Impact of Sacubitril and RAAS inhibitors on p53 expression in rat-induced heart failure. A new approach for ischemic heart disease therapy

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ABSTRACT

Background: p53 is a well-known protein that prevents cancer formation, which is recognized as the main protein in the adaptation to many harmful stimuli, like oxidative stress. Among actions of p53, studies have shown that it has an important role in the development of heart failure (HF), and arteriosclerosis. Several clinical studies were done to investigate the role of p53 in the progression of HF and with the intention to improve management of heart failure.
Objective: The purpose of this work was to investigate the mechanisms of myocardial injury that precipitates heart failure that is mediated by both β-adrenergic signaling and p53, then compare the results with administered sacubitril and angiotensin system blockers.
Methods: Thirty female albino rats (aged around 8 months weighing 230g) in average were allocated into five groups; group I: served as a control group; group II: were injected with isoproterenol for HF induction; and groups III, IV, and V (HF treated groups) whereas rats received sacubitril alone, combination of sacubitril with ramipril and combination of sacubitril with aliskiren respectively, orally on daily basis.
Results: Results revealed that rats of group II (HF induced) were significantly (P = 0.002) showed more myocardial injury and higher nuclear p53 expression compared to rats of the control group. Furthermore, rats of group III, IV, V (HF treated groups) showed significantly (P = 0.037) less myocardial injury and significantly (P = 0.015) less nuclear p53 expression compared to rats of the group II.
Conclusions: It was concluded that rats received either sacubitril alone or with combination of ramipril or aliskiren for HF treatment were alleviated myocardial injury and lower nuclear p53 expression.
It was concluded that anti-p53 approach may provide a novel therapeutic strategy for human ischemic heart diseases and myocardial infarction.

Keywords: p53, Isoprenaline, Sacubitril, Heart failure.

تاثير ساكيوباتريل و مانعات الراس علي P53 فى الفئران المحرضه لقصور القلب. نهج جديد للاستراتيجية العلاجية لأمراض نقص تروية القلب

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الخلاصة

الخلفية: ان p53 هو واحد من أكثر البروتينات شهرة و مثبط كبيرًا للأورام ، وهو معروف كجزيء رئيسي في التكيف مع مجموعة متنوعة من المحفزات الضارة ، بما في ذلك نقص الأوكسجين والجهد التأكسدي. في الجهاز القلبي الوعائي ، ظهر أن p53 له وظيفة حاسمة في تطور قصور القلب ، وتصلب الشرايين. تم إجراء العديد من الدراسات السريرية لاستقصاء وتوضيح الفسيولوجيا المرضية لـقصور القلب وتطوير علاجات فعالة.
الهدف: الهدف من هذه الدراسة هو التحقيق والتحري في آليات إصابة عضلة القلب التي تؤدي إلى قصور القلب التي يسببها كل من الإشارات الأدرينالية β-adrenergic و p53 ، ثم مقارنة النتائج مع استعمال الادويه العلاجيه Sacubitril، aliskiren و ramipril.

طرق العمل: في هذه الدراسة ، تم تخصيص 30 أنثى من فئران الوستر البيضاء في خمس مجموعات ؛ المجموعة الأولى: استخدمت كمجموعة مراقبه ضابطة وتم حقنها بمحلول ملحي متساوي التوتر ومن ثم تلقيت علاجًا باستعمال دواء وهمي ؛ المجموعة الثانية: استخدمت كمجموعة مراقبه إيجابيه ؛ اذ حقنت بمادة الايزوبرينالين (ايزوبروترينول) لتحريض قصور القلب ؛ والمجموعات الثالثة والرابعة والخامسة (المجموعات المعالجة لقصور القلب) حيث تلقت الفئران جرعات يومية عن طريق الفم من sacubitril فقط او خليط من sacubitril مع ramipril او خليط من sacubitril مع aliskiren، على التوالي.
النتائج: أظهرت النتائج أن الفئران من المجموعة الثانية (التي عرضت لـقصور القلب) اظهرت معنويا (P = 0.002) إصابة أكبر في عضلة القلب وتعبير نووي اعلى لp53 مقارنة بفئران مجموعة المراقبه. وعلاوة على ذلك ، أظهرت الفئران من المجموعة الثالثة والرابعة والخامسة (مجموعات معالجة قصور القلب) بشكل ملحوظ (P = 0.037) أقل إصابة بعضلة القلب وأقل (P = 0.015) تعبير للبروتين p53 النووي مقارنة مع الفئران من المجموعةالثانيه.
الاستنتاجات: استنتج أن الفئران التي تلقت إما السيسبيتريل sacubitril بمفرده أو مع خليط من العلاج بالراميبريل أو الأيسكيرين ramipril or aliskiren خففت من إصابة عضلة القلب وانخفاض في تعبير p53 النووي.
وكما خلصت الدراسه إلى أن انتهاج تقنية استعمال ادويه ضد السماح لتعبير P53 قد توفر استراتيجية علاجية جديدة لأمراض نقص تروية القلب البشرية واحتشاءعضلة القلب.

