

## Repeated Administration of Low Dose Isoprenaline on the Rat's Cardiovascular System

(Administrasi Berulang Isoprenalina Dos Rendah pada Sistem Kardiovaskular Tikus)

RAJASEGAR ANAMALLEY<sup>1,4</sup>, LOGESWARY RAJASSAGERAN<sup>1</sup>, YASAASWINI APPAROO<sup>1</sup>, MUHAMMAD HAFFIZ JAURI<sup>3</sup>,  
YUSOF KAMISAH<sup>2</sup>, NURHANAN MURNI YUNOS<sup>3</sup> & SATIRAH ZAINALABIDIN<sup>1,\*</sup>

<sup>1</sup>*Programme of Biomedical Science, Centre of Toxicology and Health Risk Study (CORE), Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Federal Territory, Malaysia*

<sup>2</sup>*Department of Pharmacology, Faculty of Medicine, UKMMC, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, 56000 Cheras Kuala Lumpur, Federal Territory, Malaysia*

<sup>3</sup>*Natural Products Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor Darul Ehsan, Malaysia*

<sup>4</sup>*Faculty of Health and Life Sciences, Management and Science University, University Drive, off Persiaran Olahraga, 40100 Shah Alam, Selangor Darul Ehsan, Malaysia*

*Received: 21 September 2021/Accepted: 28 December 2021*

### ABSTRACT

Isoprenaline (ISO) at high doses can cause severe stress to myocardium resulting in an infarct-like necrosis in rats. However, its effects at repeatedly low dose exposure on heart, kidney and aorta are still unclear. Hence, this study was aimed to investigate the effects of repeated administration of low dose ISO on the organs in rats by using Langendorff-perfused isolated hearts, ELISA kits, qPCR and histopathology techniques. Male Wistar rats (n=24) were randomly divided into three groups which were given 5 or 10 mg/kg/day of ISO (ISO 5 and ISO 10, respectively), or normal saline (control) subcutaneously for 14 days. Blood pressure was recorded at day-0, 7 and 14. Heart, aorta, kidneys, and blood were then collected. ISO at both doses significantly increased the heart weight and blood pressure (p<0.05), while the heart rate was significantly decreased (p<0.05). ISO also increased serum troponin and NT-pro-BNP, and decreased vascular relaxation dose-dependently. Group ISO 10 showed significantly increased cardiomyocyte area and cardiac collagen content, as well as reduced serum nitrite (p<0.05). However, ISO at both doses did not affect the cardiac mechanical function, renal oxidative stress, inflammation, as well as renal gene expressions of angiotensin-converting enzyme and angiotensin II type 1 receptor. In conclusion, repeated low dose of ISO significantly causes myocardial injury and reduces vascular function in rats. The findings imply that this rat model could be a suitable model of heart injury without the complication of renal injury.

Keywords:  $\beta$ -adrenoceptor; cardiac hypertrophy; fibrosis; isoprenaline; vascular fibrosis

### ABSTRAK

Aruhan isoprenalina (ISO) pada dos tinggi boleh menyebabkan tekanan yang teruk pada miokardium lalu mengakibatkan nekrosis pada jantung tikus. Walau bagaimanapun, kesan pendedahan pada dos rendah berulang pada jantung, ginjal dan aorta masih belum jelas diketahui. Oleh itu, penyelidikan ini bertujuan untuk mengkaji kesan pemberian ISO dos rendah secara berulang pada organ-organ tikus dengan menggunakan teknik jantung terpisah yang diperfusi Langendorff, kit ELISA, qPCR dan histopatologi. Tikus Wistar jantan (n=24) dibahagikan secara rawak kepada tiga kumpulan iaitu 5 atau 10 mg/kg/hari suntikan ISO (ISO 5 dan ISO 10), atau salina normal (kawalan) secara subkutan selama 14 hari. Tekanan darah diambil pada hari ke-0, 7 dan 14. Organ jantung, aorta, ginjal dan darah kemudian dibedah. Hasil kajian mendapati aruhan ISO pada kedua-dua dos tersebut telah meningkatkan berat jantung dan tekanan darah dengan ketara (p<0.05), sementara kadar denyutan jantung pula menurun dengan ketara (p<0.05). ISO juga meningkatkan troponin serum dan NT-pro-BNP dan penurunan relaksasi vaskular. Hanya ISO 10 berupaya mengaruh peningkatan kawasan kardiomyosit dan jumlah kolagen jantung, serta penurunan nitrit serum (p<0.05). ISO pada kedua-dua dos tersebut tidak pula mempengaruhi fungsi mekanikal jantung, tekanan oksidatif ginjal, keradangan, serta ekspresi gen penukaran enzim angiotensin dan reseptor angiotensin II Jenis 1. Kesimpulannya, aruhan dos rendah ISO yang berulang menyebabkan kecederaan miokardium dan mengurangkan fungsi vaskular pada tikus. Hasil kajian ini menunjukkan bahawa model tikus ini boleh dijadikan sebagai satu model tikus dengan kegagalan jantung tanpa sebarang komplikasi pada buah pinggang.

Kata kunci:  $\beta$  adrenoceptor; fibrosis salur darah; kardiak hipertrofi; fibrosis; isoprenalina

## INTRODUCTION

Isoprenaline (ISO) is an adrenergic synthetic drug that has positive inotropic and chronotropic effects on the heart. ISO-induced cardiotoxicity is one of the most widely studied and used model for acute and chronic heart failure (Allawadhi et al. 2018; Govindasami et al. 2020; Manjunatha et al. 2020). ISO leads to progressive cardiac damage if given in either low doses (5 or 10 mg/kg for 7-10 days) or larger doses (85 or 100 mg/kg for 2 days) for exerting acute damage. The interactions between heart, vascular and kidney are being fundamentally important in regulating physiological function in the body, partly via the beta ( $\beta$ ) receptors. There are three subtypes of  $\beta$  receptors which are  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  that can be found in different locations.  $\beta_1$ -receptor can be predominantly found in heart and kidney, while  $\beta_2$  in vascular and  $\beta_3$  in the gallbladder, urinary bladder and in brown adipose tissue (Katsarou et al. 2018).  $\beta$  receptor in vascular causes vasorelaxation while in kidney it activates the Renin-Angiotensin Aldosterone System (RAAS). Therefore, these organs are known to act as a supporting system in maintaining blood homeostasis regulated by heart. However, past studies have shown that overstimulation of  $\beta$ -receptors may lead to physiological disorders such as hypertension, atherosclerosis, and myocardial infarction. The overstimulation by ISO could induce detrimental consequences like cardiac hypertrophy and fibrosis, which subsequently lead to heart failure. In myocardial hypertrophy, inflammation plays an important role and the expression of inflammatory factors are often dysregulated in cardiomyocytes and myocardial tissues during those conditions (Zhao et al. 2017). In addition, excessive production of reactive oxygen species (ROS) by oxidative stress could also contribute to pathological cardiac hypertrophy and heart failure (Zhou et al. 2017). The effects of ISO are not limited within the cardiovascular system since its actions on  $\beta$ -adrenoreceptors are ubiquitous, such as in the vascular system and kidney.

