BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902022e20978

Article

Amlexanox Exhibits Cardioprotective Effects in 5/6 Nephrectomized Rats

Rasha Mohamed Sabry Mohamed Mohamed[1]*, Shimaa Mostafa Elshazly[2], Nevertyty Mohamed Mahmoud[1]

[1]Clinical Pharmacology Department, Faculty of Medicine, Zagazig University, Egypt
[2]Pharmacology and Toxicology Department, Faculty of Pharmacy, Zagazig University, Egypt

Cardiorenal syndrome is a life-threatening condition. The aim of the current study was to determine the cardioprotective effects of amlexanox in 5/6 nephrectomized rats. Rats were randomly assigned to three groups: sham, 5/6 nephrectomized rats, and amlexanox-treated 5/6 nephrectomized group. Amlexanox (25 mg/kg/day, i.p.) administration was started just after surgery and continued for 10 weeks. After treatment, kidney function (serum creatinine and urea) and blood pressure (systolic and diastolic) were measured. Heart weight (normalized to tibial length) and fibrosis area percentage were measured. Serum brain natriuretic peptide (BNP, heart failure marker) and cardiac levels of β1-adrenergic receptor (β1AR), β-arrestin-2, phosphatidylinositol-4,5-bisphosphate (PIP2), diacylglycerol (DAG), pS473 Akt (a survival marker), and caspase-3 activity (an apoptosis marker) were also measured. The 5/6 nephrectomy caused renal impairment, cardiac fibrosis, apoptosis, and heart failure indicated by down-regulation of cardiac β1AR down-stream signals compared with those in the sham group. Interestingly, amlexanox significantly reduced all cardiopathological changes induced after 10 weeks of 5/6 nephrectomy. Amlexanox showed potent cardiac antifibrotic and antiapoptotic effects in 5/6 nephrectomized rats, which were associated with reduced heart failure. To our knowledge, this is the first study that addresses the potent in vivo cardioprotective effects of amlexanox.

Keywords: Amlexanox. Cardiorenal syndrome. β-Arrestin-2.

INTRODUCTION

Cardiorenal syndrome (CRS) is a term used to describe the mutual effects of both cardiac and renal injury on each other (Ronco *et al.*, 2008). CRS is classified into five types, according to the organ where the injury is initiated. In the current study, we induced type 4 CRS, also known as chronic renocardiac syndrome, by performing 5/6 nephrectomies in rats (Suresh *et al.*, 2017). This type of CRS is usually associated with heart failure, cardiac hypertrophy, and cardiac remodeling (Suresh *et al.*, 2017).

The exact mechanisms by which the heart and kidney affect each other are not completely understood. Reports indicate central venous congestion (Khoury *et al.*, 2018), neuro-hormonal elaboration (Vinod *et al.*, 2017), anemia (Pallangyo *et al.*, 2017), oxidative stress (Carlstrom, Montenegro, 2019), and renal sympathetic activity (Yu *et al.*, 2017) as potential contributors to this complex syndrome. Within this context, β-blockers, angiotensin receptor blockers, or a combination of both are potential treatments for this syndrome.

β-Adrenergic receptors (βARs) are G-protein-coupled receptors that include three subtypes: β1, β2, and β3ARs (Velmurugan, Baskaran, Huang, 2019). β1 and β2ARs are the main regulators of sympathetic activity in myocardial cells through coupling with Gαs protein (Yang *et al.*, 2020). Notably, chronic stimulation of the cardiac β1AR is highly correlated with cardiomyocyte apoptosis and heart failure. Similarly, there is a strong correlation

***Correspondence:** R. M. S. M. Mohamed. Clinical Pharmacology Department. Faculty of Medicine. Zagazig University. Zagazig, 44519 Egypt. Phone: +200552303266. E-mail: rmsabry@medicine.zu.edu.eg. ORCID: 0000-0002-4173-4399

between the decreased density of the β1AR and cardiac dysfunction (Boccella, Paolillo, Perrino, 2019).

Down-regulation of βAR is mediated by a group of proteins known as G-protein-coupled receptor kinases (GRKs), which are classified into seven subtypes (GRK1 to GRK7) (Hattori, Michel 2019). GRK2 and GRK5 are the main GRKs expressed in the myocardium that mediate desensitization and down-regulation of β1- and β2-ARs, respectively. Both β1- and β2-ARs are up-regulated during heart failure (Liggett *et al*., 2008). Moreover, the process of desensitization involves translocation of cytoplasmic proteins, known as β-arrestins, to the cell membrane, resulting in uncoupling of the G-proteins and internalization of βARs (Hattori, Michel 2019). Thus, β1AR can be desensitized by the action of GRKs and β-arrestins (Hattori, Michel 2019).

β-Arrestins are classified into two types, β-arrestin-1 (major form) and -2 (minor form) (Ungerer *et al*., 1994). In the heart, the two isotypes mediate opposing effects. β-Arrestin-1 is cardiotoxic; it desensitizes β1AR and induces cardiomyocyte apoptosis and inflammation (Bathgate-Siryk *et al*., 2014). On the contrary, β-arrestin-2 does not desensitize β1AR and mediates antiapoptotic and anti-inflammatory effects (McCrink *et al*., 2017). Moreover, β-arrestin-2 competes with β-arrestin-1 for β1AR binding sites, reducing the cardiotoxic effects of β-arrestin-1 (McCrink *et al*., 2017).

Amlexanox is a pyridochromene-derived monocarboxylic acid that has anti-inflammatory, anti-allergic, and immunomodulatory effects. Amlexanox is currently used as an oral treatment for aphthous ulcers (Bell, 2005) via an unknown mechanism. Amlexanox is no longer used in the United States. On the other hand, in Japan, amlexanox is approved for the treatment of allergic diseases, based on its mast cell stabilization activity (Mucke, 2018). Recent studies have shown that amlexanox is a GRK5 inhibitor (GRK5i) (Homan *et al*., 2014) and a specific inhibitor of non-canonical inhibitory kappa B kinases (IκB kinases) (Reilly *et al*., 2013). Based on the potential role of amlexanox as a GRK5 in heart failure, we designed this study to determine the cardioprotective effects of amlexanox in 5/6 nephrectomized rats, as a model of CRS.