الكلمات المفتاحية: الفئران, قصور القلب, P53, ساكيوباتريل, ايسوبرينالين.

INTRODUCTION

The main function of the heart is to provide enough force to pump blood to various parts of our body so as oxygen and nutrients are provided. A defect in the function of the heart may cause critical negative consequences to the human body. Recent studies recognized p53, which is suppressing tumor formation, to have an important role in the progression of heart failure. The main function of p53 is that it induces a group of molecules that cause programmed cell death, arrest cell growth, and inhibit angiogenesis [1]. Heart failure (HF) is a major cause of morbidity and mortality worldwide [2]. p53 is a gene that plays a big role in the expression of many other genes. Recognizing the genes that play major roles in maintaining cardiac tissue homeostasis is considered of great importance [3]. Normally, the expression of p53 is maintained by cells at its minimum levels by a certain protein which is called mouse double minute 2 homolog (MDM2). During an acute stress, mouse double minute 2 homolog is inactivated and the enhanced p53 expression will inhibit cell division and induce programmed cell death [4]. Myocyte programmed cell death is a well-known pathological phenomenon that happens in several types of cardiac diseases [5]. p53 induces apoptosis and cell growth retardation through stress pathway [6]. Apoptosis is a process that control programmed cell death following a particular cellular challenge [7]. Isoprenaline, or isoproterenol, is a drug used for the management of heart block. It's a catecholamine that non selectively activates β-adrenoreceptor [8]. Sacubitril, after metabolism, blocks a neutral endopeptidase (neprilysin) which is responsible for terminating the action of natriuretic peptides [9] Aliskirin is acting through inhibiting of active sites of renin enzyme thereby reducing the activation of renin angiotensin aldosterone system which plays an important role in deteriorating heart failure condition, whereas ramipril is acting through inhibiting the enzyme responsible for converting angiotensin I into angiotensin II [10].

METHODS

Animals

Thirty female albino rats (5 to 6 months old, weighing 240–285 g) were used in this study. Rats were kept in rat cages in the animal facility at Hawler Medical University, rats were having free access to food and water. The animal room was programmed on half-day of light-dark cycles at 20± 5°C and 20 - 30% humidity.

Ethics Statement:

The ethics committee at the university has approved ethics measures for this work numbered 180502171.

Experimental protocol:

Study design

The rats were allocated into five groups with Completely Randomized Design (CRD) with 6 rats each (*n* = 6):
1-Group I: control group.
2-Group II: positive control group; heart failure was induced in this group of rats with intraperitoneal isoproterenol 5 mg/kg for seven days. Rats were then administered placebo for 14 days [11].
Heart failure was induced in groups III, IV, and V rats using same mentioned method that has been used for rats in group II. Then:
3-Group III: treated with sacubitril 30 mg/kg/day for two weeks [12].