Within the vascular,  $\beta_2$  adrenoceptor stimulation in arteriolar smooth muscle induces vasodilation. The presence of  $\beta_1$  and  $\beta_2$ -adrenergic receptors along the proximal, distal tubular cells and juxtaglomerular granular cells regulates norepinephrine (NE) (Chan et al. 2000). This could lead to angiotensinogen gene expression in the proximal tubular cells and initiate intrarenal renin-angiotensin-aldosterone system (RAAS) and ROS generation (De Ponte et al. 2017). The increased ROS production from prolonged RAAS can cause endoplasmic reticulum (ER) stress, inflammatory, fibrotic and apoptotic responses, predictive factors for the progression of cardiovascular and renal related diseases (Dalal et al. 2012).

Although ISO-induced model is a well-established model in heart disease research, there are still a scarce of evidence between failing heart and the response to the vascular and kidney regulation. In developing a model for heart disease, it is imperative to know if the ISO-induced myocardial injury could injure other systems. Thus, this study was aimed to assess the effect of repeated administration of low dose ISO on the rat's heart, aorta and kidney.

## MATERIAL AND METHODS

### EXPERIMENTAL DESIGN

Twenty-four male Wistar rats (200 - 250 g) were used in this study. The rats were acclimatized under standard laboratory conditions for 1 week with 12 h light/dark cycle and constant room temperature. Standard rodent pellet and tap water were provided *ad libitum*. The animal handling protocols in this study adhered to the ethical guidelines by Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC) (No. Approval: FSK/2018/SATIRAH/23-JAN/891-JAN.-2018-DEC-2018).

All rats were randomly divided into three groups, namely: (1) control (normal saline), (2) isoprenaline (ISO) given at 5 mg/kg (ISO 5) and (3) ISO given at 10 mg/kg (ISO 10). The ISO was dissolved in normal saline. The saline or ISO was administered subcutaneously daily for 14 days (Huang et al. 2014; Zhou et al. 2017). Throughout the experiment, blood pressure (BP) and heart rate were measured at day-0, 7 and 14, using the non-invasive tail cuff method (CODA™ non-invasive blood pressure system, Kent Scientific, USA). On day-15, the rats were anesthetized and sacrificed. The heart and aorta were excised freshly for Langendorff and vascular reactivity experiment, respectively. Blood and kidney were collected for further analysis.

### ISOLATED LANGENDORFF-PERFUSED RAT HEARTS

Prior to thoracic surgery, rats were anesthetized with ketamine/xylazine (1 mL/kg, i.v.) and administered with heparin (500 IU, i.p.) to prevent blood clotting. Upon loss of pedal reflex activity, the heart was rapidly excised, and placed in ice-cold perfusion buffer before subjected to retrograde perfusion via the aortic cannula of Langendorff isolated heart apparatus (ADInstruments, Australia) as previously described (Mohammed et al. 2018).

### VASCULAR REACTIVITY

Vascular functions were assessed in isolated aortic ring using a method described by Si et al. (2017). Briefly, cleaned endothelium-intact thoracic aorta rings (3-4 mm in length) were mounted in a tissue chamber

containing Krebs-Henseleit solution (in mM: NaCl 118, KCl 4.7, NaHCO<sub>3</sub> 25, KH<sub>2</sub>PO<sub>4</sub> 1.2, MgSO<sub>4</sub>·7H<sub>2</sub>O 1.2, CaCl<sub>2</sub>·2H<sub>2</sub>O 2.5 and glucose 11.7) of pH 7.4. The bath was continuously aerated with 95% O<sub>2</sub> and 5% CO<sub>2</sub> at 37 °C. The resting tension was set at an optimal tension of 1 g for ~45 min. The responses were recorded isometrically using force displacement transducer coupled to PowerLab/8sp multichannel data-acquisition system (AD Instruments, Sydney Australia), using ADI chart software (version 5.3, AD Instruments) for digital processing and data analysis. At the beginning of the experiment, the viability of the aortic ring was tested by repeated exposure to KCl solution (high K<sup>+</sup>, 80 mM). Then, further experiment on endothelium-dependent dose-response curve to ACh (10<sup>-9</sup> ~ 10<sup>-4</sup> M) was measured in PE-precontracted (10<sup>-7</sup> M) aortic rings.

#### BIOCHEMICAL ANALYSIS CARDIAC INJURY MARKERS

Serum level of troponin T and NT-pro-B-type natriuretic peptide (NT-ProBNP) was quantitatively measured using commercial ELISA kit (Elabscience Biotechnology Co., Ltd, Wuhan, China) following manufacturer's protocol. The absorbance of the reaction mixture was read spectrophotometrically at 450 nm.

#### NITRITE ASSAY

Nitrite and nitrate are stable final products of NO metabolism and may be used as indirect markers of nitric oxide (NO) presence. NO levels were measured using indirect measurement of nitrite concentration according to the Griess assay as previously described by Combet et al. (2000). The nitrite concentrations were determined by spectrophotometric analysis at 540 nm. Samples nitric oxide content were calculated from a standard curve constructed by reducing known concentration of nitrite solution. Final data were expressed as μM.

#### MATRIX METALLOPEPTIDASE 9 (MMP-9)

Serum matrix metalloproteinase-9 (MMP-9) activity was assayed using gelatin zymography method (Abcam, Cambridge, MA, USA) according to manufacturer instruction. Serum samples were denatured in non-reducing Laemmli sample buffer at a ratio of 1:1. Equal volumes equivalent to 10 μL of undiluted serum samples from 8 rats per group were then separated on 7.5% SDS-PAGE containing gelatin (4 mg/mL) without prior heating or reduction under constant voltage (60 V, 20 min; then 100 V, 80 min). After electrophoresis, SDS from the gels was removed and incubated overnight in the incubation buffer at 37 °C. Following incubation, the gel was stained with Coomassie blue and de-stained until

zones of digestion were visible as clear bands against a blue background. The area of interest was measured using ImageJ software (Bethesda, Maryland, USA) to evaluate MMP-9 activity.

#### RENAL FUNCTION TEST

Determination of BUN level was performed using the diacetyl monoxime chemical method (Patlolla et al. 2018) directly measured the chromogen formed because of condensation of urea with diacetyl monoxime. The reaction was then hydrolyzed in acidic medium to produce diacetylene which would be condensed with urea and formed a pink chromogen. The chromogen was measured at 480 nm. Serum creatinine level was determined using the method of Jaffe (Hoogwerf et al. 1986). In an alkaline medium, the creatinine in the sample reacted with picric acid producing a reddish yellow complex, which can be measured at 520 nm.

#### HISTOLOGY ANALYSIS

Apex portion of the heart, 1 cm of aorta and kidney sections were fixed in 10% formalin and processed for paraffin embedding. Tissues were sectioned into 3-μm thickness for aorta and 4-μm for the heart and kidney. The tissue sections were stained with hematoxylin and eosin (H&E). Further staining with Picrosirius Red staining was done to demonstrate fibrotic area and to estimate percentage of collagen deposition. The quantitative measurements were done using ImageJ software (Bethesda, Maryland, USA) (Ali et al. 2019).

