expression of Caspase-3 in cardiac tissue of rat

Data are expressed as Mean±SEM, (n = 6) and analyzed by one-way analysis of variance followed by Tukey's multiple range test. *p<0.05 as compared to the aortic stenosis (AS)-control group, #p<0.05 as compared to the sham group, $p<0.05 as compared to one another (lisinopril and bisacurone). Lane 1: 1000 base pair, Lane 2: Sham, Lane 3: Aortic stenosis (AS)-control, Lane 4: Lisinopril (15 mg kg^{-1}, p.o.) treated group, Lane 5-7: Bisacurone (25, 50 and 100 µg kg^{-1}, p.o.) treated rats, respectively and Lane 8: Perse treated rats. ANP: Atrial natriuretic peptide, BNP: Brain natriuretic peptide, cTnT: Cardiac troponin I, Bax: Bcl-2 associated X and Bcl-2: B-cell lymphoma 2

175.50±5.12 ms), SBP (132.00±4.74 and 122.30±2.03 mmHg), DBP (101.50±4.77 and 91.17±3.26 mmHg), LVEDP (9.17±0.48 and 8.33±0.42 mmHg), $Max_{dp/dt}$ (2851.00±125.20 and 3689.00±130.20), $Min_{dp/dt}$ (-2138.00±76.94 and -2576.00±74.70), pressure time index (21.33±1.05 and 19.50±0.62), contractility index (39.67±1.76 and 44.17±1.25) and Tau (8.67±0.80 and 7.00±0.73 ms) compared to AS control rats in Table 3.

Effect of bisacurone on cardiac SOD, GSH, MDA and NO levels in rats: The levels of cardiac MDA (5.41± 0.18 nmol/L/mg of protein) and NO (717.20±27.02 µg mg^{-1} of protein) was increased significantly (p<0.05), whereas cardiac SOD (3.96±0.49 U mg^{-1} of protein) and GSH (16.17±0.68 µg mg^{-1} protein) levels were decreased effectively (p<0.05) in AS control rats compared to sham-treated rats (MDA (2.45±0.30 nmol/L/mg of protein), NO (221.50±26.40 µg mg^{-1} of protein), SOD (10.41±0.42 U mg^{-1} of protein) and GSH (32.12±0.93 µg mg^{-1} protein). Lisinopril treatment noticeably (p<0.05) attenuated elevated cardiac MDA (3.56±0.24 nmol/L/mg of protein) and NO (327.80±26.47 µg mg^{-1} of protein), whereas cardiac SOD (9.14±0.28 U mg^{-1} of protein) and GSH (28.09±1.01 µg mg^{-1} protein) levels were increased effectively (p<0.05) as compared to AS control rats. Pressure overload-induced alterations in cardiac SOD (5.63±0.39 and 7.16±0.34 U mg^{-1} of protein), GSH (21.37±1.15 and 26.03±1.03 µg mg^{-1} protein), MDA (4.66±0.28 and 3.65±0.19 nmol/L/mg of protein) and NO (530.30±25.05 and 386.90±21.08 µg mg^{-1} of protein) levels were markedly (p<0.05) restored by bisacurone (50 and 100 µg kg^{-1}) treatment compared to AS control rats. However, lisinopril more effectively (p<0.05) inhibited pressure overload-induced alterations in cardiac SOD, GSH, MDA and NO levels compared to bisacurone treated rats in Table 4.