MATERIAL AND METHODS

Animals

Adult male Wistar rats weighing 240 to 260 g were purchased from the Faculty of Veterinary Medicine, Zagazig University, Egypt. Rats were distributed (three per cage) and acclimated for 1 week. Rats had free access to food and water. Temperature, humidity, and light/dark cycles were kept constant at 23°C ± 2°C, 60% ± 10%, and 12/12 h, respectively. All animal handling procedures were approved by the Ethical Committee for Animal Handling at the Faculty of Pharmacy, Zagazig University, Egypt, with approval no. P4-8-2017.

Drugs and experimental design

Rats were randomly divided into three groups: sham (n = 10), 5/6 nephrectomy (n = 16), and amlexanox (n = 12). In the sham group, rats were subjected to renal evacuation, decapsulation, and return of the intact kidney into the abdominal cavity performed bilaterally in a 2-week interval. In the 5/6 nephrectomy, and amlexanox groups, rats were subjected to nephrectomy as described below. After surgery, rats in the amlexanox group received amlexanox (25 mg/kg/day, i.p., Sigma, St. Louis, MO) (Mohammed *et al*., 2019) for 10 weeks beginning after surgery. Amlexanox was dissolved in dimethyl sulfoxide (DMSO, 60 mg/ml) and diluted with saline to a final volume of 2 ml/rat (5% final concentration). Also, the sham and 5/6 nephrectomy groups received 5% DMSO in the same volume and sequence as the amlexanox-treated rats (Figure 1).

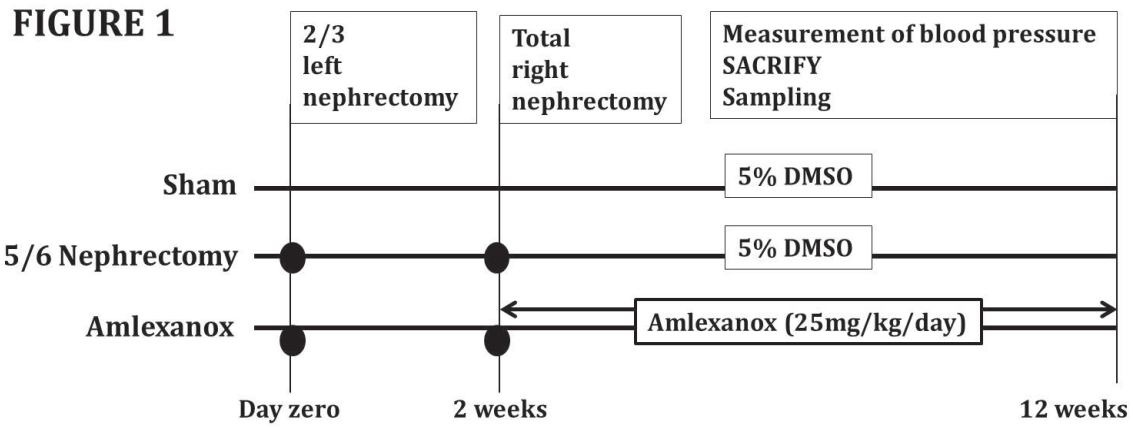

FIGURE 1 - Experimental design.

Induction of renal failure by 5/6 nephrectomy of total renal mass

The left kidney was exposed through a lateral dorsal incision and decapsulated. The renal vessels were clamped, and both poles (2/3 of the functional kidney mass) were dissected. The cut surface was treated with instant glue to stop bleeding. The vessel clamps were removed, and the renal stump was returned to the abdominal cavity. Two weeks later, the right renal vessels were ligated, and a total right nephrectomy through a lateral dorsal incision was done to achieve a 5/6 reduction. All operations in the sham and 5/6 nephrectomy groups were performed using sodium pentobarbital (100 mg/kg, i.p.) as an anesthetic. All procedures were performed under strict aseptic conditions, and special care was taken to prevent damage to the adrenals during surgery. The incisions were sutured in two layers and covered by instant glue. After each operation, rats were treated with a one-time application of antibiotic and housed individually with free access to food and water. Renal failure was significant 10 days after surgery, whereas heart failure and cardiac remodeling were significant 10 weeks after surgery (Švíglerová *et al*., 2010).

Measurement of blood pressure

Blood pressure was monitored using a tail-cuff blood pressure measuring system. Blood pressure was monitored from 10 AM to 12 noon by a non-invasive tail-cuff method (Harvard Apparatus Ltd, Edenbridge, Kent, England). Rats were trained with the instrument for 1 week before initiation of the experiment and were conscious during the measurement. Rats were placed in a heated restrainer at 37 °C ± 1°C for 10 min. Blood pressure was measured at least three times for each rat, and the average was reported.

Collection of blood and serum separation

At the end of the experiment, all animals were anesthetized with urethane (1.3 g/kg, i.p., Sigma-Aldrich, USA). Blood samples were obtained from the orbital sinus of fasted rats. Blood samples were centrifuged, and the separated serum was stored at −20°C until use.

Collection of heart samples for laboratory analysis

At the end of the experiment, rats were euthanized by decapitation. Part of the heart sample was immersed immediately in liquid nitrogen and stored at −80 °C until analysis. The other part was processed for histopathological examination.

Colorimetric assays

Serum creatinine and serum urea were measured using commercially available quantitative colorimetric assay kits obtained from Biodiagnostic Co. (Egypt). Cardiac caspase-3 activity was measured using a

caspase-3 activity assay kit (Sigma Aldrich, USA). All procedures were performed according to the manufacturers' instructions.

