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ORIGINAL PAPER

5-HT_{2B} receptor blockade attenuates β-adrenergic receptor-stimulated myocardial remodeling in rats via inhibiting apoptosis: role of MAPKs and HSPs

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Abstract Recent studies have proposed the potential role of 5-HT_{2B} receptor (5-HT_{2B}R) blockade in alleviating myocardial dysfunction; hitherto, the regulatory pathway for its protective effect has remained enigmatic. In the present study, we sought to investigate the role of SB-204741, a 5-HT_{2B}R blocker in isoproterenol-induced myocardial remodeling in rats and its cross-talk with apoptosis and mitogen activated protein kinase (MAPKs)/heat shock proteins (HSPs) pathway. To assess this hypothesis, we measured the effect of SB-204741 (0.25–1.0 mg/kg/day, i.p.) in isoproterenol (85 mg/kg/day, s.c.)-induced myocardial remodeling in rats. SB-204741 dose dependently improved hemodynamic and ventricular functions following isoproterenol-induced myocardial injury. This amelioration was well substantiated with reduced expression of 5-HT_{2B}, inflammatory proteins (NF-κBp65, IKK-β, TNF-α, IL-6, and Cox-2), MAPKs (p-p38/p38 and p-JNK/JNK ratio) accompanied with increased protein expression of HSPs (αB-crystallin, Hsp27 and Hsp70), autophagy (LC3 and Beclin-1) and p-ERK/ERK ratio. Additionally, SB-204741 inhibited apoptotic signaling pathway as there was decreased DAPI/TUNEL positivity and protein expression of cytochrome c, Bax, and caspase-3 along with increased Bcl-2 expression. Preservation of histopathological and ultrastructural components, normalization of nitric oxide level, endogenous antioxidants and myocyte injury marker enzymes were also observed. In conclusion, inhibition of apoptosis via modulation of MAPKs/HSPs is essential for 5-HT_{2B}R blockade mediated cardioprotective effect.

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Introduction

Many intracellular signaling pathways are critically involved in preventing myocardial insult from exogenous and endogenous stimuli [1, 2]. Considerable recent data suggest that pathways including mitogen activated protein kinase (MAPKs) and heat shock proteins (HSPs) have shown to mitigate myocardial ischemia–reperfusion injury through inhibiting programmed cell death. Both MAPKs (p38, ERK and JNK) and HSPs (αB-crystallin, Hsp27 and Hsp70) are multifunctional regulators that not only control myocyte differentiation, proliferation, apoptosis, autophagy and cytokine production but have also garnered significant attention in pathogenesis, progression and prognosis of heart failure [3–5]. The expressions of MAPKs and HSPs have been found to be associated in heart recovering from insults such as hypoxia, sublethal heat and ischemia. Further their expression levels correlated well with decreased levels of oxidative stress, lipid peroxidation, protein oxidation and nitric oxide in the myocardium. In fact functional interaction of MAPKs/HSPs with other signaling networks like nuclear factor-κB (NF-κB) and reactive oxygen species helps them to actively inhibit inflammatory and oxidative stress signaling pathways [3–5]. Thus MAPKs/HSPs pathway and apoptosis go hand in hand in regulating cardiomyocyte survival and demise. For instance, induction of autophagy, activation of HSPs/ERK or inhibition of p38/JNK salvage myocardium from the deleterious effect of ischemia–reperfusion injury by maintaining the cytoskeletal architecture, redox homeostasis, inhibiting pro-inflammatory cytokines,

masking of mutations and most importantly shielding the cell from spontaneous or stimulated programmed cell death [6–12].

Serotonin is regarded as a neurohormonal factor that has been linked with cardiovascular remodeling. Among 14 different known serotonin receptors, 5-HT_{2B}R antagonism has been emerged as one of the novel and putative molecular target for various cardiovascular disease conditions [13]. Intriguingly, growing body of evidence indicates that cardiac morphogenesis, hypertrophy and pulmonary hypertension are the therapeutic areas where 5-HT_{2B}R antagonism has shown substantial interest [14–16]. The idea for the development of 5-HT_{2B}R antagonism has stemmed from the fact that over expression of 5-HT_{2B}R in heart leads to abnormal mitochondrial function and cardiac hypertrophy in mice [17]. Besides, there are also experimental in vitro and in vivo data, as well as clinical evidence, which suggest that serotonin plasma level and serotonin activity are increased in cardiac hypertrophy induced by aortic constriction and in patients with heart failure [18–20]. For instance, 5-HT_{2B}R mRNA expression has been found to be significantly higher in dogs with dilated cardiomyopathy and in humans with cardiomyopathies [21, 22]. Likewise, other 5-HT_{2B}R blockers such as SB-215505 and LY-272015 have also significantly prevented isoproterenol and angiotensin-II-induced cardiac hypertrophy in mice and DOCA-salt-induced hypertension in rats respectively [15, 23].

Clubbing all these effects we hypothesized that SB-204741, a potent and selective 5-HT_{2B} receptor blocker with 135× selectivity over 5-HT_{2C}R and even higher over 5-HT_{2A}R [24] might alleviate isoproterenol-induced myocardial remodeling through modulating MAPKs/HSPs and may also affect ventricular functions, antioxidant status, cardiac injury markers and most importantly inflammatory and oxidative stress signaling pathways. Therefore, it is of considerable interest to explore interplay between MAPKs, HSPs and apoptosis by 5-HT_{2B}R blockade in ameliorating isoproterenol-induced myocardial remodeling in rats.

Materials and methods

Animals

The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (567/IAEC/2010) and all study related activities conformed to the Indian National Science Academy (INSA) Guidelines for the use and care of experimental animals in research. Male wistar rats (150–180 g) received humane care in compliance with the principles of INSA and were kept in the departmental animal house under controlled conditions of temperature at 25 ± 2 °C, relative humidity of 60 ± 5 % and light–dark cycle of 12:12 h.