#### QUANTITATIVE POLYMERASE CHAIN REACTION (qPCR)

Total RNA was extracted from the kidney cortex tissue using QIAzol lysis reagent (Qiagen, Germany). Using random primer, 2 μg of mRNA was reverse-transcribed using Quantinova Reverse Transcription Kit. Reaction mixtures containing 2 μg of mRNA, 4 μL of reverse transcription mix, 1 μL of reverse transcriptase, 2 μL of gDNA removal mix, and RNase-free water were prepared. The cDNA was then used to determine gene expression of RAS (AT1, ACE), oxidative stress (NOX2), MnSODs, fibrosis (COL3) and inflammation (IL-6) gene in real-time PCR (RT-qPCR) using Quantinova SYBR® Green (Qiagen, Germany). The ribosomal 18S gene was used as endogenous control. Finally, quantitative analysis was performed using CFX 96 Real-Time PCR Detection System (Bio-Rad, CA, USA).

#### STATISTICAL ANALYSIS

Data analyses were performed using GraphPad Prism 6. All data were expressed as mean ± SEM. Shapiro-Wilk

test was performed for the normality. Statistical analysis was performed using one-way and two-way analysis of variance (ANOVA). The Tukey post hoc test was performed, and statistical significance was set at  $p < 0.05$ .

#### RESULTS AND DISCUSSION

Our findings demonstrate that repeated administration of ISO could potentially cause heart injury and vascular dysfunction, however, there was no impairment in the kidney functions. Repeated ISO administration significantly increased the normalized heart weight (HW/TL) and left ventricle (LV/TL) ratio compared to control ( $p < 0.05$ ), probably due to fluid accumulation within the intramuscular space and necrosis of cardiac muscle fibers (Manjunatha et al. 2020). Elevation in heart weight ratio showed cardiac hypertrophy with ventricular remodeling process which include infarct expansion as well as compensatory reactive hypertrophy and dilation of the non-infarcted left ventricle (Huang et al. 2014). Cardiac remodeling process stimulates the release of biomarkers of cell death in the form of proteins (Zhong et al. 2018). Being a sensitive cardiac-specific regulatory protein, Troponin-T often serves as a gold standard in diagnosing myocardial injury (Ali et al. 2017). Besides that, increased stress on cardiac myocyte can also trigger the release of another biomarker, natriuretic peptides (NPs). Three NPs have been identified till date, which are ANP, BNP, and CNP. ANP is primarily released from the cardiac atrium in response to increased atrial pressure, whereas BNP is released mainly from the left ventricle because of ventricular wall stretch (Casserly & Klinger 2010). C-Type natriuretic peptide (CNP) another individual from natriuretic peptide family prominent in porcine cerebrum. CNP is produced by the endothelium and the heart and seems to assume a noticeable part in vascular and cardiovascular capacity, both physiologically and neurotically (Lumsden et al. 2010). Thus, in this study, troponin T and NT-pro BNP was

measured and both specific biomarkers are significantly elevated in ISO-induced group compared to control group. As reported in previous research, due to inability of maintaining cellular membrane integrity in myocardial damages injury by repeated exposure of ISO, markers such as troponin T and NT-proBNP will leak from myocardia into the circulation (Ali et al. 2017; Hasić et al. 2007; Nichtova et al. 2012). Hence, the association between NT-proBNP and troponin shows as a promising biomarker for cardiac functional injuries (Table 1).

The current study showed that there was significantly ( $p < 0.05$ ) increased of SBP and DBP in ISO-induced rats, similarly, found in previous study which stated the elevation of blood pressure could be caused by an activation of the sympathetic nervous system as a result of  $\beta_1$ -receptor stimulation by repeated exposure of low dose ISO (Ahmad et al. 2012). However, the heart rate of ISO induced rats was significantly reduced compared to control and ISO 10 group was recorded with the highest BP and lowest HR values among the groups (Table 2). These changes could reflect an overall dysfunction between myocardial contractility and relaxation (Zhou et al. 2017). Despite the altered blood pressure and heart rate measurements, the cardiac mechanical function showed no significant changes across all groups ( $p > 0.05$ ) (Figure 1). Tau is a parameter that observes the time constant for relaxation in isolated heart. The ISO-induced group shows prolonged time compared to control. Tau between ISO 5 mg and control showed a pattern of increment, although it was not statistically significant ( $p > 0.05$ ). Peak rate of contraction (+dP/dt) and peak rate of relaxation (-dP/dt) of ISO 10 mg was also tended to increase compared to control. The increment trend of Tau as well as the rate of ventricular contraction indicating impaired LV contraction due to the stiffened chamber (Mozayani & Raymon 2003). ISO could cause oxygen supply deficits, which then lead to cardiac hypoxia followed by cardiac necrosis and eventually loss of its contractility function (Allawadhi et al. 2018).

TABLE 1. Analysis of repeated ISO administration to rats body weight, heart weight, cardiac injury and kidney function indicators at the end of experiment

Group	Body weight (BW,g)	Tibia length (TL,cm)	Heart weight (HW,g)	HW/TL (g/cm)	LV/TL (g/cm)	Trop T (pg/mL)	Pro bnp (ng/mL)	Urea (mg/dl)	Creatinine (mg/dl)
Control	44.50 ± 8.75	3.53 ± 0.05	0.84 ± 0.02	0.24 ± 0.01	0.16 ± 0.01	186.2 ± 27.36	0.74 ± 0.10	51.35 ± 7.50	3.15 ± 0.15
	54.13 ± 5.79	3.61 ± 0.14	1.20 ± 0.08*	0.33 ± 0.02*	0.24 ± 0.02*	265.2 ± 28.94*	1.04 ± 0.08*	52.44 ± 6.10	3.39 ± 0.42
ISO 5	41.63 ± 5.95	3.51 ± 0.07	1.22 ± 0.04*	0.35 ± 0.01*	0.22 ± 0.01*	415.3 ± 40.72*	1.79 ± 0.12*	88.05 ± 19.27	5.23 ± 0.87

Values are presented as mean ± SEM for n = 8 per group, \* $p < 0.05$  in relative to control group

TABLE 2. The effects of repeated ISO administration to the blood pressure (BP) and heart rate (HR)