4-Group IV: treated with sacubitril 30 mg/kg/day-ramipril10mg/kg/day orally for 2 weeks [13].
5-Group V: treated with sacubitril 30 mg/kg/day-aliskiren 10 mg/kg/day orally for 2 weeks [14].
Histopathological evaluations: myocardial tissue was fixed in 10% formalin. Blocks of the specimens were prepared using paraffin and then fine slices were made using microtome (6µm) and placed on cleaned glass slides, then sections were stained with hematoxylin and eosin. The slides were examined using light microscope under different powers in histopathology laboratory at college of pharmacy, Hawler Medical University.
Semiquantitative scoring systems for myocardial injury

The severity and level of myocardial injury were observed for each case. The observations were divided into the below grades, in order to compose a range of histologic myocardial injury: (0) No change: (1) Mild - focal myocyte damage or small multifocal degeneration with slight degree of inflammation and fibrosis (2) Moderate - myofibrillar degeneration with moderate inflammatory process and fibrosis, (3) Severe - myocardial injury with diffuse inflammatory and fibrosis process [15, 16].
p53 immunoscore measurement:

The fraction of p53 positive nuclei as p53 immunohistochemical scoring system was implemented. The fraction of p53 immunoreactive cells was scored as 0 to 3+ in p53 positive regions. Nuclear p53 expression in ≥ 10% of the cells was scored as over expression, and were scored according to the 3-tiered system as following < 10% -, 10%-30% +, 31%-50% ++, and > 50% +++ [17].

Mouse Monoclonal anti-p53, Clone BP-53-12 pre dilute Antibody, Ready-To-Use, the method according to Dako recommendation was used to stain tissue by Anti- p53 antibody.

Statistical Analysis

Statistical Analysis was done by using GraphPad Software statistical package computer software. Contingency tables have been formed and built up to figure out the association between biomedical variable investigated. Fisher's exact test was used to calculate the significant differences between studied variables. A probability value of less than 0.05 was considered statistically significant [18].

RESULTS

Results revealed that rats of group II (HF induce group) significantly (P = 0.002) showed more myocardial injury compared to rats of the control group I. Two 2 rats in group 2 showed moderate myocardial injuries as well as 4 rats showed severe myocardial injury with diffuse inflammatory process. Furthermore, rats of group III, group IV, and group V, (HF treated groups) showed significantly (P = 0.037) less myocardial injury compared to the rats of the group II. Three rats of groups III, IV, and V showed mild myocardial injury, mild degree of inflammation and mild fibrosis and 3 rats showed moderate myocardial injury with moderate inflammatory process and moderate fibrosis (Figure1). These results indicate that injection rats with isoprenaline (isoproterenol) then received either sacubitril alone or with a combination of ramipril or aliskiren alleviated myocardial injury (Table 1).

Table 1. Myocardial injury scoring and extent of Severity of myocardial injury

Myocardial injury scoring	No change	Mild (1)	Moderate (2)	Severe (3)	*P value*
Control group (I)	6	0	0	0	0.002
Group (II)	0	0	2	4	
*Groups III, IV, and V	0	3	3	0	0.037

* Mean number of rats of groups III, IV, and V.

Immunohistochemical expression of p53 is presented in Table 2. Rats of group II (HF induce group) significantly (P = 0.002) showed higher nuclear p53 expression compared to the rats of the control group I. All 6 rats in group II showed (10%-30%) nuclear p53 expression compared to < 10%, for rats of control group. Moreover, rats of group III, group IV, and group V, (HF treated groups) showed significantly (P = 0.015) less nuclear p53 expression compared to the rats of the group II. Five rats showed < 10% and just one rat showed 10%-30% nuclear p53 expression, compared to 6 rats of group II with 10%-30% (Figure2). These results indicate that injection rats with isoprenaline (isoproterenol) then administered either sacubitril alone or with combination of ramipril or combination of aliskiren reduce nuclear p53 expression (Table 2).

Table 2. Immunohistochemical expression of p53.

Nuclear p53 expression	- (< 10%)	+ (10%-30%)	++ (31%-50%)	+++ (> 50%)	*P value*
Control group (I)	6	0	0	0	
Group (II)	0	6	0	0	0.002
*Groups III, IV, and V	5	1	0	0	0.015

* Mean number of rats of groups III, IV, and V.