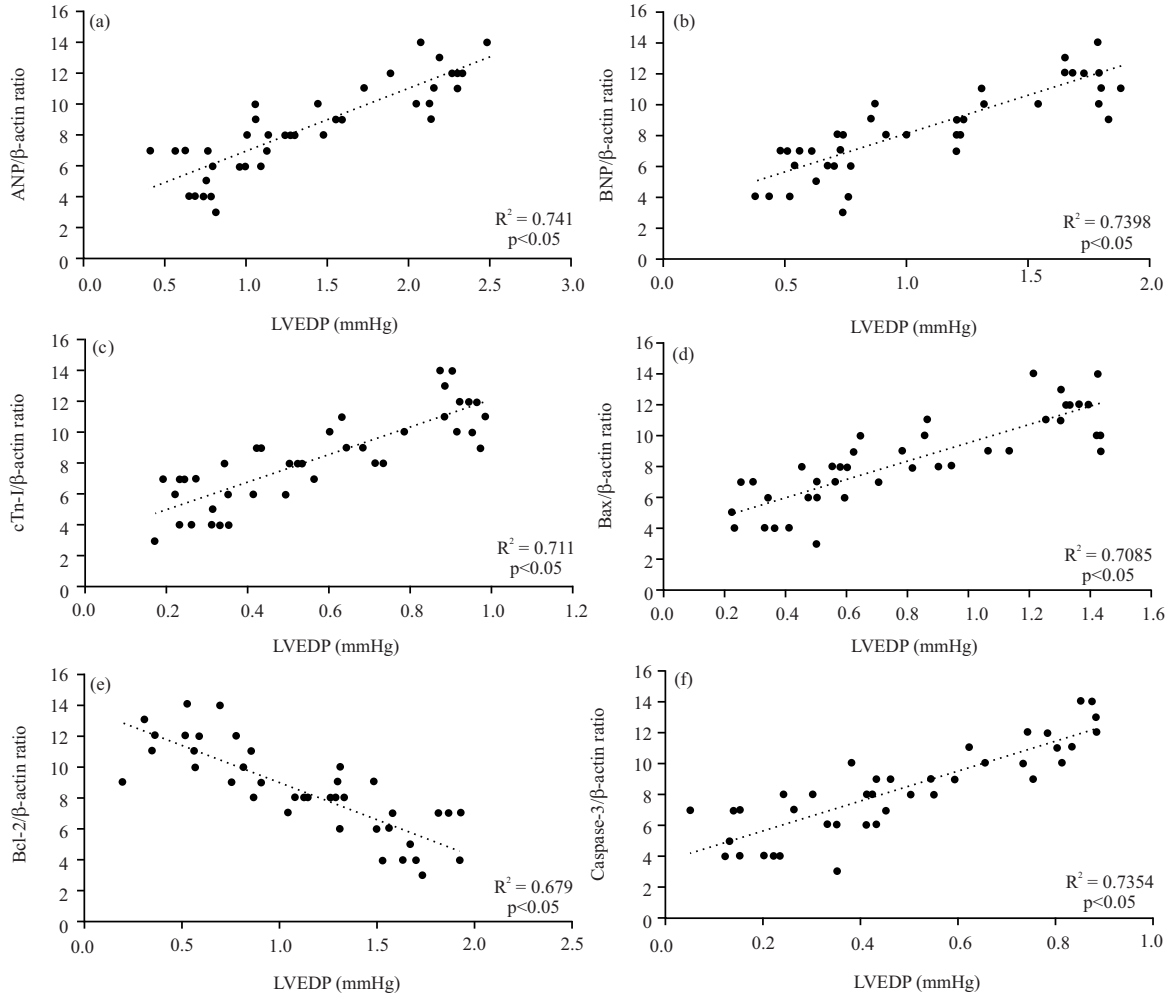

Fig. 3(a-f): Plot depicting correlation of LVEDP against, (a) ANP, (b) BNP, (c) cTn-I, (d) Bax, (e) Bcl-2 and (f) Caspase-3 in rats
ANP: Atrial natriuretic peptide, BNP: Brain natriuretic peptide, cTnT: Cardiac troponin I, Bax: Bcl-2 associated X, Bcl-2: B-cell lymphoma 2

