Congestive heart failure model representing aortic banding induced hypertrophy: A study to analyse extent of pressure overload and alteration in myocardial structure and function

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ABSTRACT

Congestive Heart failure (CHF) is a severe pathology representing a major public health problem in industrialized nations which is increasing in prevalence and incidence. The aortic banding rat model provides steady progression of cardiac dysfunction under chronic pressure overload. Present study evaluated two abdominal aortic constriction techniques including constriction of aorta above renal arteries and between renal arteries. The extent of constriction was varied with 22 G and 24 G needles and the duration for evaluation of CHF was also varied by terminating the banded animals after 6 and 8 weeks of banding. Various hemodynamic, ECG and tissue parameters were evaluated after banding to see the progression of CHF. The findings revealed that the constriction of the aorta above both renal arteries with 24 G needle is a better technique amongst other tested banding techniques as the rate of progression of CHF was found to be maximum with it. On the basis of above study, it was concluded that, aortic banding above both renal arteries with 24 G needle is a better technique for induction of pressure overload and for further observation in transition of the cardiac compensatory to decompensatory phase, the duration of the model needs to be prolonged.

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1. Introduction

Heart failure represents a major public health problem in industrialized nations. Congestive heart failure (CHF) is a condition that can result from any structural or functional cardiac disorder that impairs the ability of the heart to fill with or pump sufficient amount of blood through the body. It appears to be the only common cardiovascular condition that is increasing in prevalence and incidence. According to the American Heart Association update on heart disease and stroke statistics, 15 million patients are believed to have symptomatic heart failure worldwide. According to the 44 years follow up of the Framingham Heart Study, CHF incidence approaches 10 per 1000 populations per year after age 65 years [1]. Besides high blood pressure, valvular heart diseases, metabolic disorders like thyroid abnormalities, vitamin deficiencies (e.g., thiamine, ascorbic acid) and endocrine abnormalities (e.g. acromegaly, pheochromocytoma) are also contributing factors for CHF [2]. CHF is a clinical syndrome in which pathophysiologic

underpinnings include left ventricular (LV) dysfunction, remodeling, and increased neurohormonal activation. Experimental models are required to better understand the pathophysiology and progression of the disease and to screen and evaluate new test drugs [3]. Aortic banding model in rats produce CHF by causing pressure overload is one such way to understand the disease pathophysiology [4]. In this model, the CHF is associated with multi organ dysfunction as seen in human CHF pathophysiology [5]. The advantages of this model are that the mortality rate of the banding procedure is relatively low and that there is a high success of induction of consistent LV hypertrophy [6].

2. Materials and methods

2.1. Experimental animals

Inbred Wistar male rats were obtained from the animal facility of Torrent Research Centre. All the animals were of 5–7 weeks of age with the body weight range from 150 to 240 gm. All the protocols used in the study were approved by Institutional Animal Ethics Committee. Animals were maintained in Individual Ventilated Cage (IVC) system with well controlled supplied air, humidity

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(30–70%) and temperature (19–25 °C) with 12 h day and night cycle. Animals were provided with autoclaved standard rat chow (Pranav Agro Ltd, Maharashtra) adlibitum and autoclaved water. The animal cages and bedding material (Shree sagar rice mill, Ahmedabad) were changed regularly twice in a week.

2.2. Materials

Isoflurane (Forane, Abott chemicals Ltd.), Buprenorphine HCL (Buprigesic, Neon Laboratories Ltd.), Ketamine (Parke Davis India Ltd.), Xylazine (Aarambh Life Sciences), Benzyl Penicillin (Benzyl Penicillin Injection, Alembic Ltd.), Povidone-Iodine (Betadine, Win-Medicare Pvt. Ltd.), Heparin (Beparine, Biological E. Ltd.), Eosin Y disodium salt (Fluka), Specimen embedding medium (Cryochrome), Sodium chloride AR (S D Fine-Chem Ltd.), Terg-A-Zyme (Alconox, Inc.), Nonabsorbable silk sutures (2–0, 3–0, 4–0), anaesthesia induction cone, gloves, glass petridish, cotton buds, cotton gauze, cotton, bloating paper, tissue roll, syringe (1.0 ml, 2.0 ml), hypodermic needles (22 G, 24 G, 26 G), etc. were procured and used as per the experimental need.