Reagents

Isoproterenol and SB-204741 were purchased from the Sigma Chemicals (St. Louis, MO, USA). 5-HT_{2B} (SC-15080), LC3 (SC-28266), αB-crystallin (SC-22744), p-p38 (SC-7973), IL-6 (SC-1265) and Cox-2 (SC-1746) antibodies were purchased from Santa Cruz, USA. SAPK/JNK (#9252S), phospho-SAPK/JNK (Thr183/Tyr185) (#9251S), p44/42 MAPK (ERK½) (137F5) (#4695S), phospho-p44/42 MAPK (ERK½) (Thr202/Tyr204) (#4370S), Hsp27 (#2442S), Hsp70 (#4872S), caspase-3 (#9662S) and IKK-β (#2678S) antibodies were purchased from Cell Signaling Technology, USA. NF-κBp65chip grade (Ab7970), cytochrome c (Ab110325), Bax (Ab32503), Bcl-2 (Ab7973), p38 (Ab7952), Beclin-1 (Ab55878) and β-actin (Ab52614) antibodies were procured from Abcam Technologies, USA. Secondary antibodies were purchased from Merck GeNei, India and Santa Cruz, USA. Creatine Kinase isoenzyme-MB (CK-MB) (Spinreact, Spain), Rat Tumor necrosis factor-alpha (TNF-α) (DiacloneTepnel Company, UK) and lactate dehydrogenase (LDH) isoenzyme (Logotech, Delhi, India) kits were used. For the purpose of administration SB-204741 was freshly prepared and dissolved in DMSO and propylene glycol (2:1) whereas isoproterenol was dissolved in normal saline.

Experimental protocol

All the animals were divided into six groups with eight animals in each group. The animals were either injected intraperitoneally (i.p.) into the lower left quadrant of the abdomen or subcutaneously (s.c.) into the dorsolateral areas of neck.

Group 1 (Sham): Rats were administered DMSO and propylene glycol (2:1) (1.5 mL/kg/day, i.p.) for a period of 28 days. Concomitantly, on 27th and 28th day the experimental animals were administered normal saline (1.5 mL/kg/day, s.c.).

Group 2 (ISO): Rats were administered DMSO and propylene glycol (2:1) (1.5 mL/kg/day, i.p.) for a period of 28 days. Concomitantly, on 27th and 28th day the experimental animals were administered isoproterenol (85 mg/kg/day, s.c.).

Group 3-5 (SB 0.25 + ISO, SB 0.5 + ISO and SB 1.0 + ISO): 5-HT_{2B}R blocker, SB-204741 (SB, 0.25, 0.5 and 1.0 mg/kg/day, i.p.) was administered for 28 days. Concomitantly, on 27th and 28th day the experimental animals were administered isoproterenol (85 mg/kg/day, s.c.).

Group 6 (SB 1.0 per se): SB-204741 (1.0 mg/kg/day, i.p.) was administered for 28 days. Concomitantly, on 27th and 28th day the experimental animals were administered normal saline (1.5 mL/kg/day, s.c.).

The doses of the test drug were selected based on the previous literature [25].

Surgical procedures

Rats were anesthetized with pentobarbitone sodium (60 mg/kg, i.p.). Atropine was administered to reduce the broncho-tracheal secretions during the course of the surgery. The neck was opened with a ventral midline incision and tracheostomy was performed. The rats were ventilated with room air from a positive pressure ventilator (Inco, Ambala, India) using compressed air at a rate of 70 strokes/min and a tidal volume of 10 mL/kg. The left jugular vein was cannulated with polyethylene tube for continuous infusion of 0.9 % saline solution. The right carotid artery was cannulated with a cannula filled with heparinized saline and connected via pressure transducer to record hemodynamic variables. The animals were then allowed to stabilize for 15 min before recording the basal hemodynamic variables viz. systolic (SAP), diastolic (DAP), mean arterial pressure (MAP) and heart rate (HR). Furthermore, left thoracotomy was performed between the fifth and sixth intercostal space and the pericardium was opened to expose the heart. A wide bore (1.5 mm) sterile metal cannula connected to a pressure transducer (Gould Statham P231D, USA) was inserted into the cavity of left ventricle from the posterior apical region of heart for recording left ventricular pressure dynamics using Biopac system software BSL 4.0 MP36. Left ventricular function variables viz. +LVdP/*dtmax* (rate of contraction), −LVdP/*dtmax* (rate of relaxation) and increased LVEDP (preload) were recorded following the surgical procedure. Animals were then sacrificed for evaluating heart weight (HW), biochemical, molecular, immunohistochemical, electron microscopy and histopathological studies.

Biochemical studies

A 10 % heart homogenate was prepared in ice-chilled phosphate buffer (0.1 M, pH 7.4) and from that an aliquot was used for the estimation of reduced glutathione (GSH) [26] and thiobarbituric acid substances (TBARS) [27]. Moreover, supernatant obtained at 3,000 g for 20 min at 4 °C was used to measure superoxide dismutase (SOD) [28], nitrite (NO) [29] and LDH levels. Moreover, TNF-α and creatine kinase-MB (CK-MB) levels were measured spectrophotometrically in serum.

4′,6-Diamidino-2-phenylindole (DAPI) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

DAPI and TUNEL assays were performed using kit from Vector Laboratories, USA and in situ cell death detection kit, POD (Roche, Germany) respectively following the manufacturer's instructions and were quantified using Image J software.

Histopathological and ultrastructural analysis

The brief protocol for histopathological and ultrastructural analysis has been described in our previous paper [30].

Western blot analysis

Left ventricular heart tissue protein samples (40 µg) were separated by SDS-PAGE, transferred to nitrocellulose membrane which was blocked for 2 h with 5 % bovine serum albumin or non-fat dried milk and incubated for 24 h at 4 °C with a primary antibody. The primary antibody was detected with horse radish peroxidase (HRP)-conjugated secondary antibody anti-rabbit/anti-goat/anti-mouse. Furthermore, the bands were visualized using Enhanced chemiluminescent luminol (ECL) Kit (Thermo Scientific) under FluorChem M Protein imaging System (Bucher Biotec AG, Basel, Switzerland) and were quantified by Bio-Rad Quantity One 4.4.0 software (BIO-RAD, Hercules, CA, USA). The brief protocol for western blot procedure has been described in our earlier study [31].

Immunohistochemistry (IHC) analysis

VECTOR ABC KIT, CA, USA was used to perform IHC. The brief protocol for the IHC procedure has been described in our previous study [31].

Statistical analysis

The data were expressed as mean ± S.D. One way ANOVA followed by post hoc Bonferroni test was done SPSS software package Version 11.5. The value of $P < 0.05$ was considered as statistically significant.

Results

SB 1.0 per se group did not show any significant change on all the parameters evaluated

There were no significant changes observed on hemodynamic, biochemical, molecular, histopathological and ultrastructural parameters as compared to sham group on the

highest administered dose of SB-204741 (1.0 mg/kg/day) (Figs. 1, 2, 3, 4, 5, 6; Table 1).

SB-204741 did not showed any significant change on heart weight (HW), body weight (BW) and HW/BW ratio

Administration of SB-204741 (0.25–1.0 mg/kg/day) for 28 days did not exhibit any significant change on HW, body weight (BW) and HW/BW ratio in failing and recovered myocardium (Fig. 1a–c).