Enzyme-linked immunosorbent assays (ELISA)

Serum levels of brain natriuretic peptide (BNP, Peninsula Laboratories, Bachem Group, USA), cardiac levels of β1-adrenergic receptor (β1AR, Biomatik, Ontario, Canada), β-arrestin-2 (Life Span Biosciences, Inc.), phosphatidylinositol-4,5-bisphosphate (PIP2, Echelon Biosciences, Inc., Salt Lake City, UT), diacylglycerol (DAG, Wuhan EIAab Science Co., Ltd, China), and phosphorylated AKT S473 (Kit-3997, DRG International, Inc., USA) were measured using rat ELISA kits. All procedures were performed according to the manufacturers' instructions.

Histopathology

Part of the heart was harvested, fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 5-µm sections. Sections were stained with Mallory trichrome staining. Sections were assessed and quantified by digital image analysis using the computer software Scion Image Beta 4.03 (Scion Corporation, Frederick, MD, USA) to detect the extent of fibrosis.

Statistics

Data are presented as mean ± SE. Statistical analysis was performed using GraphPad Prism version 5 (GraphPad Software, Inc., CA, USA). Groups were compared using one-way ANOVA and post-hoc Tukey test. P-values <0.05 were considered significant.

RESULTS

Effects of amlexanox on survival rate, kidney functions, and diastolic and systolic blood pressures in 5/6 nephrectomized rats

As presented in Table I, amlexanox improved the 10-week survival of 5/6 nephrectomized rats. As shown in Figure 2 and Table II, serum creatinine (0.98 ± 0.02 vs. 0.57 ± 0.01 mg/dl after 10 weeks of surgery) and urea (125.4 ± 6.5 vs. 24.17 ± 2.2 mg/dl after 10 weeks of surgery) increased significantly in the 5/6 nephrectomized rats than in the sham group. Interestingly, both creatinine (19% decrease, Figure 2A) and urea (27% decrease, Figure 2B) were significantly lower in amlexanox-treated rats than in 5/6 nephrectomized rats. Moreover, both diastolic and systolic blood pressures (8% and 23% decrease, Figures 2C and 2D, respectively) were significantly lower in amlexanox-treated rats than in 5/6 nephrectomized rats.

TABLE I - Rat survival by the end of the experiment

Groups	Rat survival	
	Day 0	10th week
Sham	10	10
5/6-nephrectomized rats	16	13
Amlexanox	12	11

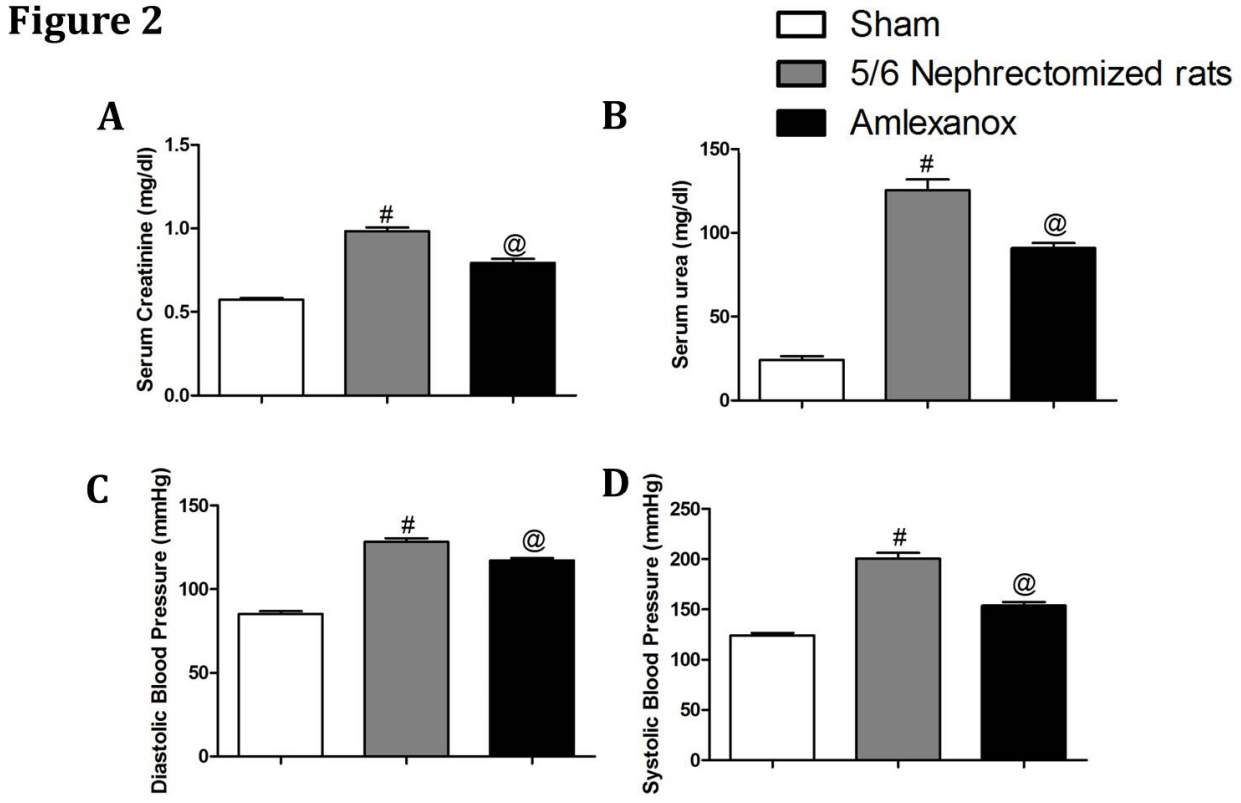

FIGURE 2 - Changes in kidney function and blood pressure 10 weeks after surgery. Quantitative analysis of serum creatinine **(A)** and serum urea **(B)**. Graphical presentation of diastolic blood pressure **(C)** and systolic blood pressure **(D)**. Amlexanox (25 mg/kg/day) was injected i.p. for 10 weeks, starting just after 5/6 nephrectomy. Groups were compared using one-way ANOVA and post-hoc Tukey's test. Values are presented as mean ± SE (n = 6). #P < 0.05 compared with the sham group, @P < 0.05 compared with the 5/6 nephrectomy group.