Group	Blood pressure (mmHg)									Heart rate (bpm)		
	Systolic blood pressure			Diastolic blood pressure			Mean arterial pressure					
	(SBP)			(DBP)			(MAP)					
Day	0	7 <sup>th</sup>	14 <sup>th</sup>	0	7 <sup>th</sup>	14 <sup>th</sup>	0	7 <sup>th</sup>	14 <sup>th</sup>	0	7 <sup>th</sup>	14 <sup>th</sup>
Control	124.40 ±1.93	124.86 ±2.01	124.32 ±0.86	85.93 ±2.06	86.29 ±2.34	86.54 ±1.52	98.75 ±1.92	99.14 ±2.16	99.14 ±1.26	366.64 ±15.77	346.67 ±18.66	369.33 ±17.22
ISO 5	127.82 ±2.98	137.62 ±5.01	162.14 ±3.26*	88.97 ±2.92	103.43 ±4.88	121.64 ±4.21*	101.92 ±2.87	114.83 ±4.65	135.14 ±3.62*	383.89 ±15.85	298.05 ±30.92	245.50 ±25.52*
ISO 10	126.03 ±2.06	141.84 ±4.54*	179.12 ±10.44*	88.33 ±2.41	102.79 ±3.40*	133.14 ±8.73*	100.90 ±2.27	115.81 ±3.59*	148.47 ±8.93*	387.33 ±9.04	281.27 ±7.28*	256.38 ±35.97*

The measurement was taken in intervals of 7 days (0 to 14 days). n = 8 per group. Values expressed as Mean ± SEM, \*p < 0.05 in relative to control group

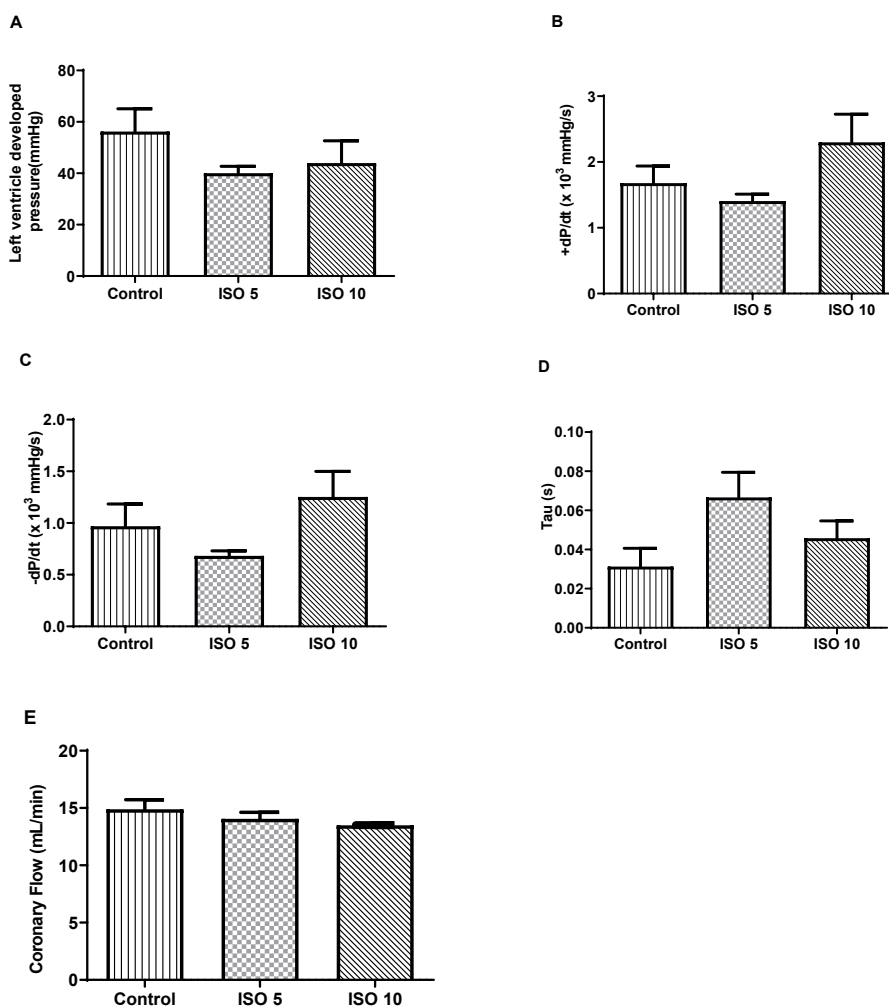


FIGURE 1. Analysis of cardiac mechanical function in isolated perfused rat hearts. (A) LVDP, (B) LVdP/dt<sub>max</sub>, (C) LVdP/dt<sub>min</sub>, (D) Tau, and (E) coronary flow. Values are expressed as the mean ± SEM (n=4 or 5 per group)

As for the vascular reactivity, there was a significant ( $p < 0.05$ ) reduction in acetylcholine (ACh) dependent vascular relaxation in ISO-induced rats (Figure 2). ACh failed to induce a normal endothelium-dependent relaxation in the aorta, which indicates that the endothelium layer could be damaged by the free radicals produced from the overstimulation of  $\beta$  receptors by ISO administration. This result was also in parallel with the significantly low nitric oxide (NO) content in ISO-induced rat serum (Figure 2). Nitric oxide, which is an unstable molecule, will be easily inactivated by the free radicals produced from the  $\beta$  overstimulation. The low NO bioavailability in the serum will cause the vascular tendency to contract and could possibly contribute to the elevated blood pressure which was noted in this study (Rajendran et al. 2013).

The vascular dysfunction was further investigated, and the result showed there was not any endothelial injury or structural damage in the aorta of ISO induced rat compared with control rats (Figure 5(A)-5(C)). This suggests that the free radicals produced by the overstimulation of  $\beta$ -receptor were yet to cause any cellular or vascular damage. This result was further supported by the unaltered level of matrix metalloproteinase 9 (MMP-9) activity across the groups (Figure 5(D)). However, there was a trend of increased MMP-9 activity in ISO 10 group. MMP-9 is an enzyme that will be secreted by endothelial cells if there is any injury in blood vessels (Magid et al. 2003; Yu et al. 2008). The increment of MMP-9 activity in ISO 10 showed that this enzyme can be potential marker for the endothelial injury in this model.

Histopathology study was conducted to study on the structure damages and fibrosis deposition in heart, aorta and kidneys by repeated ISO administration. ISO 10 group showed significant enlargement of cardiomyocytes area after 14 days as observed through haematoxylin and eosin-stained heart section (Figure 3(A)-3(C)). This is further supported by quantification of the cardiomyocytes area size whereby ISO 10 group were statistically significant compared to control group (Figure 3(D)). The repeated exposure of low dose ISO rats showed myocardial damages with hypertrophy, and this was further supported by quantification of the cardiomyocytes area size, similar observations as in previous studies (Allawadhi et al. 2018; Krenek et al. 2009; Nichtova et al. 2012). Cardiac fibrosis which resulted from myocardial remodeling is characterized by the over deposition of myocardial interstitial collagen and altered cardiac function which could contribute to heart failure development (Che et al. 2019). In this study, ISO 10 mg/kg group showed marked collagen deposition percentage compared to control group (Figure 4(A)-4(C)), and this was also further supported by the quantification of heart section that showed significant higher amount of collagen deposition in ISO induced rats compared to control rats (Figure 4(D)). Earlier studies have reported an association between increased oxidative stress and enhanced fibrosis in ISO induced cardiac hypertrophy. According to Ali et al. (2019), ISO-induced rats experienced loss of cardiomyocytes due to myocardial necrosis that was accompanied by inflammation and cardiac fibrosis.