2.3. Study design

Animals were randomly divided in 6 groups before surgery (I: Aortic banding at suprarenal portion with 22 G needle (Supra 22) (n = 7), II: Aortic banding at suprarenal portion with 24 G needle (Supra 24) (n = 7), III: Sham for aortic banding at suprarenal portion (Sham supra) (n = 7), IV: Aortic banding between renal arteries with 22 G needle (Inter 22) (n = 6), V: Aortic banding between renal arteries with 24 G needle (Inter 24) (n = 7) and VI: Sham for aortic banding between renal arteries (Sham inter) (n = 7)). From the 41 animals, 8 animals were found dead during first week after surgery. Each group was left with six animals except Inter 22 (n = 4) and Inter 24 (n = 5) half of which were sacrificed at 6th week except Inter 24 where out of 5 animals, two were sacrificed at 6th week. Rest of the animals in each group were sacrificed at 8th week.

2.4. Experimental protocol

Male Wistar rat was standardized for aortic banding model. A dose of Buprenorphine (0.8 mg/kg, s.c.) and Benzylpenicillin (30000 IU/rat, i.m.) were administered prior to surgery. Body weight, food consumption and water intake were recorded before surgery. Abdominal aortic banding was performed in order to induce CHF (Vogel et al. 1965). The surgery was performed under isoflurane anaesthesia followed by maintenance between 1 and 3 % (as per requirement) in medical oxygen with help of anaesthesia station. The body temperature of animal was maintained at 37 °C. Abdomen was opened to take out intestine and 0.5 cm segment of abdominal aorta was dissected free either above or between renal arteries for respective groups and a 4–0 silk ligature (Ethicon) was passed under the aorta. Blunted needle was placed on the anterior surface of the aorta. Constriction was made by 22 G (0.7 mm, o.d.) or 24 G (0.5 mm, o.d.) needle for respective groups of animals. A ligature was tied around both the aorta and the needle. The needle was removed, leaving the internal diameter of the aorta approximately equal to that of the needle. The intestine was placed back into the abdominal cavity. Simple interrupted stitches were taken with 2–0 and 3–0 nonabsorbable silk sutures (Diamond) for muscle and skin respectively. Animals in Sham group underwent same surgical procedure as mentioned above with an untied ligature placed in the same location. The surgeon variation was avoided throughout the study keeping the same surgeon to ascertain the method of needle holding and pressure gradient during banding process remains homogenous throughout all groups. Animals were

transferred to the cages initially on blotting paper and then shifted to normal paddy husk bedding upon adequate recovery. Doses of buprenorphine (0.8 mg/kg, s.c.) and Benzylpenicillin (30,000 IU/rat, i.m.) was administered as a part of postoperative care. Animals were inspected daily for level of activity, healing of surgical wounds and clinical signs of illness like abnormal movement, hunched posture, paralysis of hind limbs, piloerection, faecal output, urination and dyspnea. Body weight, food consumption and water intake were recorded daily up to 7 days of surgery and from the 8th day till termination, the measurement was taken every alternate day.

2.5. Measurement of blood pressure

Non-invasive blood pressure (NIBP) was measured using tail cuff method before surgery and at the 2, 4, 6 and 8 weeks after surgery. The animals were acclimatized for 30 min before taking the readings. The instrument had 6 channels so at a time 6 animals were placed and systolic blood pressure (SBP) recording was obtained simultaneously for all the 6 rats. For each animal the SBP was measured on 3 consecutive days. On each day of recordings, 5 readings were obtained for each animal and data from day 2 and day 3 were averaged and was expressed as the mean SBP for the respective animal.

2.6. ECG measurement

The electrocardiogram (ECG) was recorded with a Bio Amp device and PowerLab System (PowerLab 8/30; AD Instruments). ECG measurement was performed after ketamine (75 mg/kg) and Rompun (0.75 mg/kg xylazine) administration subcutaneously. The limb lead II ECG was recorded from needle electrodes inserted subcutaneously into the right forelimb (negative), left hind limb (positive) and right hind limb (ground). The signal was acquired for about 5 min using Chart 5 software. A representative 15 s segment of the recording was averaged to obtain the signal averaged ECG. The recorded files were then analyzed by Chart 5-Pro software for parameters like QRS interval and QT interval.