SB-204741 improves hemodynamic and ventricular dysfunction in isoproterenol-induced myocardial remodeling in rats

As anticipated, in comparison to sham group, ISO group exhibited significant ($P < 0.001$) hemodynamic impairment as observed by reduced SAP, DAP and MAP (Fig. 1d–f). In accordance with hemodynamic findings, we also observed significant ($P < 0.001$) ventricular dysfunction viz. decreased +LVdP/*dtmax* (rate of contraction), −LVdP/*dtmax* (rate of relaxation) and increased LVEDP (preload) as compared to sham group (Fig. 1g–i). Intriguingly, SB-204741 abolishes the isoproterenol-induced myocardial remodeling as there was significantly dose dependent improvement in hemodynamic (SAP, DAP and MAP) and ventricular functions (±LVdP/*dtmax* and LVEDP) though the effect was most pronounced ($P < 0.01$) at 1.0 mg/kg/day as compared to other two doses (Fig. 1d–i). Moreover, change in HR was statistically insignificant in all the groups (Fig. 1j).

SB-204741 bolsters endogenous anti-oxidant enzymes activities, improves cardiac injury markers, NO level and lipid peroxidation level and attenuated TNF-α level in isoproterenol-induced myocardial remodeling in rats

As shown in Fig. 2, isoproterenol-challenged myocardium resulted in significant ($P < 0.001$) depletion in anti-oxidant

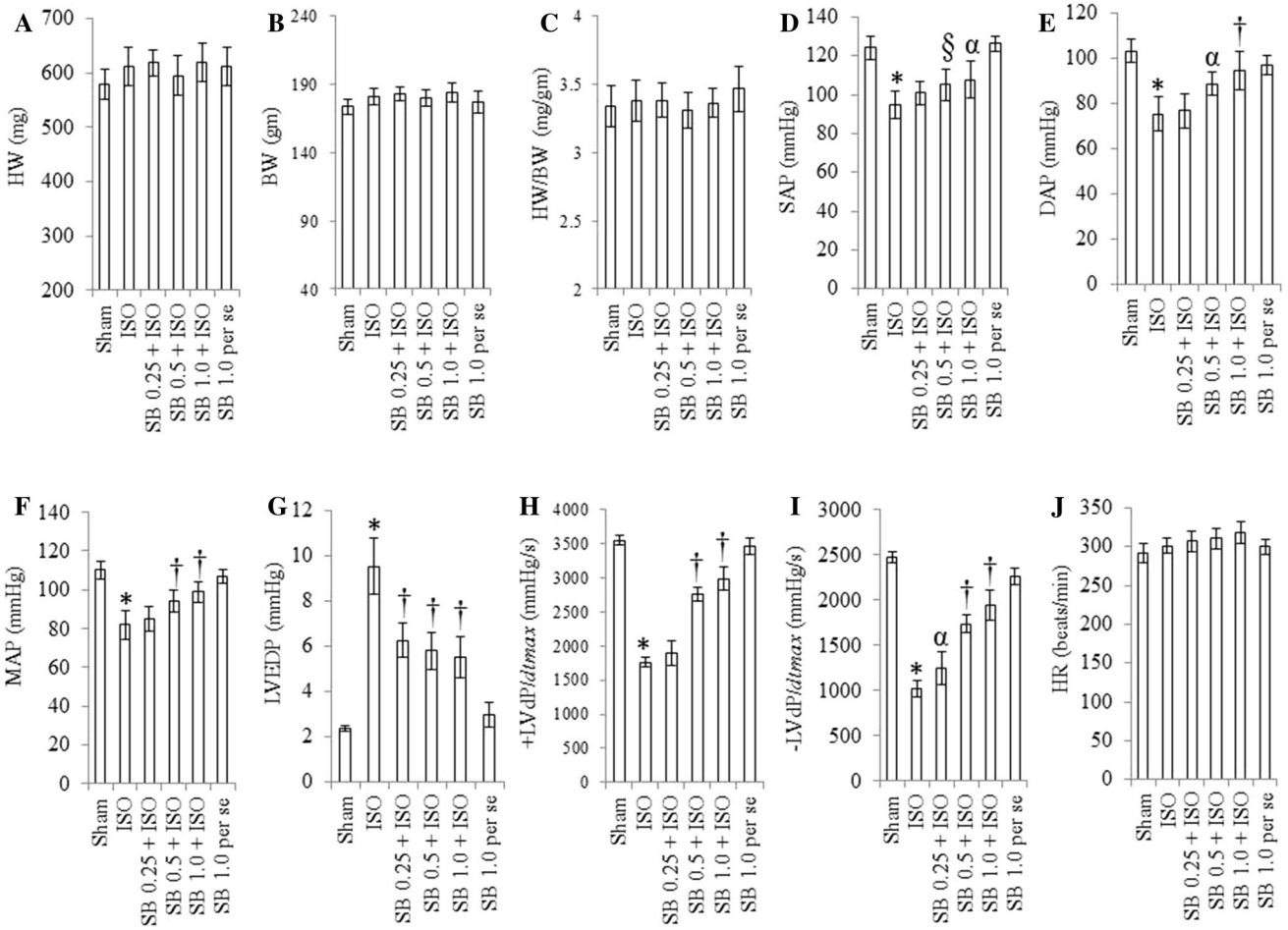

Fig. 1 Effect of SB-204741 on heart weight, body weight, heart weight body weight ratio and hemodynamic parameters following isoproterenol-induced myocardial remodeling. **a** *HW* heart weight; **b** *BW* body weight; **c** HW/BW ratio; **d** *SAP* systolic arterial pressure; **e** *DAP* diastolic arterial pressure; **f** *MAP* mean arterial pressure; **g** *LVEDP* left ventricular end diastolic pressure; **h** +LVdP/*dtmax* maximal positive rate of left ventricular pressure; **i** −LVdP/*dtmax* maximal negative rate of left ventricular pressure and **j** *HR* heart rate. All values are expressed as mean ± S.D (n = 8/group).*$P < 0.001$ versus sham and $^{\S}P < 0.05$, $^{\alpha}P < 0.01$, $^{\dagger}P < 0.001$ versus ISO