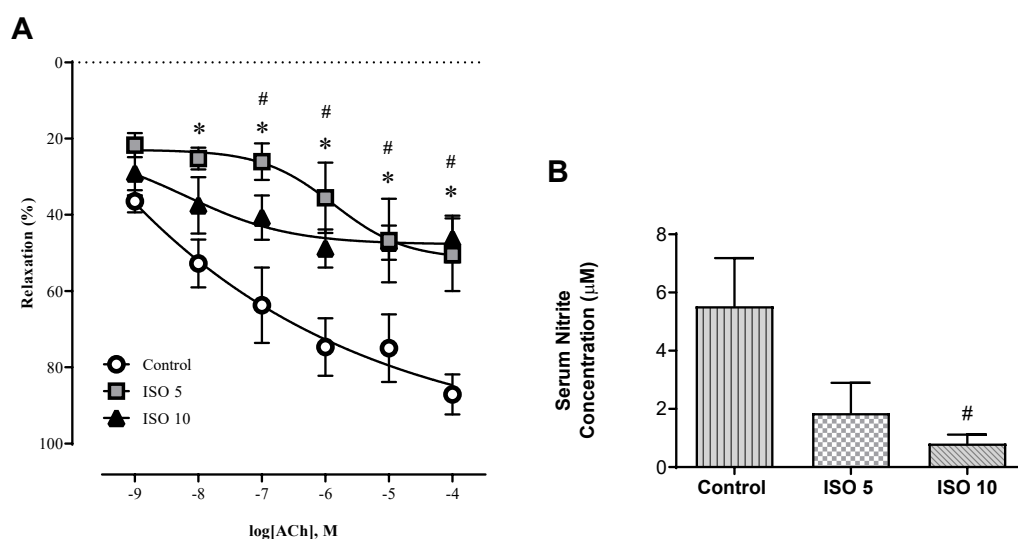


FIGURE 2. Aortic concentration-response curve to the (A) endothelium-dependent vasodilator ACh and (B) serum nitrite concentration. Values are expressed as mean  $\pm$  S.E.M ( $n = 5-7$ ) \* $p < 0.05$  in relative to control group using two-way ANOVA, # $p < 0.05$  in relative to control group

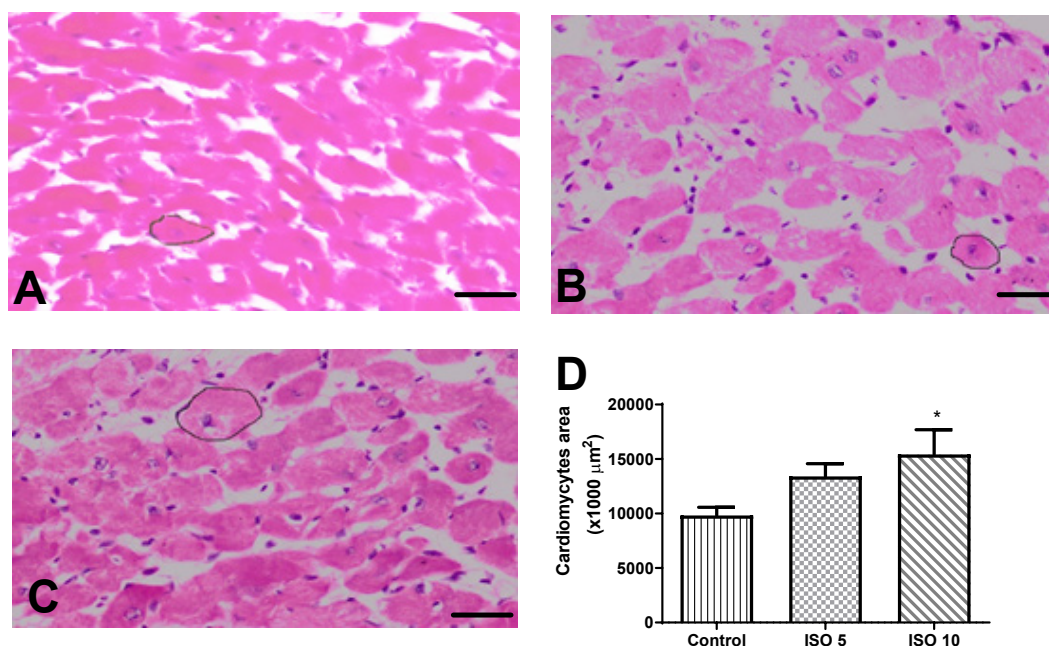


FIGURE 3. Representative images of H&E-stained left ventricle sections; 40 $\times$  magnification; scale bar 50  $\mu\text{m}$ . Representative cardiomyocytes circled in black are showing cardiomyocytes area measured (A: Control, B: ISO 5 and C: ISO 10). (D) The measurements of cardiomyocyte cross-sectional areas in H&E-stained left ventricle sections. Values are expressed as mean  $\pm$  S.E.M, where  $n = 5-7$ . \* $p < 0.05$  in relative to control group using one-way ANOVA