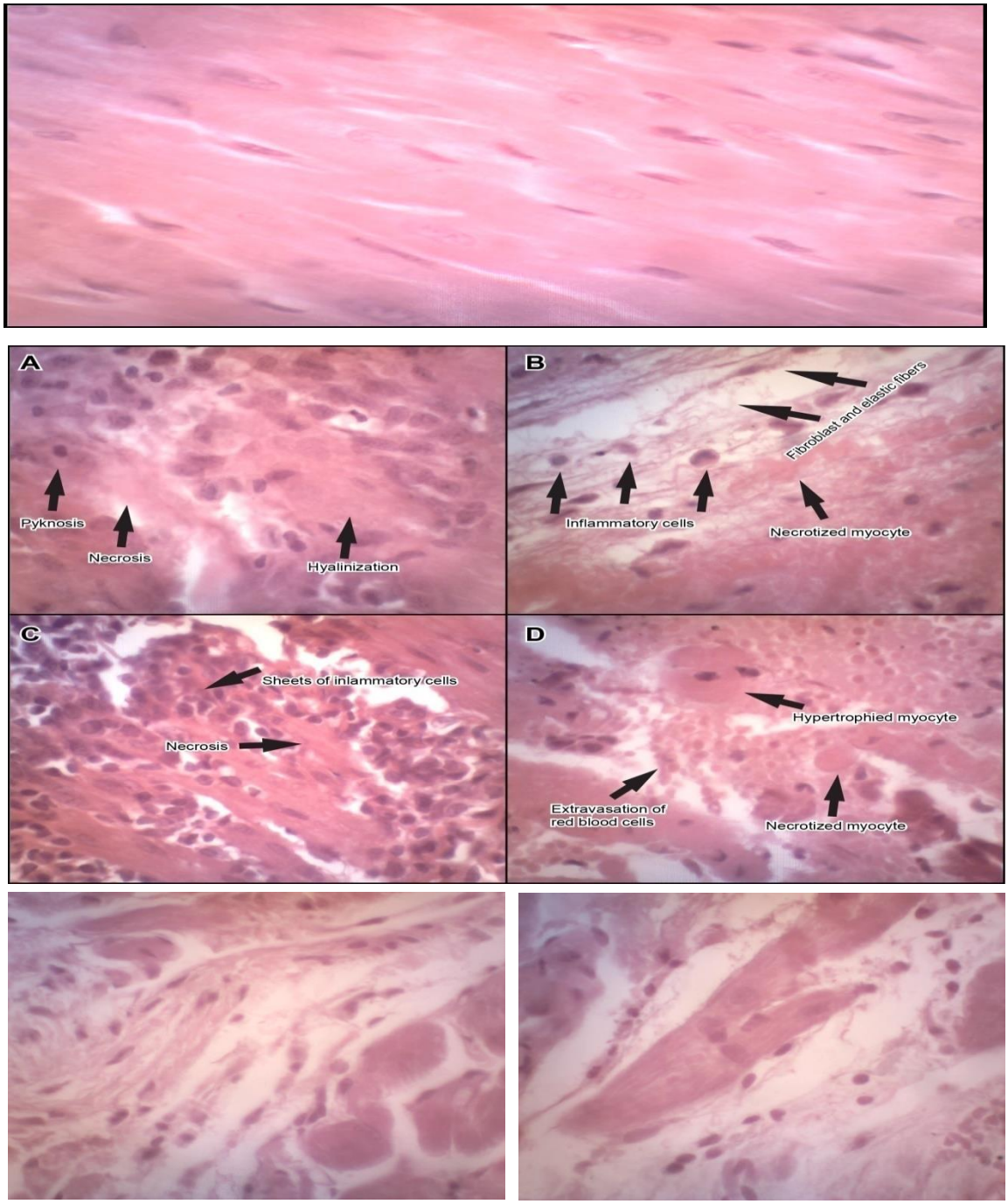

Figure1: Histological examination of myocardial tissue, hematoxylin and eosin stain. (A) group I (control group)showed normal architecture (B,1,2,3,4) group II (HF induce group) showed myocardial cells death and degeneration ,hyalinization of muscle fibers ,inflammatory cells infiltration and fibrosis (C) group III, group IV, and group V, (HF treated groups) showed reduced myocardial damage ,less inflammatory cells infiltration and remodeling of the fibrotic change with cardioprotective effect (400x)