2.7. Measurement of hemodynamic parameters

Millar pressure volume system (MPVS-300) was used to measure cardiac hemodynamic parameters at the time of termination. A Mikro-Tip P-V catheter (SPR 838) was connected with the pressure transducer connector and volume transducer connector. The catheter tip was soaked in saline solution at 37 °C for 30 min prior to insertion into the animal to get the catheter temperature near to body temperature. Two-point pressure and volume calibration procedure were carried out using the electronic calibration settings on the MPVS-300. Ventilator was connected with anaesthesia station to maintain anaesthesia (between 1 and 3% isoflurane) along with respiration during the experiment. The rate of ventilator volume was set at 10 ml/kg body weight.

2.8. Insertion procedure to use Mikro-Tip P-V catheter

Animal was anesthetized followed by a midline neck incision extending from the mandible to the sternum for endotracheal intubation. The thin muscle layer was bluntly dissected around the throat to expose the trachea. A small cut about halfway through the trachea was made and the intubation tube was inserted into the trachea and secured with suture. The intubation tube was attached to the small animal ventilator connected with the anaesthesia station. Isoflurane concentration was maintained between 1 and 3 % (as per requirement) in medical oxygen to maintain a respiratory rate between 60 and 90 breaths/min.

The left jugular vein was cannulated for infusion of hypertonic saline (20%). The right carotid artery was isolated and the proximal end of the artery was ligated with the help of suture (proximal to the head) (Fig. 1). Steady state recording for the animal was taken with switching off the ventilator and as steady loops were obtained (min. of 8–10 loops), the ventilator was started immediately. The recording was stopped after getting the steady state and saline loops and catheter was removed. The recorded files were analyzed to obtain hemodynamic parameters like aortic SBP and diastolic blood pressure (DBP), heart rate (HR), left ventricular end systolic volume (LVESV), left ventricular end diastolic volume (LVEDV), left ventricular end systolic pressure (LVESP), left ventricular end diastolic pressure (LVEDP), stroke volume (SV), ejection fraction (EF), cardiac output (CO), dP/dtmax and dP/dtmin.

2.9. Morphometry of the organs

The animals were euthanized and heart was first isolated followed by lungs and kidneys. All the organs were weighed. The photographs of the heart were taken. The heart was cut from atrioventricular groove to separate the auricles and ventricles. Left ventricle was separated and weighed. The ventricles were again cut at 2 mm distance from atrio-ventricular groove for sectioning. With the help of Cryostat instrument (Thermo electron corporation, UK), 10 μm thick section was taken on the slide followed by staining with Eosin Y disodium salt (1% solution in water) for 5–7 s and washing softly with water. The water was removed from the slide before scanning. The left ventricular weight, wall thickness and area were measured using Image Pro Plus software from the scanned slides.

2.10. Measurement of biochemical parameters

The urine and blood samples were collected before surgery (basal) and at the second, forth, sixth and eighth week of surgery. The animals were placed in the metabolic cage for 24 h and urine volume was measured and urine samples were analyzed for creatinine level. The 0.5 ml of Blood was collected each time by sublingual route under isoflurane anaesthesia in eppendroff tube containing 2 μl heparin of 5000 IU/ml concentration to separate the plasma. The plasma samples were tested for the estimation of Urea, Creatinine, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT). The urine samples were estimated for Creatinine. The estimations were carried out in Biochemistry analyzer (Olympus).

2.11. Statistical analysis

Results were expressed as mean ± SEM. Results from each banded group at particular week were compared with that of respective week sham control group. All comparisons were performed on Graph pad PRISM software, version 3.02 using Student *t*-test. Statistical significance was set at p < 0.05.