TABLE II - Effects on kidney functions and blood pressure after 10 days of 5/6 nephrectomy

	Serum Creatinine (mg/dl)	Serum Urea (mg/dl)	Diastolic blood pressure (mmHg)	Systolic blood pressure (mmHg)
		Before Surgery		
Sham	0.55±0.01	24.18±1.9	83.67±3.2	122.8±2.4
5/6-nephrectomized rats	0.56±0.01	25.00±1.7	85.00±3.9	121.2±1.9
Amlexanox	0.53±0.02	25.00±0.9	85.67±2.4	124.0±2.1
10 Days after Surgery				
Sham	0.52±0.02	25.25±2.5	92.00±2.1	118.3±2.8
5/6-nephrectomized rats	0.87±0.03#	110.1±6.1#	123.7±3.5#	179.2±4.1#
Amlexanox	0.83±0.03	102.5±3.5	117.7±3.2	159.5±2.0

Values are expressed as mean± SE (n= 6). Amlexanox (25mg/kg/day) was given i.p. Statistical analysis using Two-way ANOVA, followed by Bonferroni's Post-test. #P < 0.05 vs. sham

Effects of amlexanox on cardiac hypertrophy and fibrosis in 5/6 nephrectomized rats

Heart weight normalized to tibial length (427.7 ± 7.8 vs. 283.3 ± 4.9 mg/cm, Figure 3A) and cardiac fibrosis area percentage (blue-stained area, 23.5 ± 1.5 vs. 2.5 ± 0.28, Figures 3B and 3C) increased significantly in 5/6 nephrectomized rats than in the sham group. In harmony with the improved diastolic and systolic blood pressures, cardiac hypertrophy, and fibrosis (13% and 55% decrease, respectively) were significantly lower in the amlexanox-treated rats compared with blood pressures in the 5/6 nephrectomized rats.

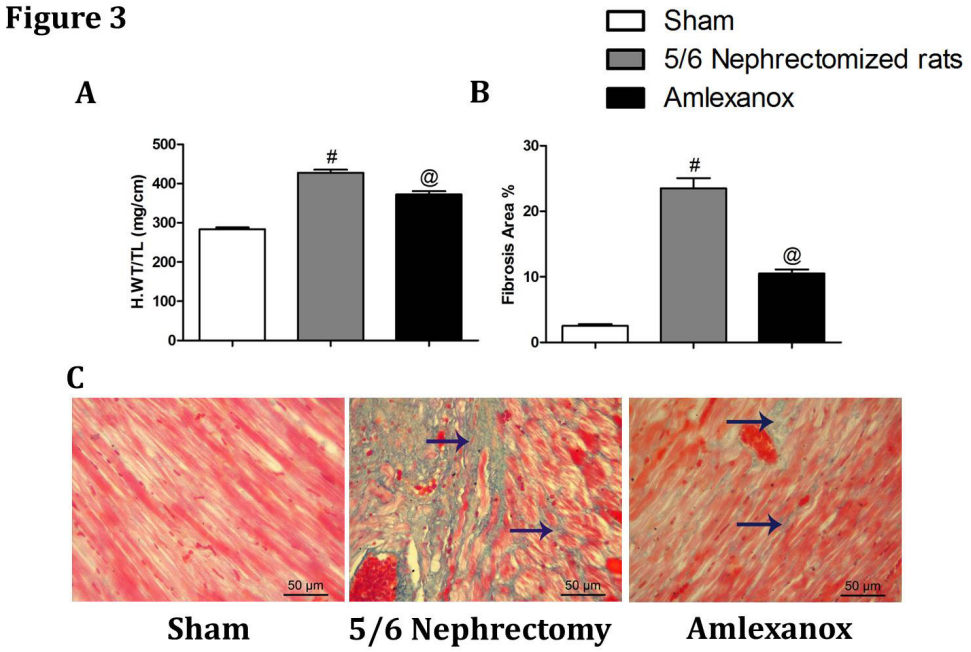

FIGURE 3 - Changes in heart weights and fibrosis area percentage. Graphical presentation of heart weight normalized to tibial length (H. WT/TL) **(A)** and fibrosis area % **(B)**. Representative photomicrographs of cardiac tissues stained with Mallory trichrome **(C)**. Amlexanox (25 mg/kg/day) was injected i.p. for 10 weeks, starting just after 5/6 nephrectomy. Groups were compared using one-way ANOVA and post-hoc Tukey's test. Values are represented as mean ± SE (n = 6). #P < 0.05 compared with the sham group, @P < 0.05 compared with the 5/6 nephrectomy group.

Effects of amlexanox on serum BNP and cardiac β1-adrenergic receptor (β1AR) down-stream signals in 5/6 nephrectomized rats

As depicted in Figure 4, 5/6 nephrectomy significantly increased serum BNP levels (2.05 ± 0.07 vs. 0.25 ± 0.02 ng/ml, Figure 4A) but significantly decreased cardiac levels of β1AR (13.8 ± 0.85 vs. 38.02 ± 2.2 pg/mg, Figure 4B), β-arrestin-2 (82.3 ± 4.5 vs. 166.2 ± 7.96 pg/mg, Figure 4C), and PIP2 (16.5 ± 1.19 vs. 47.29 ± 2.69 nmol/mg, Figure 4D) compared with levels in the sham group. Interestingly, serum BNP levels (40% decrease, Figure 4A) were significantly lower and cardiac β1AR (230%, Figure 4B), β-arrestin-2 (32%, Figure 4C), and PIP2 (100%, Figure 4D) levels were significantly higher in amlexanox-treated rats compared with the levels in 5/6 nephrectomized rats.