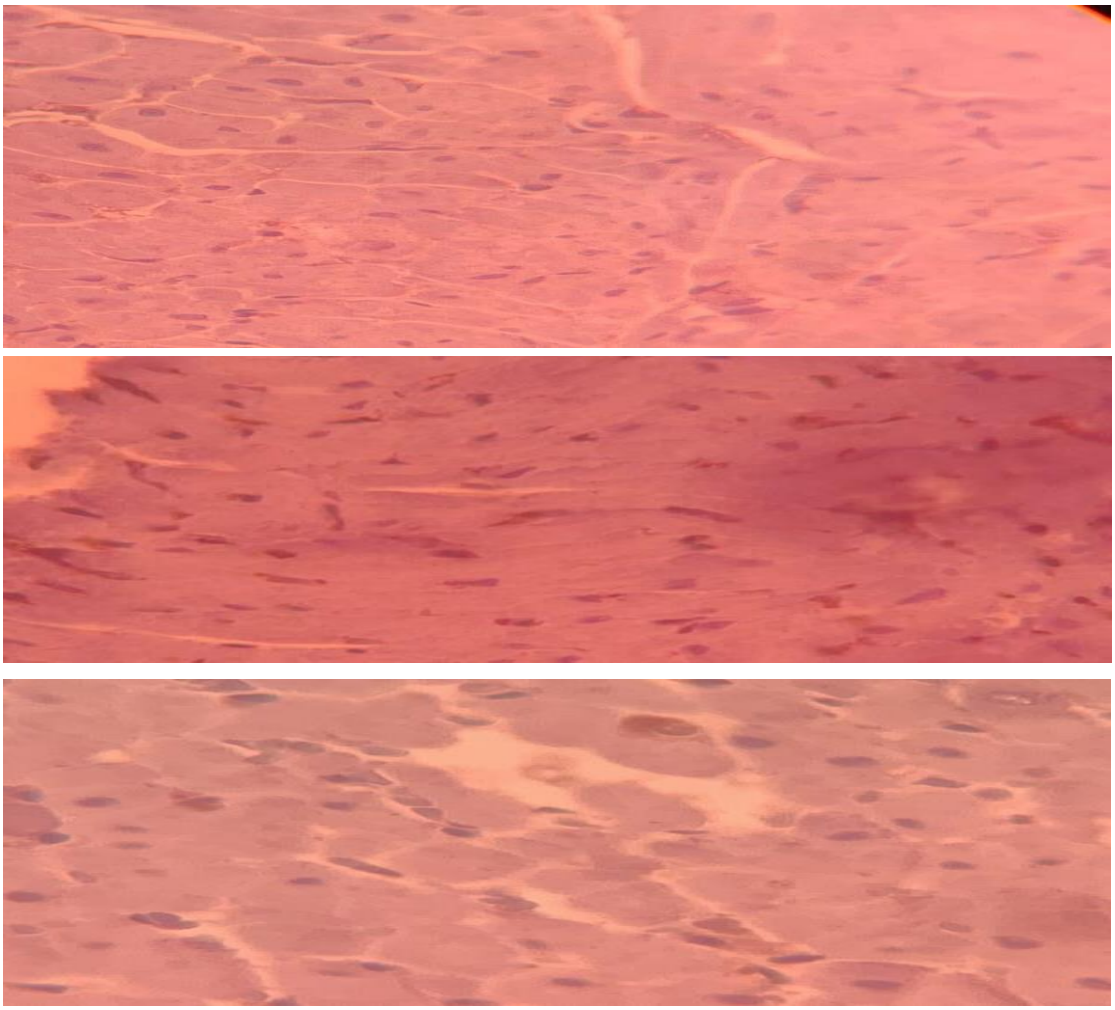

Figure2: Expression of P53determined by immunoreactivity in representative sections of myocardial tissue. (A) group I (control group)showed nuclear p53 expression scored < 10%(-) (B) group II (HF induce group) showed nuclear p53 expression (10%-30%) (+) (C) group III, group IV, and group V, (HF treated groups) showed nuclear p53 expression scored < 10%(-) (400x).