3. Results

3.1. Body weight, food consumption, water intake and biochemical parameters after 6 and 8 weeks of aortic banding:

Food consumption and water intake was found similar in banded groups in comparison to respective sham groups throughout course of the study. Although a slight decrease in body weight was observed in banded groups compared to sham, the difference was not significant in any of the groups either at 6 or at 8 weeks.
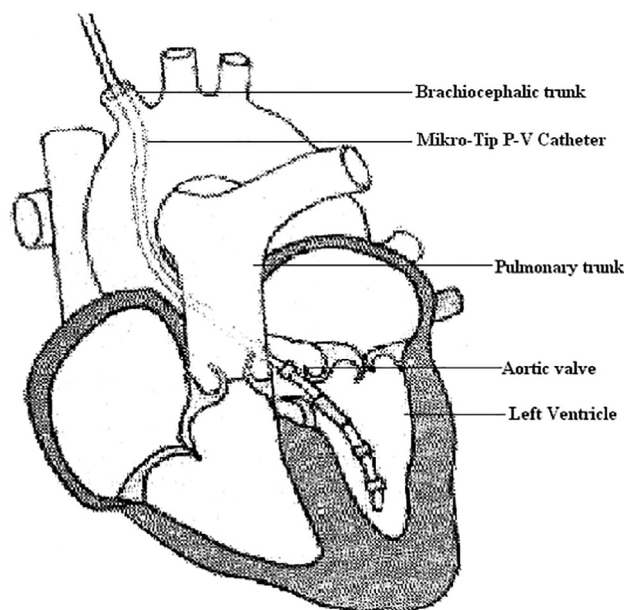

Fig. 1. Catheter setting for Hemodynamic measurements.

Biochemical estimation was carried out at 0, 2, 4, 6 and/or 8 weeks to measure plasma parameters like urea, AST, ALT, Cr and urine parameters like CrCl and urine volume. There was no significant difference found in the plasma and urinary biochemical parameters in any of the banded groups throughout the course of the study.

3.2. Hemodynamic measurement

3.2.1. Effect on systolic blood pressure after 6 and 8 weeks of aortic banding

There was significant rise in SBP in supra 22 and supra 24 (Fig. 2a) groups of animals compared to sham supra group at the end of 6 weeks of aortic banding. Amongst the animals terminated at the 8 weeks of aortic banding, only supra 24 group of animals showed significant rise in SBP (Fig. 2a) in comparison to supra sham group.

In comparison, when SBP was measured in conscious rats by NIBP, neither banded group showed significant difference at the 6th week period. Since blood pressure measurement using Millar is an invasive technique in stable anaesthetized animals, the results obtained seems to be more accurate whereas NIBP is non-invasive technique, so the chances of variation are more in the results obtained with NIBP. Therefore, SBP measured by NIBP, at the end of 6 weeks in supra groups were not statistically significant compared to respective sham control. At the end of 8 weeks, supra 24 group (Fig. 2b) showed significant increase in SBP compared to sham supra as seen in the results using millar. This may be due to the large magnitude of difference in SBP observed in supra 24 group at the end of 8 weeks of aortic banding. Thus, the difference was significant in the results obtained with NIBP.

3.2.2. Effect on diastolic blood pressure measured by Millar PV system after 6 and 8 weeks of aortic banding

The mean DBP was significantly higher in supra 24 (Fig. 3) group compared to respective sham group at the end of 6 weeks of aortic banding when measured by Millar PV system.