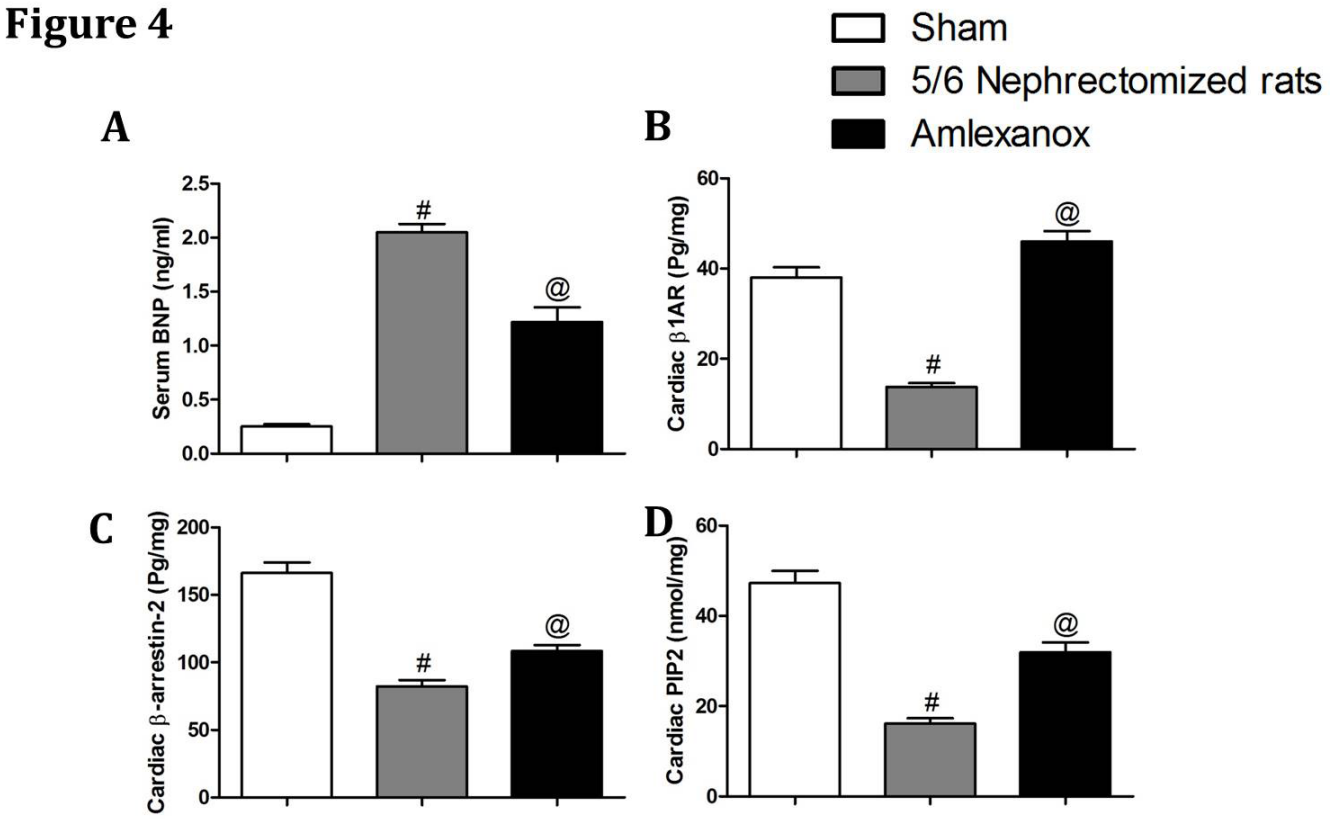

FIGURE 4 - Changes in serum brain natriuretic peptide (BNP) and cardiac adrenergic signaling pathways. **(A)** Quantitative analysis of serum BNP. **(B)** Quantitative analysis of β1-adrenergic receptor β1AR. **(C)** Quantitative analysis of β-arrestin-2. **(D**) Quantitative analysis of phosphatidylinositol-4,5-bisphosphate (PIP2). Amlexanox (25 mg/kg/day) was injected i.p. for 10 weeks, starting just after 5/6 nephrectomy. Groups were compared using one-way ANOVA and post-hoc Tukey's test. Values are represented as mean ± SE (n = 6). #P < 0.05 compared with the sham group, @P < 0.05 compared with the 5/6 nephrectomy group.

Effects of amlexanox on cardiac diacylglycerol (DAG) levels and apoptosis signals in 5/6 nephrectomized rats

Cardiac DAG (5.3 ± 0.33 vs. 1.64 ± 0.12, Figure 5A) and caspase-3 activity (451.4 ± 26.3 vs. 78.08 ± 8.09 U/g, Figure 5B) levels increased significantly but cardiac pS473 Akt levels (1.77 ± 0.13 vs. 5.2 ± 0.25 ng/mg, Figure 5C) decreased significantly in 5/6 nephrectomized rats compared with the levels in the sham group. Notably, cardiac DAG and caspase-3 activity levels were significantly lower (38% and 53% decrease, Figures 5A and 5B, respectively) and cardiac pS473Akt levels were significantly higher (44%, Figure 5C) in amlexanox-treated rats than in 5/6 nephrectomized rats.