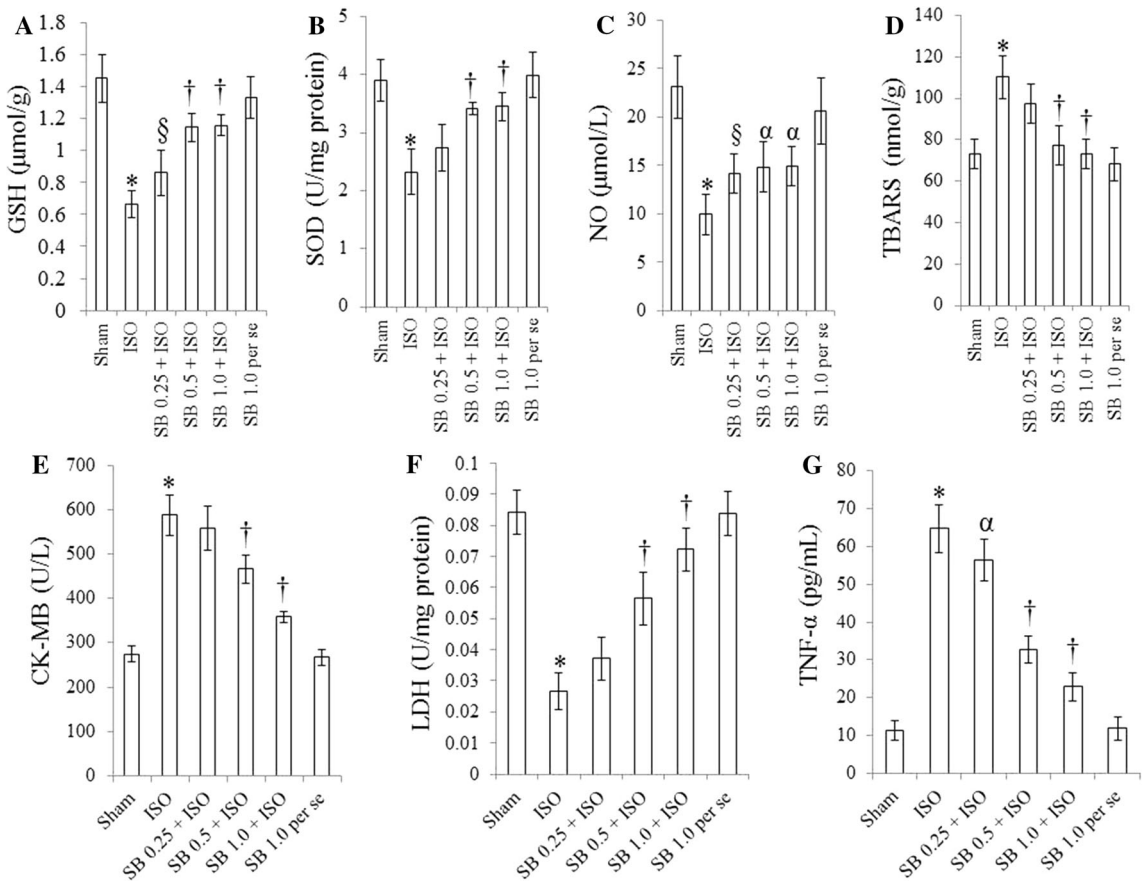

Fig. 2 Effect of SB-204741 on biochemical findings following isoproterenol-induced myocardial remodeling. **a** *GSH* reduced glutathione; **b** *SOD* superoxide dismutase; **c** *NO* nitric oxide; **d** *TBARS* thiobarbituric acid reactive substances; **e** *CK-MB* creatine Kinase-MB; **f** *LDH* LDH and **g** *TNF-α* tumor necrosis factor-alpha. All values are expressed as mean ± S.D (n = 8/group). *$P < 0.001$ versus sham and $^{\S}P < 0.05$, $^{\alpha}P < 0.01$, $^{\dagger}P < 0.001$ versus ISO

enzymes activities such as GSH and SOD and NO level from myocardium as compared to sham group (Fig. 2a–c). Similarly, there was also markedly ($P < 0.001$) increase in TBARS level, a marker of lipid peroxidation, as compared to sham group (Fig. 2d). Pre-treatment with SB-204741 (0.5 and 1.0 mg/kg/day) for 28 days significantly amplified NO ($P < 0.01$) level and GSH ($P < 0.001$) and SOD ($P < 0.001$) activities and attenuated TBARS ($P < 0.001$) level following isoproterenol-induced myocardial remodeling (Fig. 2a–d). As per TNF-α level and cardiac injury marker enzymes are concerned, treatment with SB-204741 dose dependently and significantly ($P < 0.001$) decreased serum TNF-α and CK-MB levels and augmented tissue LDH level as compared to ISO group (Fig. 2e–g).

SB-204741 inhibited inflammatory protein expression, upregulated autophagy and HSPs protein expressions in isoproterenol-induced myocardial remodeling in rats

To delineate the molecular role of SB-204741 we studied various protein expression changes in isoproterenol-insulted myocardium. Isoproterenol administration significantly ($P < 0.001$) amplified 5-HT_{2B} protein expression as confirmed by western blotting and immunohistochemistry analysis. Conversely, SB-204741 at all the doses significantly ($P < 0.001$) attenuated 5-HT_{2B} protein expression (Fig. 3a, 5A2–F2). Furthermore, reduced HSPs expression (αB-crystallin, Hsp27 and Hsp70), autophagy (Beclin-1 and LC3) and elevated inflammation (NF-κBp65, IKK-β, IL-6 and Cox-2) were also significantly ($P < 0.001$) normalized by SB-204741 treatment in the recovered myocardium as compared to the failing myocardium (Fig. 3a–d).

Moreover, to determine whether this cardioprotection was MAPKs dependent, we next assessed the phosphorylation of these proteins. Interestingly, we did not observe any significant change in p38, JNK and ERK protein expressions in any of the groups, though we observed significantly ($P < 0.01$) elevated expressions of p-ERK and p-ERK/ERK ratio and decreased expressions of p-p38, p-p38/p38 ratio, p-JNK and p-JNK/JNK ratio (Fig. 4a–c).