However, though increased DBP was observed in supra 24 group at the end of 8 weeks of aortic banding, it was not statistically significant because of higher variability of this parameter
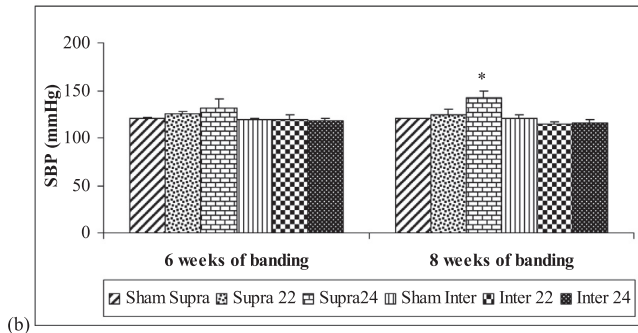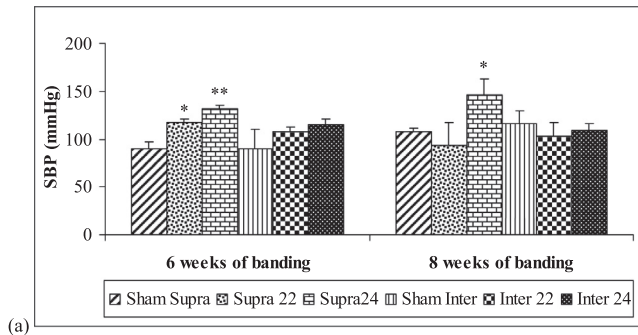
Fig. 2. (a) Average SBP measured by Millar PV system and (b) Average SBP measured by NIBP after 6 and 8 weeks of aortic banding.
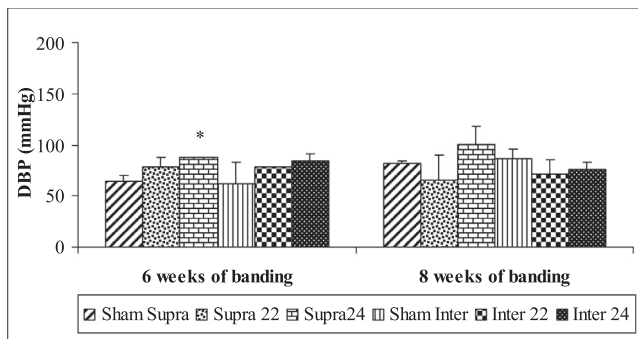

Fig. 3. Average DBP measured by Millar PV system after 6 and 8 weeks of aortic banding.

Fig. 4. Average HR measured by ECG after 6 and 8 weeks of aortic banding.
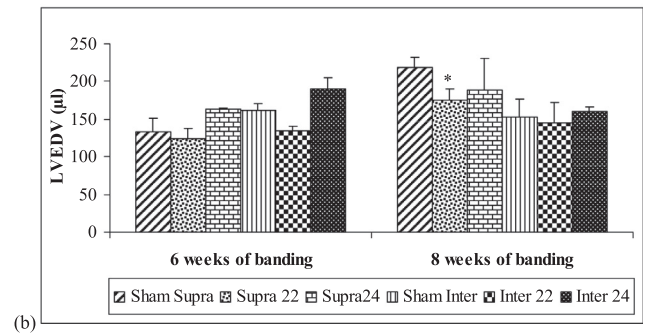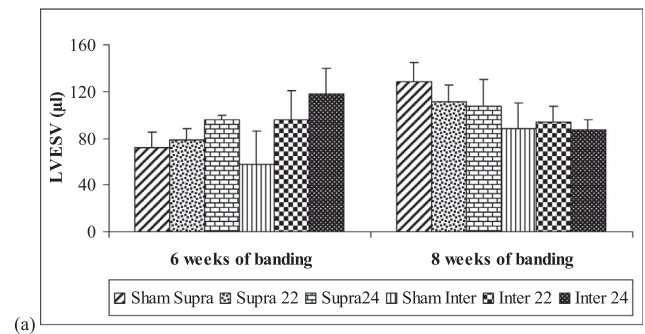
Fig. 5. (a) Average LVESV and (b) Average LVEDV after 6 and 8 weeks of aortic banding.

within animals of this group. Other banded groups showed decrease in DBP as compared to sham. Since, NIBP method was found to be inaccurate in giving the results of DBP, the measurement was carried out only by Millar PV system.

3.2.3. Effect on heart rate measured by ECG after 6 and 8 weeks of aortic banding

Results of ECG at the 6 weeks of aortic banding showed no significant difference in HR in any of the banded groups when compared to their respective sham groups. At the 8 weeks of aortic banding there was significant decrease in HR in supra 24 group (Fig. 4) when compared with sham supra.

3.2.4. Effect on left ventricular end systolic volume and left ventricular end diastolic volume after 6 and 8 weeks of aortic banding

LVESV was increased in all the banded groups at the 6 weeks of aortic banding. However, 8 weeks of aortic banding caused decrease in LVESV in all the banded groups (Fig. 5a).