Figure 5

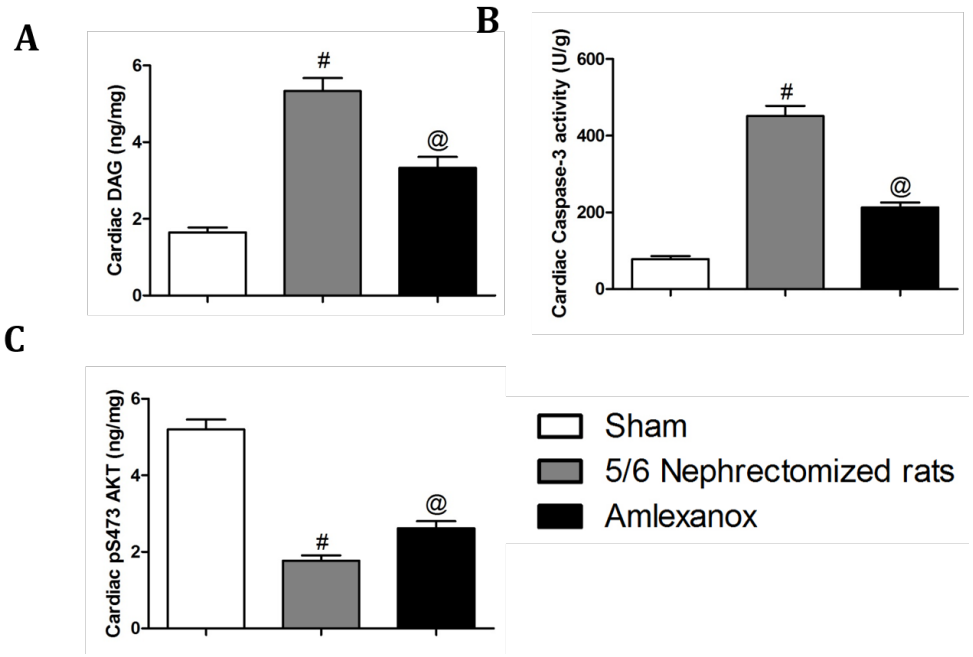

FIGURE 5 - Changes in cardiac diacylglycerol (DAG) and apoptosis signals. **(A)** Quantitative analysis of diacylglycerol (DAG). **(B)** Quantitative analysis of caspase-3 activity. **(C)** Quantitative analysis of pS473AKT. Amlexanox (25 mg/kg/day) was injected i.p. for 10 weeks, starting just after 5/6 nephrectomy. Groups were compared using one-way ANOVA and post-hoc Tukey's test. Values are represented as mean ± SE (n = 6). #P < 0.05 compared with the sham group, @ P < 0.05 compared with the 5/6 nephrectomy group.

DISCUSSION

Chronic kidney disease (CKD) is a public health problem with high economic burden, morbidity, and mortality rates. Patients suffering from CKD are at high risk of cardiovascular complications, a condition known as Cardiorenal syndrome CRS (Ronco *et al.*, 2008). The molecular mechanisms of CRS are not well understood. In this study, we investigated the cardioprotective effect of amlexanox, which was recently classified as a GRK5i, in a model of CRS generated after 10 weeks of 5/6 nephrectomy in rats.

One of the potential mechanisms of cardiac dysfunction in 5/6 nephrectomized rats is sympathetic over-activity (Yu *et al.*, 2017). Indeed, 5/6 nephrectomy is a stress factor that induces the release of stress hormones, such as catecholamines, which increase blood pressure and induce hypertrophic, fibrotic, and apoptotic signals in the heart through over-stimulation of βARs (Liggett *et al.*, 2008; Zipursky *et al.*, 2017). In time, chronic activation of cardiac βARs leads to their down-regulation as a compensatory mechanism, subsequently leading to heart failure (Santulli and Iaccarino 2016).

In agreement with previously described pathological changes, our study showed that 5/6 nephrectomy in rats induced renal impairment and elevated diastolic and systolic blood pressures, cardiac hypertrophy, and fibrosis 10 weeks after surgical interference. These data are also consistent with the findings of Linssen *et al.* (2018), who showed that serum BNP levels, a marker of heart failure, were significantly increased in 5/6 nephrectomized rats.

Consistent with these pathological changes, cardiac levels of β1AR and its down-stream signals also decreased. Interestingly, cardiac levels of β-arrestin-2 also decreased in the nephrectomized mice. To our knowledge, this is the first study to address this point. In harmony with these changes in cardiac β1AR and β-arrestin-2 levels, PIP2 levels significantly decreased

in 5/6 nephrectomized rats than in the sham group. PIP2 is a down-stream signal for β-arrestins (Nelson *et al.*, 2008). Cardiac PIP2 regulates actin dynamics and T-tubule functions in cardiomyocytes. PIP2 deficiency is associated with T-tubule disruption and remodeling and defects in calcium cycling, which may contribute to the progression of heart failure (Ibrahim *et al.*, 2020).

Cardiac DAG levels significantly increased in 5/6 nephrectomized rats compared with levels in the sham group. DAG, through activation of PKC, mediates activation of inflammatory and oxidative stress pathways (Hsu *et al.*, 2018; Lučić, Truebestein, Leonard, 2016). Oxidative stress, in turn, impairs calcium handling and activates inflammatory, apoptotic, and fibrotic pathways in cardiomyocytes, leading to cardiac hypertrophy and fibrosis secondary to cardiac fibroblast hyperproliferation (Rababa'h *et al.*, 2018). We also detected decreased pS473Akt levels (survival signal) and increased caspase-3 activity (apoptotic signal) in the heart in nephrectomized rats, findings consistent with those previously reported (Mohamed *et al.*, 2016).

Interestingly, treating rats with amlexanox beginning after surgery and continuing for 10 weeks prevented most of the pathological changes induced by 5/6 nephrectomy. The highest cardioprotective effects of amlexanox were observed at the level of cardiac fibrosis and apoptosis. To our knowledge, this is the first study to demonstrate that amlexanox can ameliorate the detrimental effect of CRS on both renal and cardiac functions.