DISCUSSION

Recognizing the factors that regulate and preserve cardiac tissue function is of clinical and scientific importance. It was reported that induction of cardiomyopathy by isoprenaline intraperitoneally revealed Cardiomyocyte injury, inflammatory response with a predominance of macrophages within rat myocardial tissue [19, 20], which is similar to our result which showed that isoproterenol caused moderate to severe myocardial injuries and inflammatory changes and also agreed with Heraldo *et al* results [15]. Other report also found that isoproterenol induced myocardial ischemia, cardiotoxicity and cardiac fibrosis [21].

The result of this study found that rats of group II (HF induce group), where rats injected with isoproterenol showed cardiac hypertrophy, which is in agreement with others who found that Neuroglobin affects cardiac hypertrophy though including p53-mediated apoptosis [7]. Furthermore, interstitial fluid was also observed in rats of group II while less interstitial fluid was notice in HF treated groups.

This result was similar to that reported earlier [22]. Also, it was noticed in this investigation that interstitial fibrosis with collagen fibers were more prominent in group II, in contrast to myocytes from HF treated groups (Group III, IV, and V), where rats had myofibrils interspersed with few collagen bundles indicating remodeling effect, which confirms other report [23]. On the other hand, study indicated that *sacubitril combined with* other medications improved the imbalance between the renin and natriuretic peptide systems, which may due to the inhibition of inflammation response of macrophages which might have a cardioprotective effect [24]. It was reported that sacubitril and valsartan protected myocardial infarction through inhibiting neprilysin in isoproterenol- induced myocardial infarction ameliorating oxidative damage in rats [25].

Result of this study revealed that p53 protein was minimally stained in control group of less than 10% of the cardiomyocytes. In contrast, p53 positive stained nuclear was significantly increased in group II cardiomyocytes (HF induced group) of rats with severe inflammatory changes, while p53 expression reduced to less than 10% in HF treated groups III, IV, and V. Similar finding was found [26]. Moreover, p53 was shown to be upregulated when coexists with isoproterenol in rat cultured myocardial tissue [27]. Protein level of p53 is also kept low in myocardial tissue but it increased when cardiac cells exposed to hypoxia [28]. p53 blocks cell growth during stress by modifying gene transcription. Another vital question that could be raised is how the level of p53 activity could play a role in the protection against external stressing events in addition to provide a possible novel therapeutic technique [3]. Increased p53 expression is related with myocardial tissue programmed cell death in advanced heart failure [25]. Other study suggested that deletion or inhibition of p53 activity can prevent or reduce the occurrence of heart failure [29].

So it means that there is an association between p53 upregulation and human HF [30]. Su et al findings revealed a novel regulatory mechanism of ROCK1/p53/NOXA signaling in modulating myocardial apoptosis in vitro and with congenital cardiac defects in vivo [31]. Recently, the roadblocks in charge of adult myocyte cell cycle cessation located at the center of developing regenerative therapies for cardiovascular diseases. Therefore, it is suggested that blockade of p53/Mdm2-regulated mitochondrial RNAs would promote the expression of cell cycle activators leading to proliferation of adult murine cardiomyocytes [32]. It was stated that the cell deaths of differentiated cells, directly influence tissue function. This may lead to a clue for a novel therapy working through counter acting apoptosis processes [33]. Apoptosis constitutes an important event in the pathogenesis of HF [34]. Similarly, it has been suggested that anti-p53 strategy would be effective in preventing ischemic injury and in myocardial infarction treatment [35]. Enhancing the effect of anti-p53 mediates a beneficial effect on myocardial function. and inhibiting cell death in failing myocardium is considered as an approach to prevent and manage HF [36].

CONCLUSIONS

This study confirmed the suggestion that p53 acts as a regulator protein for cardiac function and structure. It was concluded that anti-p53 approach may provide a novel therapeutic strategy for human ischemic heart diseases and myocardial infarction.

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