Effect of bisacurone on cardiac Na-K-ATPase and Ca-ATPase levels in rats: The cardiac Na-K-ATPase (2.65±0.27 μmol mg^{-1} of protein) and Ca-ATPase (1.98±0.26 μmol mg^{-1} of protein) levels decreased ($p<0.05$) significantly in AS control rats (5.40±0.29 and 3.82±0.28 μmol mg^{-1} of protein) compared to sham-treated rats. Administration of lisinopril notably ($p<0.05$) increase cardiac Na-K-ATPase (5.13±0.34 μmol mg^{-1} of protein) and Ca-ATPase (3.35±0.21 μmol mg^{-1} of protein) levels compared to AS control rats. Pressure overload-induced diminished levels of cardiac Na-K-ATPase (4.05±0.34 and 5.05±0.24 μmol mg^{-1} of protein) and Ca-ATPase (2.32±0.35 and 3.01±0.16 μmol mg^{-1} of protein) were effectively ($p<0.05$) increased by bisacurone (50 and 100 μg kg^{-1}) compared to AS control rats. Administration of lisinopril showed a more effective ($p<0.05$) increase in cardiac Na-K-ATPase and Ca-ATPase levels than bisacurone treated rats in Table 4.

Effect of bisacurone on the cardiac ANP, BNP, cTn-I, Bax, Bcl-2 and Caspase-3 mRNA expressions in rats: The mRNA expressions of ANP, BNP, cTn-I, Bax and Caspase-3 were noticeably ($p<0.05$) up-regulated in cardiac tissue of AS control rats (2.27±0.05, 1.76±0.03, 0.94±0.02, 1.36±0.02 and 0.83±0.02) compared to sham-treated rats(0.67±0.06, 0.54±0.06, 0.27±0.03, 0.32±0.04 and 0.20±0.03) in Fig. 2(a-e). Whereas cardiac Bcl-2 mRNA expression was down-regulated in AS control rats (0.38±0.05) compared to sham-treated rats (1.75±0.06) in Fig. 2f and g. Treatment with lisinopril showed significant ($p<0.05$) amelioration in up-regulated cardiac ANP (1.04±0.07), BNP (0.78±0.04), cTn-I (0.42±0.03), Bax (0.54±0.03) and Caspase-3 (0.39±0.02) mRNA expressions whereas it effectively ($p<0.05$) up-regulated cardiac Bcl-2 mRNA (1.40±0.05) expression as compared to AS control rats. Pressure overload-induced modification in cardiac ANP (1.51±0.06 and 1.10±0.03), BNP