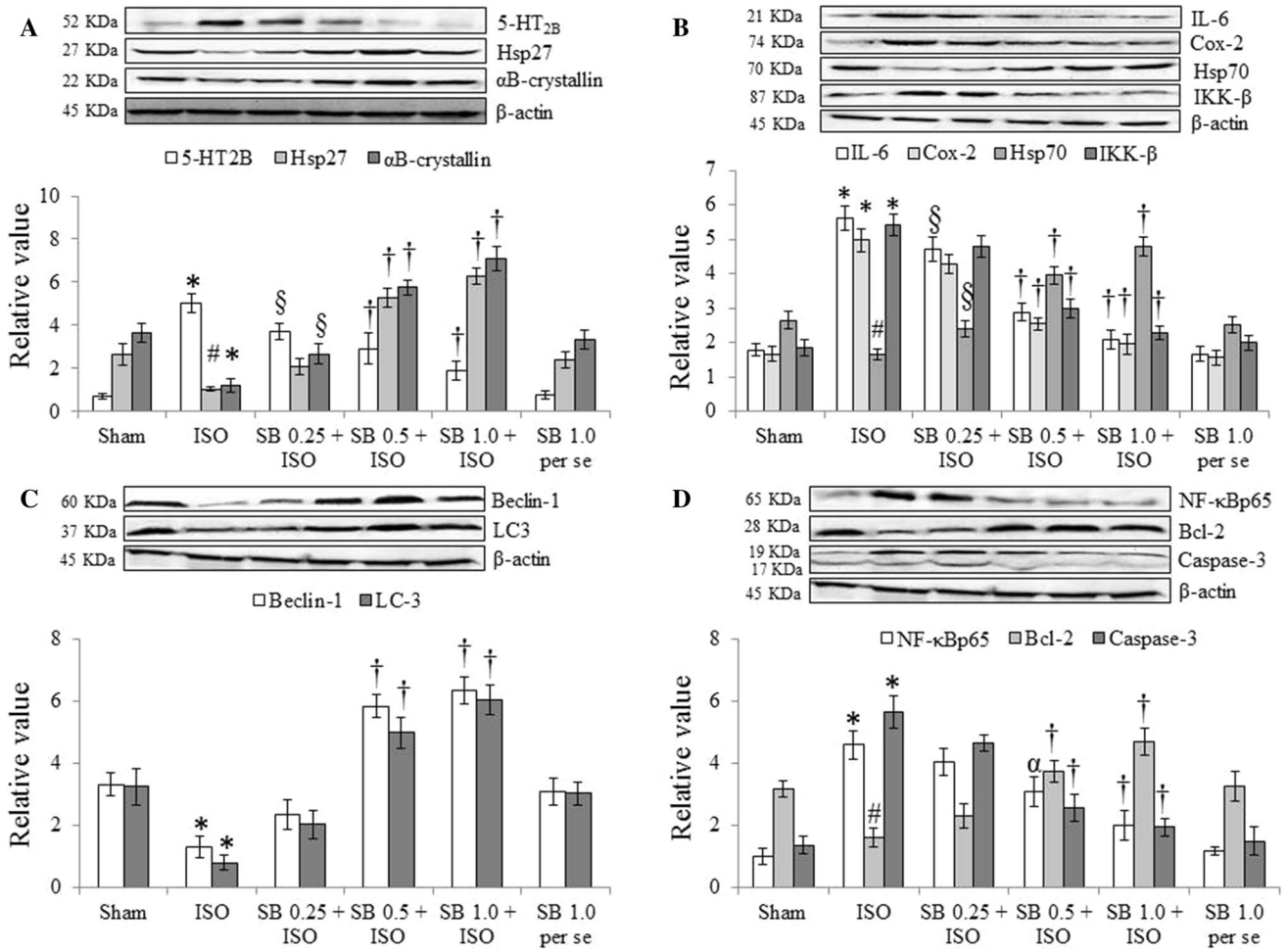

Fig. 3 Effect of SB-204741 on protein expressions following isoproterenol-induced myocardial remodeling. **a** 5-HT2B, Hsp27 and αB-crystallin; **b** IL-6, Cox-2, Hsp70 and IKK-β; **c** Beclin-1 and LC3 and **d** NF-κBp65, Bcl-2 and caspase-3. All values for protein expressions are expressed as mean ± S.D (n = 3/group). $^{\#}P < 0.01$, $^{*}P < 0.001$ versus sham and $^{\S}P < 0.05$, $^{\alpha}P < 0.01$, $^{\dagger}P < 0.001$ versus ISO

SB-204741 inhibits apoptosis in isoproterenol-induced myocardial remodeling in rats

Figures 3d, 4d and 6 illustrates that protein expression of cytochrome c, Bax and caspase-3 and cardiomyocyte DAPI/TUNEL positivity were markedly ($P < 0.001$) increased whereas protein expression of Bcl-2 was significantly ($P < 0.001$) decreased in isoproterenol-challenged failing hearts in comparison to sham group. Treatment with SB-204741 dose dependently normalized the aforementioned protein expressions and DAPI/TUNEL positivity in recovered myocardium though the maximum inhibition was achieved at the highest dose.

SB-204741 improves myocardial architecture in isoproterenol-induced myocardial remodeling in rats

To assess the tissue viability, effect of SB-204741 on histopathological and ultrastructural changes in rat myocardium were done. Figure 5A1 exhibited normal architecture of the myocardial fibers. Likewise, myocardial architecture of the per se group also did not show any myocardial damage (Fig. 5F1). Contrastingly, ISO group displayed focal and confluent necrosis of muscle fibers with inflammatory cell infiltration and edema along with extravasation of red blood cells (Fig. 5B1). Interestingly, SB-204741 (0.25–1.0 mg/kg/day) dose dependently improved myonecrosis with less edema and inflammatory cells and preserved architecture of myocardial fibers (Fig. 5C1–E1; Table 1).

Figure 5A3–F3 shows the effect of SB-204741 on ultrastructural changes in isoproterenol-induced myocardial remodeling. Sham group and per se group presented intact and integrated myofibrils with normal mitochondria and prominent Z bands (Fig. 5A3, F3). Isoproterenol-challenged rats resulted in mitochondrial dysfunction, disruption of cristae with vacuolation, distorted Z bands and nucleus exhibited chromatin condensation (hallmark feature of apoptosis) (Fig. 5B3). Treatment with SB-