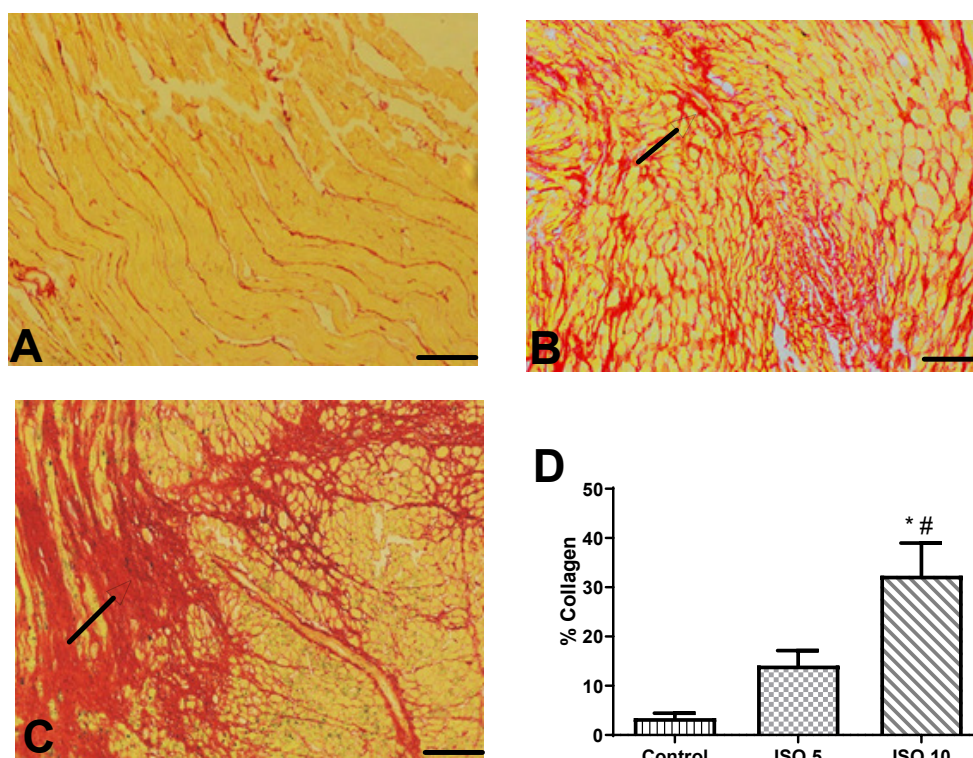


FIGURE 4. Representative images of Picosirius red-stained left ventricle sections; 100 $\times$  magnification; scale bar 50  $\mu\text{m}$ . Arrows show deposition of collagen in red stain. (A: Control, B: ISO 5 and C: ISO 10). (D) The measurement of collagen deposition (%) in Picosirius red-stained left ventricle sections. Values are expressed as mean  $\pm$  S.E.M, where  $n = 8$ . \* $p < 0.05$  in relative to control group, # $p < 0.05$  for ISO 5 vs. ISO 10 using one-way ANOVA

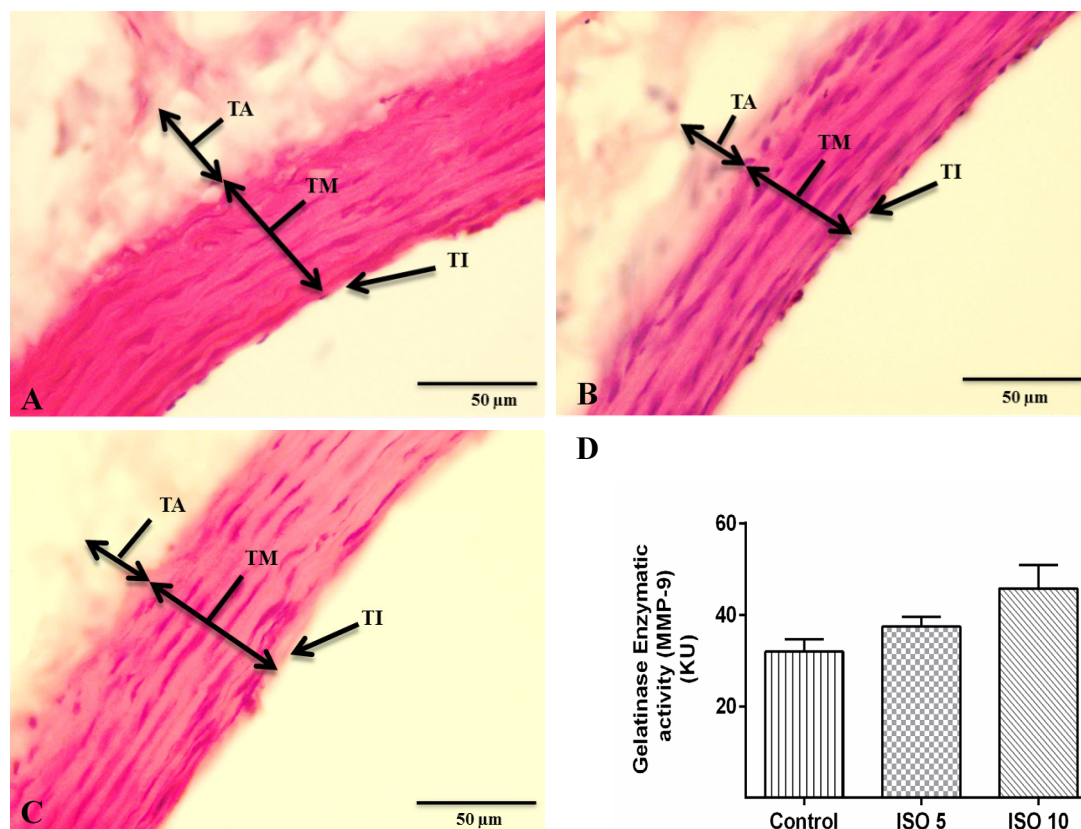


FIGURE 5. Representative images of H&E-stained aortic wall structure following 14 days of ISO administration in (A) control, (B) ISO 5, (C) ISO 10. (D) Measurement of matrix metalloproteinase-9 (MMP-9) activity were measured in the serum (D) Magnification of 400X; scale bar 50  $\mu$ m. TA – Tunica adventitia, TM – Tunica Media, TI – Tunica Intima

The direct and indirect effects of heart failure are identified as leading to acute kidney injury and dysfunction as reported in previous research (Colombo et al. 2012; De Ponte et al. 2017). Previous research has also shown that repeated  $\beta$ -stimulation will lead to increase in oxidative stress and inflammation which cause the kidney injury (De Ponte et al. 2017). However, in contrary, there was not any injury or dysfunctions of kidneys was found in this study. Serum creatinine, which is the breakdown product of creatinine phosphate from the muscle, and urea, the byproduct of protein metabolism was measured for kidney function indicator. These end products are used clinically to monitor the progression of the kidney disease. Based on our findings, there was no significant kidney impairment, although there was an increment trend of both creatinine and urea observed especially in the ISO 10 mg/kg group (Table 1).

Several gene expressions of RAAS related to kidney impairment were investigated in this current research. The results showed that the genes for angiotensin converting enzyme (ACE) and angiotensin II type 1 receptor (AT1)

remained unchanged between all the experimental groups (Table 3). According to Palipoch (2013), activation of NOX due to ANG II stimulation and formation of ROS has been shown to be involved in kidney injury and SOD would act as a defense mechanism to scavenge the ROS activity. However, in our research, there were no significant changes in the oxidative stress markers NADPH oxidase 2 (NOX2), a pro-oxidative, and mitochondrial superoxide dismutase (MnSOD), although ISO has the potential to auto oxidize itself into toxic compound and react with oxygen to form reactive oxygen species (ROS) (Hasić et al. 2007; Palipoch 2013). Apart from that, interleukin-6 (IL-6) gene expression was measured as a marker of inflammation and there was no indication of marked activity between all groups (Table 3). Further studies on other markers such as tumor necrosis factor- $\alpha$ , interferon and IL-12 could be warranted to investigate further into possible mechanism of inflammation.

Morphological studies on the kidneys also showed that there were no observable changes in the control group and ISO 5 group (Figure 6(A) & 6(B)). There was no



infiltration of leukocytes seen as well in any of the groups, suggesting that there was no inflammation. However, vacuolation with reduction in Bowman capsule size in rats treated with ISO 10 mg/kg group was observed (Figure 6(C)). Besides that, marker for fibrosis collagen 3 (Col2) gene expression was unaltered too (Figure 6(D)). This suggests that low dose of ISO treatment for 14 days did not cause any obvious morphological changes in kidney. It was previously shown that structural changes and

fibrosis would only occur at later stage of kidney injury (Colombo et al. 2012; Patlolla et al. 2018). Although this study has shed light on the effects of repeated exposure of low dose ISO on hemodynamic parameters and histopathological changes on cardiovascular, the cellular and molecular mechanism causing subacute cardiac remodeling was not studied in this research. Future studies are needed to determine the effects of exposure of low dose ISO through the cellular and molecular mechanism especially in cardiovascular system.