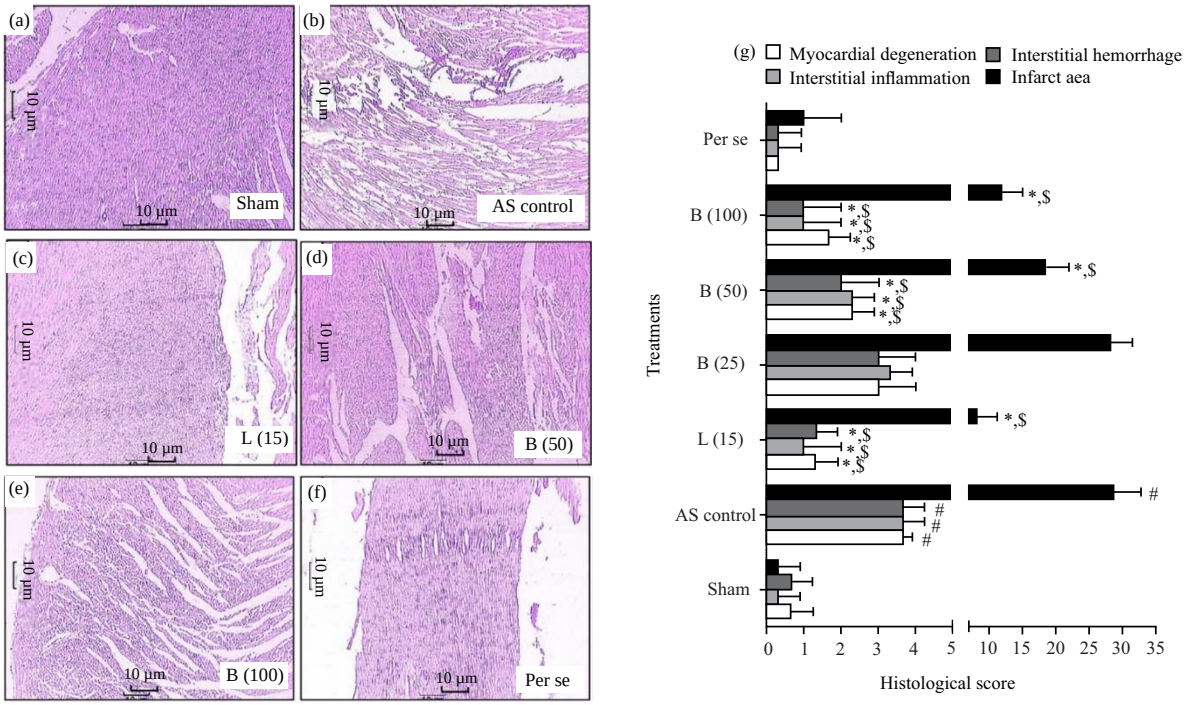

Fig. 4(a-g): Effect of bisacurone on pressure overload-induced alterations in cardiac histopathology in rats, (a) Photomicrograph of sections of the heart of sham, (b) Aortic stenosis (AS)-control group, (c) Lisinopril (15 mg kg^{-1}, p.o.) treated group, (d) B (50): Bisacurone (50 µg kg^{-1}, p.o.) treated group, (f) B (100): Bisacurone (100 µg kg^{-1}, p.o.) treated rats and (g) Per se treated group

H and E stain and Images at 40×. The quantitative representation of histological score (G). Data are expressed as Mean±SEM (n = 3) and one-way ANOVA followed by the Kruskal-Wallis test was applied for *post hoc* analysis. *p<0.05 as compared to the aortic stenosis (AS)-control group, #p<0.05 as compared to the sham group

(1.25±0.02 and 0.85±0.04), cTn-I (0.70±0.02 and 0.52±0.02), Bax (0.96±0.05 and 0.68±0.04), Bcl-2 (0.88±0.04 and 1.21±0.05) and Caspase-3 (0.58±0.02 and 0.41±0.04) mRNA expressions was markedly (p<0.05) inhibited by bisacurone (50 and 100 µg kg^{-1}) treatment compared to AS control rats.

Linear correlation analysis suggested that pressure overload-induced variations in ANP (R^2 = 0.741), BNP (R^2 = 0.7398), cTn-I (R^2 = 0.711), Bax (R^2 = 0.7085), Bcl-2 (R^2 = 0.679) and Caspase-3 (R^2 = 0.7354) in Fig. 3(a-f) were significantly correlated (p<0.05) with LVEDP.

Effect of bisacurone on pressure overload-induced alterations in cardiac histopathology of rats: Figure 4a and f showed normal cardiac tissue architecture from sham control and per se treated rats. Histological analysis of cardiac tissue from sham control and per se treated rats showed minimal interstitial inflammation (0.33±1.87 and 0.33±0.65), haemorrhage (0.67±1.87 and 0.33±0.65) and myocardial degeneration (0.67±1.87 and 0.33±0.57). However, chronic constriction of the left renal artery aorta caused significant induction of cardiac hypertrophy reflected by marked (p<0.05) myocardial degeneration (3.33±2.11), elevation in interstitial inflammation (3.67±2.11) and haemorrhage (3.67±2.11) in AS control rats in Fig. 4b. Administration of lisinopril effective (p<0.05) inhibited pressure overload-induced alteration in myocardial histology reflected by mild interstitial inflammation (1.00±2.25), haemorrhage (1.33±2.25) and myocardial degeneration (1.33±2.25) in Fig. 4c compared to AS control rats. Figure 4d and e depicted the histological architecture from bisacurone (50 and 100 µg kg^{-1}) treated rats showed a significant (p<0.05) reduction in pressure overload-induced alteration in myocardial damage including myocardial degeneration (2.33±2.70 and 1.67±1.43), interstitial inflammation (2.33±2.70 and 1.00±1.43) and haemorrhage (2.00±2.70 and 1.00±1.43) in Fig. 4g.