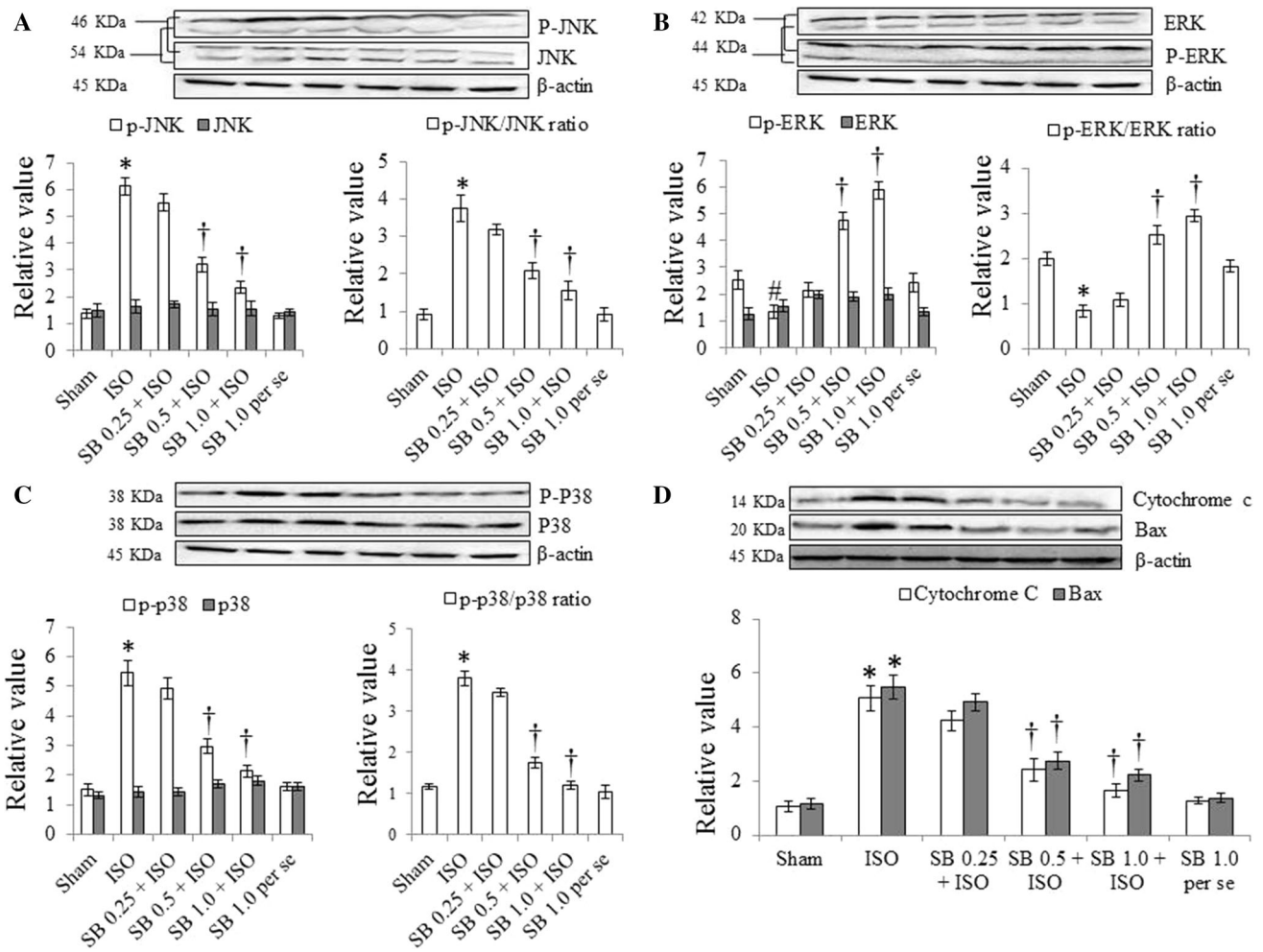

Fig. 4 Effect of SB-204741 on protein expressions following isoproterenol-induced myocardial remodeling. **a** p-JNK, JNK and p-JNK/JNK ratio; **b** p-ERK, ERK and p-ERK/ERK ratio; **c** p-p38, p38 and p-p38/p38 ratio and **d** cytochrome c and Bax. All values for protein expressions are expressed as mean ± S.D (n = 3/group). $^{\#}P < 0.01$, $^{*}P < 0.001$ versus sham and $^{\dagger}P < 0.001$ versus ISO

Table 1 Effect of SB-204741 on grading of histopathological changes in different experimental groups

Treatment groups	Myonecrosis	Inflammatory cells	Edema
Sham	−	−	−
ISO	+++	+++	++++
SB 0.25 + ISO	++	++	++
SB 0.5 + ISO	++	+	+
SB 1.0 + ISO	+	+	+
SB 1.0 per se	−	−	−

Score (−): absence of any myonecrosis, edema and inflammation; Score (+): focal areas of myonecrosis, edema and inflammation; Score (++): patchy areas of myonecrosis, edema and inflammation; Score (+++): confluent areas of myonecrosis, edema and inflammation; Score (++++): massive areas of myonecrosis, edema and inflammation (n = 8/group)

204741 (0.25–1.0 mg/kg/day) displayed milder myonecrosis, swelling, vacuolation and lesser nuclear condensation along with improved myofibril ultrastructure (Fig. 5C3–E3).

Discussion

This exploration into the role of SB-204741, a 5-HT_{2B}R blocker mediated cardioprotection reports a number of novel findings in abrogating the harmful effects of myocardial remodeling ensuing from reduced autophagy to augmented apoptosis, inflammation and oxidative stress. Intriguingly, administration of SB-204741 for 28 days