TABLE 3. Relative gene expression of RAAS (AT<sub>1</sub>, ACE), oxidative stress (NOX2, MnSOD) and inflammation (IL-6) in kidney cortex

Group	AT <sub>1</sub> (AT1/18S)	ACE (ACE/18S)	NOX2 (NOX2/18S)	MnSOD (MnSOD/18S)	IL-6 (IL-6/18S)
Control	1.00 ± 0.36	1.00 ± 0.22	1.00 ± 0.27	1.00 ± 0.29	1.00 ± 0.36
ISO 5	2.05 ± 0.96	1.67 ± 0.50	1.83 ± 0.65	1.94 ± 1.02	1.45 ± 0.35
ISO 10	0.76 ± 0.71	0.78 ± 0.24	0.22 ± 0.11	0.49 ± 0.32	1.82 ± 0.08

The results are normalized to 18S housekeeping gene after 14 days of ISO administration. Values are expressed as mean ± S.E.M, where n = 5-7

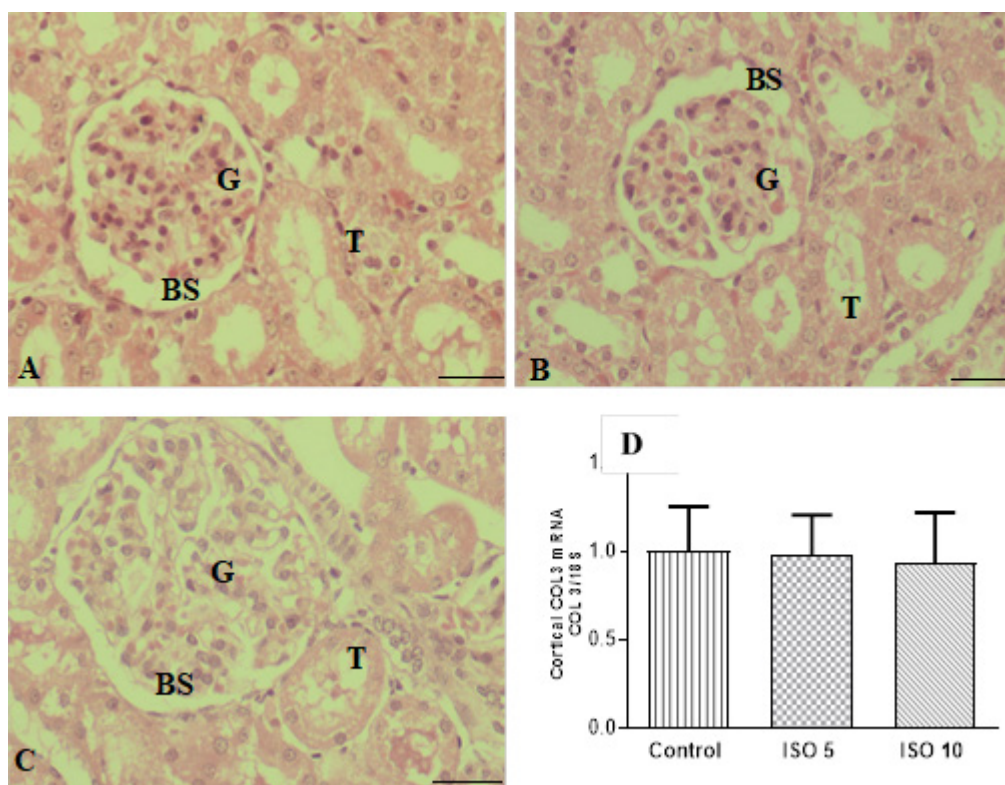


FIGURE 6. Representative images H&E-stained kidney cortex after 14 days of ISO administration in (A) control, (B) ISO 5, (C) ISO 10. (D) Gene expression of COL3 was measured in the kidneys. Magnification of 400X; scale bar 50  $\mu$ m; G – Glomerulus, BS – Bowman's space, T – Tubule. Values are expressed as mean ± S.E.M, where n= 5-7

## CONCLUSION

In conclusion, repeated exposure of low dose ISO 10 mg/kg group showed significant changes in hemodynamic parameters which could be triggered by reduction in the bioavailability of nitric oxide and vascular reactivity. The ISO dosage also caused myocardial injury as well as hypertrophied cardiomyocytes and fibrosis deposition, in spite of the unaltered cardiac functions from the Langendorff apparatus. Repeated beta receptors stimulation did not cause injury or inflammation to the kidneys. Overall, this rat model could be proposed as an animal model for investigation on subacute cardiac remodelling without any complication to the kidney function and structure.

## ACKNOWLEDGEMENTS

This study received financial support from the Ministry of Higher Education, Malaysia (Grant No.: FRGS/1/2017/SKK10/NRE/02/1 and FF-2018-127).