DISCUSSION

Left ventricular hypertrophy has been suggested as an independent cardiovascular risk factor in patients with hypertension[20,36]. Numerous studies suggested that activation of the Renin-Angiotensin-Aldosterone System (RAAS) plays an

important role in regulating vascular remodelling induced hypertension and thus cardiac and renal functions[37]. Constriction of the abdominal aorta above the renal arteries caused pressure overload-induced progression of cardiac remodelling and hypertension, leading to heart failure[2,7]. Furthermore, many studies have reported that during myocardial ischemia, the release of several pro-inflammatory cytokines, including tumour necrosis factor α (TNF-α) and interleukins (IL-1β and IL-6), caused massive inflammatory influx, which further contributes to cardiac damage[19,38-40]. In the current study, altered cardiovascular parameters increased myocardial apoptosis and oxidative stress suggested induction of cardiac hypertrophy due to constriction of the abdominal aorta. However, administration of bisacurone ameliorated pressure overload-induced cardiac hypertrophy via inhibition of oxidative stress (SOD, GSH, MDA and NO) and apoptosis (Bax, Bcl-2 and Caspase-3).

In recent years, left ventricular functions have been suggested as a vital parameter during cardiovascular disease[41-43]. As the pressures and volumes are dependent on various stages of the heart cycle, the alterations in these pressures and volumes depicted the damage to the cardiac tissue. Experimentally, Left Ventricular end-diastolic Pressure (LVEDP) provides insight into the total volume and related pressure present in the left ventricle[44]. Additionally, sustained elevated blood pressure has been suggested as an important pathophysiological factor in developing ventricular hypertrophy[2,20,23,45]. In the present investigation, pressure overload resulted in morphological perturbation in cardiac tissue depicted by elevated relative heart weight, LVEDP and decreased $Max_{dP/dt}$, $Min_{dP/dt}$ and contractility index, suggesting cardiac hypertrophy. In contrast, administration of bisacurone resulted in inhibition of development of LV hypertrophy reflected by restoration of left ventricular contractile functions. The observations of the present investigation are in good agreement with findings of previous studies, which reported amelioration of cardiac hypertrophy via administration of bisacurone[31].

Studies have reported that aorta constriction resulted in anaerobic glycolysis, which further reduces the permeability of the myocardial cell membrane[46-49]. Alteration in membrane permeability passively releases various myocardial biomarkers, including LDH, CK-MB and AST, into blood[9,43]. Thus, elevated levels of these biomarkers in the blood have been used as a useful diagnostic tool by an investigator to determine the progress and magnitude of myocardial and cellular damage[50]. In the present investigation, increased levels of LDH asT and CK-MB after ischemia suggested myocardial damage, which is in line with the findings of cardiac function alternations histopathological observations of cardiac tissue from the AS control group. Nevertheless, bisacurone treatment markedly attenuated elevated levels of LDH, CK-MB and AST, suggesting its cardioprotective potential. Results of the present study corroborate the findings of previous investigators where administration of bisacurone reduced LDH and CK-MB levels[31].