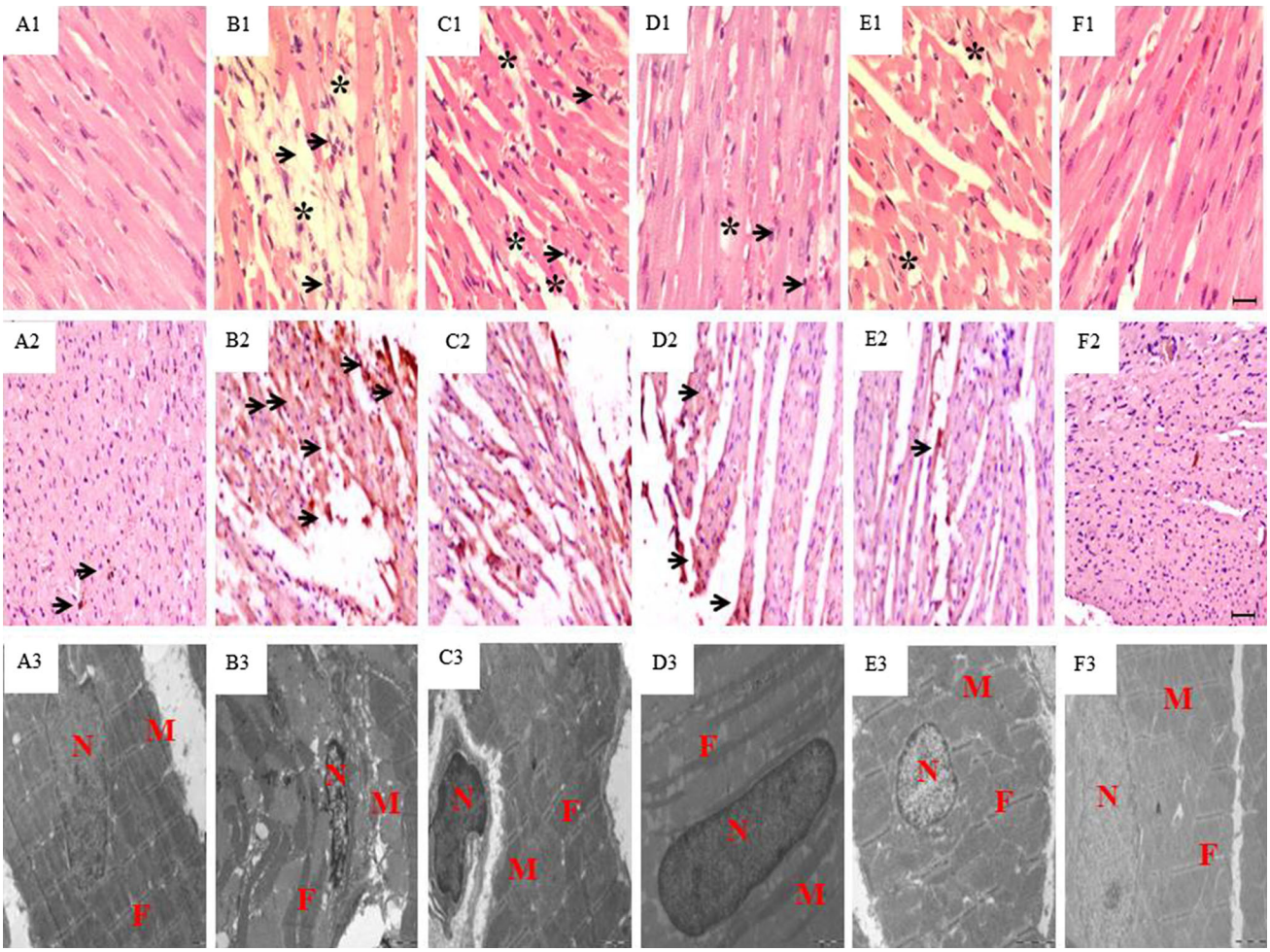

Fig. 5 Effect of SB-204741 on **A**1–**F**1 Light microscopic changes (40×, *Scale bar* 100 μm, *arrow* indicates inflammatory cells, *asterisk* indicates edema), **A**2–**F**2 5-HT_{2B} immunohistochemistry (10×, *Scale bar* 50 μm, *arrow* indicate protein expression); **A**3–**F**3 electron microscopic changes (4000×, *Scale bar* 1 μm, *N* nucleus; *M* mitochondria; *F* myofibrils) in different experimental groups. Sham group (**A**1–**A**3); ISO (**B**1–**B**3); SB 0.25 + ISO, SB 0.5 + ISO, SB 1.0 + ISO respectively (**C**1–**C**3, **D**1–**D**3 and **E**1–**E**3); and SB 1.0 per se (**F**1–**F**3)

negatively modulated the myocardial remodeling as substantiated through alleviated myocardial dysfunction (contractility, relaxability and preload), oxidative stress markers (TBARS, GSH and SOD), cardiac injury markers (CK-MB and LDH) and inflammatory markers (NF-κBp65, IKK-β, IL-6, Cox-2 and TNF-α). Of note, we also observed that this cardioprotective role of SB-204741 might be strongly attributed through inhibition of apoptosis via modulation of MAPKs/HSPs pathways.

In the present study and also in consonance with previous studies of our lab confirmed that isoproterenol, a synthetic catecholamine and β-adrenergic agonist insulted myocardium resulted in compromised hemodynamic and ventricular dysfunction [30, 32, 33]. Isoproterenol and 5-HT_{2B}R stimulation has shown to increase cytosolic calcium overload resulting in degradation of cardiac troponin T and I and subsequently contractile dysfunction [34–36]. Moreover, it has been proposed that overexpression of αB-crystallin, Hsp27 and Hsp70 regulates actin cytoskeleton dynamics, inhibits cytosolic calcium overload and improves ventricular filling and resistance to cardiac dysfunction in mice [4, 5, 8–10]. Intriguingly, in the present study SB-204741 significantly upregulated αB-crystallin, Hsp27 and Hsp70 protein expression in the recovered myocardium which might be one of the strong plausible mechanisms for its improved cardiac output. Apart from this, other probable pathways which might also shed a light on 5-HT_{2B}R blocker mediated improved ventricular dysfunction are direct inhibition of calcium channel by SB-204741, anti-hypertensive effect of SB-204741 and 5-HT_{2B}R blockade mediated regulation of blood pressure [16, 25, 37]. Notably, isoproterenol administration is linked with decreased autophagy which in part plays a major contributing factor towards isoproterenol-induced failing myocardium [33]. In our study, SB-204741