## REFERENCES

- Ahmad, A., Sattar, M.Z., Rathore, H.A., Khan, S.A., Lazhari, M.A., Hashmi, F., Abdullah, N.A. & Johns, E.J. 2012. Impact of isoprenaline and caffeine on development of left ventricular hypertrophy and renal hemodynamic in Wistar Kyoto rats. *Acta Poloniae Pharmaceutica* 72(5): 1015-1026.
- Ali, S.S., Mohamed, S.F.A., Rozalei, N.H., Boon, Y.W. & Zainalabidin, S. 2019. Anti-fibrotic actions of Roselle extract in rat model of myocardial infarction. *Cardiovascular Toxicology* 19(1): 72-81.
- Allawadhi, P., Khurana, A., Sayed, N., Kumari, P. & Godugu, C. 2018. Isoproterenol-induced cardiac ischemia and fibrosis: Plant-based approaches for intervention. *Phytotherapy Research* 32(10): 1908-1932.
- Casserly, B. & Klinger, J. 2010. The clinical utility of brain natriuretic peptide in pulmonary arterial hypertension. *PVRI Review* 2(2): 85.
- Chan, J.S., Wang, T.T., Zhang, S.L., Chen, X. & Carrière, S. 2000. Catecholamines and angiotensinogen gene expression in kidney proximal tubular cells. *Molecular and Cellular Biochemistry* 212(1): 73-79.
- Che, Y., Shen, D.F., Wang, Z.P., Jin, Y.G., Wu, Q.Q., Wang, S.S. & Yuan, Y. 2019. Protective role of berberine in isoprenaline-induced cardiac fibrosis in rats. *BMC Cardiovascular Disorders* 19(1): 1-11.
- Colombo, P.C., Ganda, A., Lin, J., Onat, D., Harxhi, A., Iyasere, J.E., Uriel, N. & Cotter, G. 2012. Inflammatory activation: Cardiac, renal, and cardio-renal interactions in patients with the cardiorenal syndrome. *Heart Failure Reviews* 17(2): 177-190.
- Combet, S., Miyata, T., Moulin, P., Pouthier, D., Goffin, E. & Devuyst, O. 2000. Vascular proliferation and enhanced expression of endothelial nitric oxide synthase in human peritoneum exposed to long-term peritoneal dialysis. *Journal of the American Society of Nephrology* 11(4): 717-728.
- Dalal, S., Foster, C.R., Das, B.C., Singh, M. & Singh, K. 2012. B-adrenergic receptor stimulation induces endoplasmic reticulum stress in adult cardiac myocytes: Role in apoptosis. *Molecular and Cellular Biochemistry* 364(1): 59-70.
- De Ponte, M.C., Casare, F.A.M., Costa-Pessoa, J.M., Cardoso, V.G., Malnic, G., Mello-Aires, M., Volpini, R.A., Thieme, K. & Oliveira-Souza, M. 2017. The role of B-Adrenergic overstimulation in the early stages of renal injury. *Kidney and Blood Pressure Research* 42(6): 1277-1289.
- Govindasami, S., Uddand Rao, V.V., Raveendran, N. & Sasikumar, V. 2020. Therapeutic potential of biochanin-A against isoproterenol-induced myocardial infarction in rats. *Cardiovascular & Hematological Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Cardiovascular & Hematological Agents)* 18(1): 31-36.
- Hasić, S., Jadrić, R., Kiseljaković, E., Mornjaković, Z. & Winterhalter-Jadrić, M. 2007. Troponin T and histological characteristics of rat myocardial infarction induced by Isoproterenol. *Bosnian Journal of Basic Medical Sciences* 7(3): 212-217.
- Hoogwerf, B.J., Laine, D.C. & Greene, E. 1986. Urine C-peptide and creatinine (Jaffe method) excretion in healthy young adults on varied diets: Sustained effects of varied carbohydrate, protein, and meat content. *The American Journal of Clinical Nutrition* 43(3): 350-360.
- Huang, D., Ke, J., Zhao, A., Yang, Z., Liang, D., Pan, J. & Chen, J. 2014. Establishment and evaluation of isoproterenol induced chronic heart failure and cardiac remodeling model in rats: An experimental study. *International Journal of Cardiovascular and Cerebrovascular Disease* 2(5): 45-50.
- Katsarou, M.S., Karathanasopoulou, A., Andrianopoulou, A., Desiniotis, V., Tzinis, E., Dimitrakis, E. & Drakoulis, N. 2018. Beta 1, beta 2 and beta 3 adrenergic receptor gene polymorphisms in a southeastern European population. *Frontiers in Genetics* 9: 560.
- Krenek, P., Kmecova, J., Kucerova, D., Bajuszova, Z., Musil, P., Gazova, A. & Kyselovic, J. 2009. Isoproterenol-induced heart failure in the rat is associated with nitric oxide-dependent functional alterations of cardiac function. *European Journal of Heart Failure* 11(2): 140-146.
- Lumsden, N.G., Khambata, R.S. & Hobbs, A.J. 2010. C-type natriuretic peptide (CNP): Cardiovascular roles and potential as a therapeutic target. *Current Pharmaceutical Design* 16(37): 4080-4088.
- Magid, R., Murphy, T. & Galis, Z.S. 2003. Expression of matrix metalloproteinase-9 in endothelial cells is differentially regulated by shear stress role of c-Myc. *Journal of Biological Chemistry* 278(35): 32994-32999.
- Manjunatha, S., Shaik, A.H., Al Omar, S.Y., Mohammad, A. & Kodihela, L.D. 2020. Combined cardio-protective ability of syringic acid and resveratrol against isoproterenol induced cardio-toxicity in rats via attenuating NF- $\kappa$ B and TNF- $\alpha$  pathways. *Scientific Reports* 10(1): 1-13.
- Mohammed Yusof, N.L., Zainalabidin, S., Mohd Fauzi, N. & Budin, S.B. 2018. *Hibiscus sabdariffa* (Roselle) polyphenol-rich extract averts cardiac functional and structural abnormalities in type 1 diabetic rats. *Applied Physiology, Nutrition, and Metabolism* 43(12): 1224-1232.

- Mozayani, A. & Raymon, L. 2003. *Handbook of Drug Interactions: A Clinical and Forensic Guide*. Springer Science & Business Media.
- Nichtova, Z., Novotova, M., Kralova, E. & Stankovicova, T. 2012. Morphological and functional characteristics of models of experimental myocardial injury induced by isoproterenol. *Gen. Physiol. Biophys.* 31(2): 141-151.
- Palipoch, S. 2013. A review of oxidative stress in acute kidney injury: Protective role of medicinal plants-derived antioxidants. *African Journal of Traditional, Complementary and Alternative Medicines* 10(4): 88-93.
- Patlolla, A.K., Kumari, S.A., Madhusudhanachary, P., Turner, T. & Tchounwou, P.B. 2018. Biochemical and histopathological evaluation of Al<sub>2</sub>O<sub>3</sub> nanomaterials in kidney of Wistar rats. *Current Topics in Biochemical Research* 19: 1-12.
- Rajendran, P., Rengarajan, T., Thangavel, J., Nishigaki, Y., Sakthisekaran, D., Sethi, G. & Nishigaki, I. 2013. The vascular endothelium and human diseases. *International Journal of Biological Sciences* 9(10): 1057-1069.
- Si, L.Y.N., Yusof, K., Ramalingam, A., Lim, Y.C., Budin, S.B. & Zainalabidin, S. 2017. Roselle supplementation prevents nicotine-induced vascular endothelial dysfunction and remodelling in rats. *Applied Physiology, Nutrition, and Metabolism* 42: 765-772.
- Yu, F., Kamada, H., Niizuma, K., Endo, H. & Chan, P.H. 2008. Induction of MMP-9 expression and endothelial injury by oxidative stress after spinal cord injury. *Journal of Neurotrauma* 25(3): 184-195.
- Zhao, L., Wu, D., Sang, M., Xu, Y., Liu, Z. & Wu, Q. 2017. Stachydrine ameliorates isoproterenol-induced cardiac hypertrophy and fibrosis by suppressing inflammation and oxidative stress through inhibiting NF- $\kappa$ B and JAK/STAT signaling pathways in rats. *International Immunopharmacology* 48: 102-109.
- Zhong, J., Guo, D., Chen, C.B., Wang, W., Schuster, M., Loibner, H., Penninger, J.M., Scholey, J.W., Kassiri, Z. & Oudit, G.Y. 2011. Prevention of angiotensin II-mediated renal oxidative stress, inflammation, and fibrosis by angiotensin-converting enzyme 2. *Hypertension* 57(2): 314-322.
- Zhou, R., Ma, P., Xiong, A., Xu, Y., Wang, Y. & Xu, Q. 2017. Protective effects of low-dose rosuvastatin on isoproterenol-induced chronic heart failure in rats by regulation of DDAH-ADMA-NO pathway. *Cardiovascular Therapeutics* 35(2): e12241.

\*Corresponding author; email: satirah@ukm.edu.my