It has been well documented that pressure overload to cardiac tissue is associated with elevated production of Reactive Oxygen Species (ROS) (such as superoxide anion, hydrogen peroxide, hydroxyl radical and singlet oxygen) as well as reactive nitrogen species that diminished endogenous antioxidant defence system[32,51]. These altered antioxidant levels activate the inflammatory pathway, accompanied by local and systematic tissue damage via lipid peroxidation. The report suggested that malondialdehyde (MDA) is an index to evaluate the extent of lipid peroxidation that destroys structural and cellular proteins[52]. However, GSH, a glutamate tripeptide, plays a decisive role in converting MDA to their corresponding alcohols. Furthermore, superoxide dismutase (SODs) and enzymes scavenge superoxide radicals and convert them into hydrogen peroxide via catalytic reaction. NO, an important signalling molecule in the cardiovascular system, has been reported to elevate peroxynitrite production, contributing to myocardial dysfunction[32]. In the present investigation, constriction of the abdominal aorta significantly induces an influx of ROS followed by elevated oxido-nitrosative stress reflected by decreased SOD and GSH levels in cardiac tissue. However, the administration of bisacurone significantly ameliorated pressure overload-induced cardiac damage via attenuation of elevated oxido-nitrosative stress. The previous investigator also documented the protective efficacy of bisacurone by virtue of its antioxidant potential[29,31]. The present investigation findings are following the previous investigator[29,31].

Researchers have well documented the role of excessive intracellular calcium influx, activation of intracellular proteolysis, inflammatory infiltration and endothelium dysfunction during the pathogenesis pressure overload cardiotoxicity[53,54]. Accumulated evidence suggests that chronic constriction of the abdominal aorta caused exquisite equilibration for the production and release of Reactive Oxygen Species (ROS), leads to mitochondrial apoptosis reflected by elevated Bax (Bcl-2 Associated X) and Caspase-3 expressions resulted in structural perturbation in protein and DNA which contributes to ventricular dysfunction via its

apoptosis and hypertrophy[32,55]. The mitochondrial apoptotic pathway is regulated by the Bcl-2 family, which is a pro-apoptotic factor that interacts with Bax, thus neutralizingit[25,27]. Our present study shows increased apoptosis in AS control rats reflected by elevated Bax and Caspase-3 expression in cardiac tissue. A positive correlation between the apoptotic factors (Bax and Caspase-3) and LVEDP suggested the potential role of apoptosis in the pathogenesis of cardiac hypertrophy. Conversely, bisacurone treatment inhibited up-regulated expressions of Bax and Caspase-3, suggesting its antiapoptotic potential during cardiac hypertrophy. Recently, Cui *et al.*[31] also reported the cardioprotective potential of bisacurone via inhibition of up-regulated apoptotic expressions. The results of the present investigation are in line with the findings of the previous researchers[31].

CONCLUSION

In summary, the present investigation findings showed that constriction of the abdominal aorta resulted in alteration of electrocardiographic, hemodynamic and left ventricle contractile functions, increased cardiac oxidative stress and apoptosis suggesting induction of cardiac hypertrophy. Nevertheless, bisacurone treatment inhibited pressure overload-induced progressive cardiovascular dysfunctions and cardiac hypertrophy. Bisacurone exerts its beneficial effect through its inhibitory potential against oxidative stress (SOD, GSH, MDA and NO) and apoptosis (Bax, Bcl-2 and Caspase-3). Thus, bisacurone can be considered as an important therapeutic moiety in the management of congestive heart failure.

SIGNIFICANCE STATEMENT

To the best of our knowledge, findings of the present study, first time reported putative mechanism of action of bisacurone against pressure overload-induced cardiac hypertrophy in experimental rats. The content of the present manuscript is discussed about pharmacological action of bisacurone against pressure overload-induced cardiac hypertrophy that may assist in the upcoming drug designing. Furthermore, this article also emphasizes frontiers to find alternative healthcare products to manage congestive heart failure. This article will deliver valuable information to researchers and physicians to find an alternative healthcare product to manage cardiomyopathy in pressure overload patients.

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