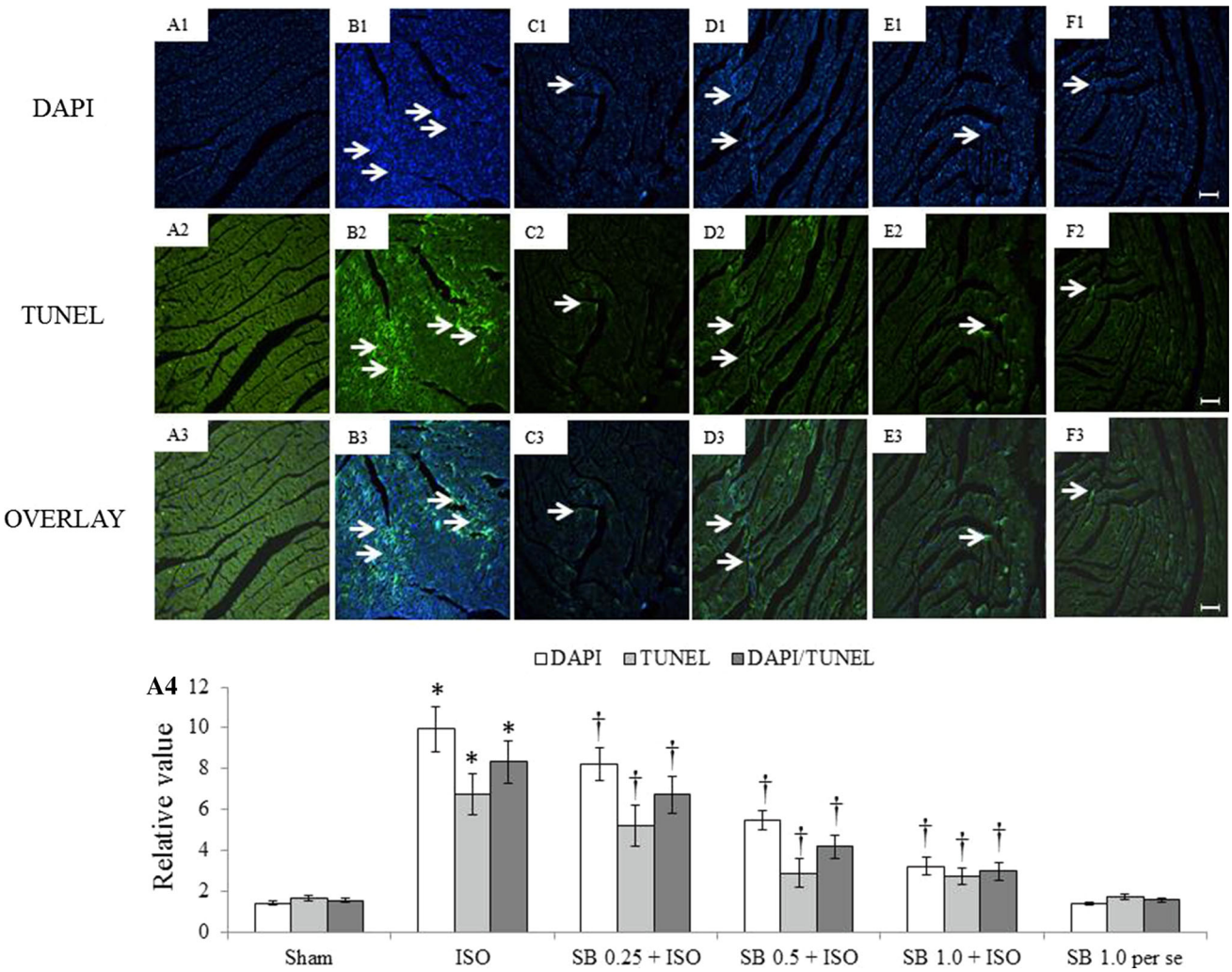

Fig. 6 Effect of SB-204741 on **A**1–**F**1 DAPI (20×, *Scale bar* 100 μm); **A**2–**F**2 TUNEL positivity (20×, *Scale bar* 100 μm); **A**3–**F**3 DAPI/TUNEL overlay (20×, *Scale bar* 100 μm) in different experimental groups. Sham group (**A**1–**A**3); ISO (**B**1–**B**3); SB 0.25 + ISO, SB 0.5 + ISO and SB 1.0 + ISO (**C**1–**C**3, **D**1–**D**3 and **E**1–**E**3 respectively); and SB 1.0 per se (**F**1–**F**3) and **A**4 quantification of cardiomyocyte DAPI and TUNEL positive nuclei. All values are expressed as mean ± S.D (n = 8/group). *Arrow* indicates apoptotic nuclei. *$P < 0.001$ versus sham and †$P < 0.001$ versus ISO

significantly increased the expression of autophagy markers (Beclin-1 and LC3), thereby amplifying the myocyte survival and conserving the ventricular functions [11, 12]. Thus, SB-204741 preserved myocardial performance not solely by improving myocardial contraction, preload and afterload reduction but correlation between MAPKs/HSPs and autophagy also contributes in overall functional improvement.

Crosstalk between MAPKs/HSPs, apoptosis and inflammatory signaling pathways has been well documented [3–10, 38, 39]. Administration of isoproterenol resulted in significant increase in IL-6/TNF-α/Cox-2 and NF-κBp65/IKK-β protein expression along with decrease in αB-crystallin, Hsp27 and Hsp70 protein expressions. Moreover, isoproterenol-induced stress, also initiates apoptosis pathway as the pro-apoptotic Bax outcompetes anti-apoptotic Bcl-2, thereby resulting in cytochrome c release from mitochondrial membrane to cytosol which in turn forms complex with apoptotic protease activating factor 1 and finally triggering caspase-3 [39]. Likewise, we have also found significantly increased protein expression of cytochrome c, Bax, and caspase-3 and decreased expression of Bcl-2, thereby, validating the apoptotic signaling pathway in isoproterenol insulted myocardium. SB-204741 administration significantly normalized the aforementioned markers in a dose dependent fashion thereby, making the myocardium less susceptible to apoptotic and inflammatory insult. The underlying reason could be due to fact that upregulation of HSPs/p-ERK or inhibition of p-p38/p-JNK by SB-204741 attenuates the recruitment of

inflammatory cytokines and mitigates apoptosis thus, resulting in reduced IL-6/TNF-α/Cox-2 and NF-κBp65/IKK-β protein expression and DAPI/TUNEL positivity. Furthermore, induction of HSPs or modulation of MAPKs by SB-204741 impedes the initiation of apoptotic signaling pathway which could otherwise lead to opening of mitochondrial permeability transition pore resulting in cytochrome c release and triggering the other pro-apoptotic factors such as caspase-9 and caspase-3 [3–10]. Consistently, direct inhibition of calcium influx by SB-204741 also constrains calcium dependent endonucleases resulting in reduced DNA fragmentation and apoptotic body formation, as evidenced by reduced DAPI/TUNEL positivity in our study, thereby resulting in increased myocardial survival [25]. In accordance with our study, Jaffré and colleagues has reported that isoproterenol-induced cytokine production has been abolished by 5-HT_{2B}R blocker/5-HT_{2B}R-knockout fibroblasts [40].

Apart from displaying anti-apoptotic potential, SB-204741 also amplified autophagy (increased protein expression of LC3 and Beclin-1) which was shown to be decreased by isoproterenol administration [33]. LC3 and Beclin-1 are two essential markers of autophagy that are critically involved in the recognition of autophagosomes and participate in autophagosomes formation. By doing this, they not only accelerate autophagy, a process of programmed cell survival, but parallely inhibit apoptotic body formation. In fact activation of autophagy by both these proteins have shown to inhibit apoptosis and NF-κBp65 and TNF-α transcriptionally [11, 12, 41, 42]. Likewise, our results also demonstrated inhibition of apoptosis and inflammation and induction of autophagy through SB-204741 mediated cardioprotection.

Notably, another important observation in this report is that SB-204741 dose dependently ameliorated myocardial dysfunction via inhibiting lipid peroxidation, bolstering NO level and anti-oxidant defense system through upregulation of GSH and SOD activities. The mechanistic approach behind the anti-oxidant effect could be due to inhibition of p-p38/p-JNK and induction of HSPs/p-ERK or autophagy which results in shutting down the central transcriptional signaling pathway that initiates oxidative stress, dismutation of superoxide radicals, and precluding the formation of hydroxyl and peroxynitrite radicals [3–10, 42, 43]. Importantly, per se effect of SB-204741 in upregulating the GSH and SOD could also be an apparent mechanism behind the cardioprotective effect as these proteins plays a significant role in maintaining the redox status of the myocardium [15, 25].

In summary, present study support the hypothesis that cross-talk between apoptosis and MAPKs/HSPs by SB-204741 is essential in salvaging isoproterenol-induced myocardial remodeling in rats. However, further clinical data is warranted to decipher the exact role of 5-HT_{2B}R blockade in myocardial remodeling.

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Conflict of interest None declared.

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