Recently, amlexanox was classified as a GRK5i and a specific IκB kinase inhibitor (Homan *et al.*, 2014; Reilly *et al.*, 2013). Notably, GRK5 is up regulated during heart failure and its deficiency mediates protective effects (Liggett *et al.*, 2008). In the same context, GRK5 mainly mediates phosphorylation and desensitization of β2AR and, to a lesser extent, β1AR (Chen *et al.*, 2001). Furthermore, GRK5 activates inflammatory and apoptotic pathways through direct activation of nuclear factor κ-B (NF-κB) (Patial *et al.*, 2011). Therefore, inhibition of GRK5 may contribute to the cardioprotective effects of amlexanox. Also, the direct inhibitory action of amlexanox on IκB kinase cannot be excluded (Reilly *et al.*, 2013).

In this study, we show for the first time, to our knowledge, that amlexanox has potent in vivo antifibrotic and antiapoptotic effects that mediate cardiac protection (Figure 6).

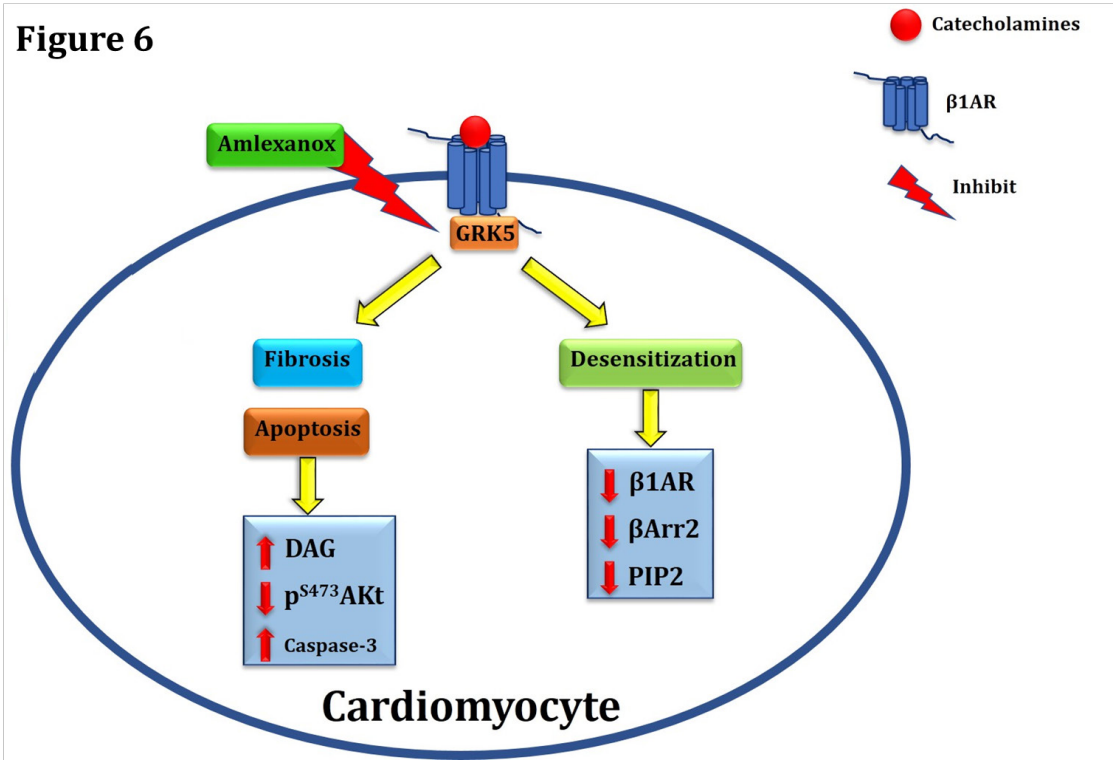

FIGURE 6 - Possible mechanisms for the cardioprotective effect of amlexanox.

AUTHOR CONTRIBUTIONS

RM, NM, and SE conceived and designed the research.

REFERENCES

Bathgate-Siryk A, Dabul S, Pandya K, Walklett K, Rengo G, Cannavo A, et al. Negative impact of β-arrestin-1 on post-myocardial infarction heart failure via cardiac and adrenal-dependent neurohormonal mechanisms. Hypertension. 2014;63(2):404-412.

Bell J. Amlexanox for the treatment of recurrent aphthous ulcers. Clin Drug Investig. 2005;25(9):555-566.

Boccella N, Paolillo R, Perrino C. Epac1 inhibition as a novel cardioprotective strategy: lights and shadows on GRK5 canonical and non-canonical functions. Cardiovasc Res. 2019;115(12):1684-1686.

Carlstrom M, Montenegro M. Therapeutic value of stimulating the nitrate-nitrite-nitric oxide pathway to attenuate oxidative stress and restore nitric oxide bioavailability in cardiorenal disease. J Intern Med. 2019;285(1):2-18.

Chen EP, Bittner HB, Akhter SA, Koch WJ, Davis RD. Myocardial function in hearts with transgenic overexpression of the g protein-coupled receptor kinase 5. Ann Thorac Surg. 2001;71(4):1320-1324.

Hattori Y, Michel MC. G protein-coupled receptor kinases (GRKS) and β-arrestins: New insights into disease regulators. Front Pharmacol. 2019;10:1654.

Homan KT, Wu E, Cannavo A, Koch WJ, Tesmer JJ. Identification and characterization of amlexanox as a g protein-coupled receptor kinase 5 inhibitor. Molecules. 2014;19(10):16937-16949.

Hsu HT, Tseng YT, Wong WJ, Liu CM, Lo YC. Resveratrol prevents nanoparticles-induced inflammation and oxidative stress via downregulation of PKC-α and NADPH oxidase in lung epithelial a549 cells. BMC Complement Altern Med. 2018;18(1):1-13.

Ibrahim WS, Ibrahim IA-H, Mahmoud MF, Mahmoud AA. Carvedilol diminishes cardiac remodeling induced by high-fructose/high-fat diet in mice via enhancing cardiac β-arrestin2 signaling. J Cardiovasc Pharmacol Ther. 2020; 25(4):354-363.