At the 6 weeks of aortic banding, supra 24 and inter 24 groups showed increase in LVEDV whereas other two banded groups
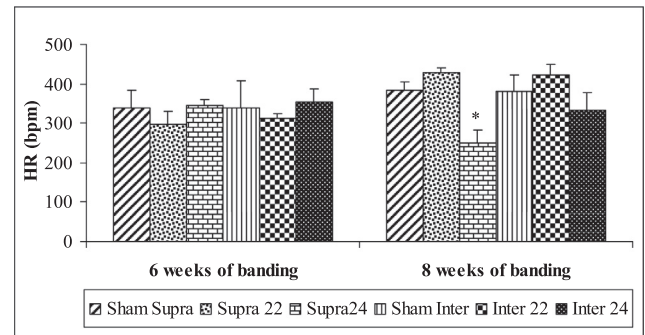
showed decreased LVEDV. However, at the 8 weeks of aortic banding all the banded groups showed decrease in LVEDV and the difference was significant in supra 22 group (Fig. 5b) when compared against sham supra group.

3.2.5. Effect on left ventricular end systolic pressure and left ventricular end diastolic pressure after 6 and 8 weeks of aortic banding

Aortic banding after 6 weeks caused a significant increase in LVESP in supra 24 group (Fig. 6a) when compared against respective sham group whereas all other banded groups showed an increase in LVESP. At the end of 8 weeks, significant increase in LVEDP was observed in inter 22 group (Fig. 6b) compared to sham inter group.

3.2.6. Effect on ejection fraction and cardiac output after 6 and 8 weeks of aortic banding

After 6 weeks of aortic banding, a reduction in EF was observed in all the four banded groups. However, EF was found to be similar to control at the 8 weeks of aortic banding period (Fig. 7a).

Insignificant difference in CO was observed in all the banded groups at 6 and 8 weeks of aortic banding in comparison to respec-
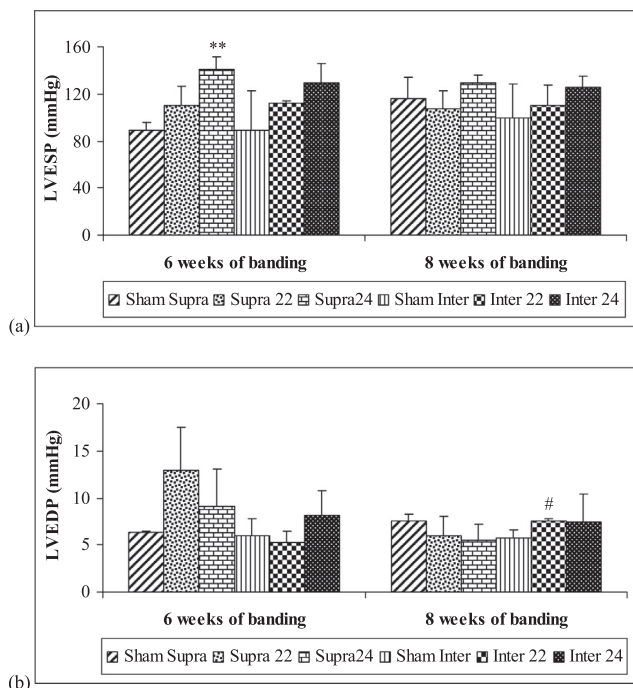
Fig. 6. (a) Average LVESP and (b) Average LVEDP after 6 and 8 weeks of aortic banding.
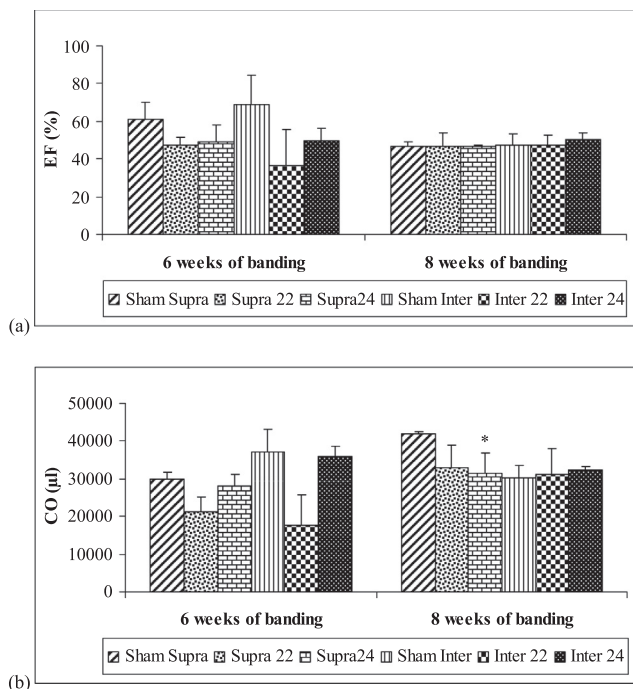