Khoury S, Steinvil A, Gal-Oz A, Margolis G, Hochstatd A, Topilsky Y, et al. Association between central venous pressure as assessed by echocardiography, left ventricular function and acute cardio-renal syndrome in patients with st segment elevation myocardial infarction. Clin Res Cardiol. 2018;107(10):937-944.

Liggett SB, Cresci S, Kelly RJ, Syed FM, Matkovich SJ, Hahn HS, et al. A grk5 polymorphism that inhibits β-adrenergic receptor signaling is protective in heart failure. Nat Med. 2008;14(5):510-517.

Linssen G, Jaarsma T, Hillege H, Voors A,van Veldhuisen D. A comparison of the prognostic value of bnp versus nt-probnp after hospitalisation for heart failure. Neth Heart J. 2018;26(10):486-492.

Lučić I, Truebestein L, Leonard TA. Novel features of DAG-activated PKC isozymes reveal a conserved 3-d architecture. J Mol Biol. 2016;428(1):121-141.

McCrink KA, Maning J, Vu A, Jafferjee M, Marrero C, Brill A, et al. B-arrestin2 improves post–myocardial infarction heart failure via sarco (endo) plasmic reticulum Ca2+-ATPase–dependent positive inotropy in cardiomyocytes. Hypertension. 2017;70(5):972-981.

Mohamed RM, Morimoto S, Ibrahim IAH, Zhan DY, Du CK, Arioka M, et al. Gsk-3β heterozygous knockout is cardioprotective in a knockin mouse model of familial dilated cardiomyopathy. Am J Physiol Heart Circ Physiol. 2016;310(11):H1808-H1815.

Mohammed SG, Ibrahim IAH, Mahmoud MF,Mahmoud AA. Carvedilol protects against hepatic ischemia/reperfusion injury in high-fructose/high-fat diet-fed mice: Role of g protein-coupled receptor kinase 2 and 5. Toxicol Appl Pharmacol. 2019;382:114750.

Mucke HA. Drug repurposing patent applications April–June 2018. Assay Drug Dev Technol. 2018;16(7):420-6.

Nelson CD, Kovacs JJ, Nobles KN, Whalen EJ, Lefkowitz RJ. B-arrestin scaffolding of phosphatidylinositol 4-phosphate 5-kinase iα promotes agonist-stimulated sequestration of the β2-adrenergic receptor. J Biol Chem. 2008;283(30):21093-21101.

Pallangyo P, Fredrick F, Bhalia S, Nicholaus P, Kisenge P, Mtinangi B, et al. Cardiorenal anemia syndrome and survival among heart failure patients in Tanzania: A prospective cohort study. BMC Cardiovasc Disord. 2017;17(1):59.

Patial S, Shahi S, Saini Y, Lee T, Packiriswamy N, Appledorn DM, et al. G□protein coupled receptor kinase 5 mediates lipopolysaccharide□induced nfκb activation in primary macrophages and modulates inflammation in vivo in mice. J Cell Physiol. 2011;226(5):1323-1333.

Rababa'h AM, Guillory AN, Mustafa R, Hijjawi T. Oxidative stress and cardiac remodeling: An updated edge. Curr Cardiol Rev. 2018;14(1):53-59.

Reilly SM, Chiang SH, Decker SJ, Chang L, Uhm M, Larsen MJ, et al. An inhibitor of the protein kinases TBK1 and IKK-ε improves obesity-related metabolic dysfunctions in mice. Nat Med. 2013;19(3):313-321.

Ronco C, Haapio M, House AA, Anavekar N, Bellomo R. Cardiorenal syndrome. J Am Coll Cardiol. 2008;52(19):1527-1539.

Santulli G, Iaccarino G. Adrenergic signaling in heart failure and cardiovascular aging. Maturitas. 2016;93:65-72.

Suresh H, Arun B, Moger V, Swamy M. Cardiorenal syndrome type 4: A study of cardiovascular diseases in chronic kidney disease. Indian Heart J. 2017;69(1):11-16.

Švíglerová J, Kuncova J, Nalos L, Tonar Z, Rajdl D, Štengl M. Cardiovascular parameters in rat model of chronic renal failure induced by subtotal nephrectomy. Physiological research. 2010;59(Suppl 1):S81-S88.

Ungerer M, Parruti G, Böhm M, Puzicha M, DeBlasi A, Erdmann E, et al. Expression of beta-arrestins and beta-adrenergic receptor kinases in the failing human heart. Circ Res. 1994;74(2):206-213.

Velmurugan BK, Baskaran R,Huang C-Y. Detailed insight on β-adrenoceptors as therapeutic targets. Biomed Pharmacother. 2019;117:109039.

Vinod P, Krishnappa V, Chauvin AM, Khare A, Raina R. Cardiorenal syndrome: Role of arginine vasopressin and vaptans in heart failure. Cardiol Res. 2017;8(3):87-95.

Yang HQ, Zhou P, Wang LP, Zhao YT, Ren YJ, Guo YB, et al. Compartmentalized β1-adrenergic signalling synchronizes excitation–contraction coupling without modulating individual ca2+ sparks in healthy and hypertrophied cardiomyocytes. Cardiovasc Res. 2020;116(13):2069-2080.

Yu L, Huang B, Wang Z, Wang S, Wang M, Li X, et al. Impacts of renal sympathetic activation on atrial fibrillation: The potential role of the autonomic cross talk between kidney and heart. J Am Heart Assoc. 2017;6(3):e004716.

Zipursky RT, Press MC, Srikanthan P, Gornbein J, McClelland R, Watson K, et al. Relation of stress hormones (urinary catecholamines/cortisol) to coronary artery calcium in men versus women (from the multi-ethnic study of atherosclerosis [mesa]). Am J Cardiol. 2017;119(12):1963-1971.

Received for publication on 09th January 2021
Accepted for publication on 14th July 2021