Fig. 7. (a) Average EF and (b) Average CO after 6 and 8 weeks of aortic banding.

tive sham control except for 8 weeks supra 24 group which showed significant difference in CO (Fig. 7b) in comparison to its respective sham control group.

3.2.7. Effect on dP/dt max and dP/dt min after 6 and 8 weeks of aortic banding

Maximum rate of ventricular pressure rise during systole is denoted by dP/dt max which was increased at the 6 weeks period

of banding. Maximum rate of ventricular pressure reduction during diastole is represented by dP/dt min which was decreased after 8 weeks of banding except for inter 24. No significant difference was observed in dP/dt min in all the banded groups at 6 and 8 weeks of aortic banding (Fig. 8a and 8b).

3.3. ECG parameters

3.3.1. Effect on QRS interval and QT interval after 6 and 8 weeks of aortic banding

QRS interval represents ventricular depolarization. QRS interval was significantly increased in supra 24 group (Fig. 9a) compared to respective sham control, after 6 weeks of aortic banding. At the end of 8 weeks, inter 22 group showed decrease in QRS interval value whereas other banded groups showed values near to control animals.

QT Interval represents ventricular activation and recovery. In all the banded groups at 6 weeks, QT Interval was increased insignificantly. After 8 weeks of aortic banding, only supra 24 group showed significant prolongation (Fig. 9b) in QT Interval compared to respective sham control.

3.4. Morphometric analysis

3.4.1. Effect on lung and kidney weight after 6 and 8 weeks of aortic banding

All the banded groups showed increase in lung weight after 6 weeks of aortic banding. At the 8 weeks period of aortic banding, both the supra groups showed increased lung weight whereas results in inter groups were similar to sham control (Fig. 10a).

At the 6 weeks of aortic banding, except for inter 22, all other banded groups showed decrease in kidney weight and the difference was significant in supra 22 group (Fig. 10b) compared against sham supra group. At the 8 weeks period of aortic banding, the significant decrease was observed in both the supra groups (Fig. 10b) when compared against respective sham group.

3.4.2. Effect on heart weight after 6 and 8 weeks of aortic banding

Abdominal aortic banding resulted in significant rise in heart weight in supra 24 group (Fig. 11) after 6 weeks when compared against respective sham group. Heart weight increased significantly in all the banded groups (Fig. 11) when compared against respective sham groups at 8th week, except for supra 22 group. Photographs of normal and hypertrophied heart are shown in Fig. 12.

3.4.3. Effect on LV weight after 6 and 8 weeks of aortic banding

Significant increase in LV weight was observed upon abdominal aortic banding in supra 24 group (Fig. 13) after 6 weeks. Banded groups showed significant increase in LV weight as compared to respective sham groups at 8th week.

3.4.4. Effect on LV wall thickness after 6 and 8 weeks of aortic banding

Sections of the ventricles were taken to measure left ventricular wall thickness. Sections of normal and hypertrophied heart are shown in Fig. 14. At the 6 weeks of aortic banding, only inter 24 group (Fig. 15) showed significant increase in LV wall thickness compared to respective sham control. At the 8 weeks of aortic banding, supra 24 and inter 22 groups (Fig. 15) showed significant increase in LV wall thickness.

4. Discussion

Abdominal aortic banding has been widely used, for induction of pressure overload model of experimental congestive heart fail-
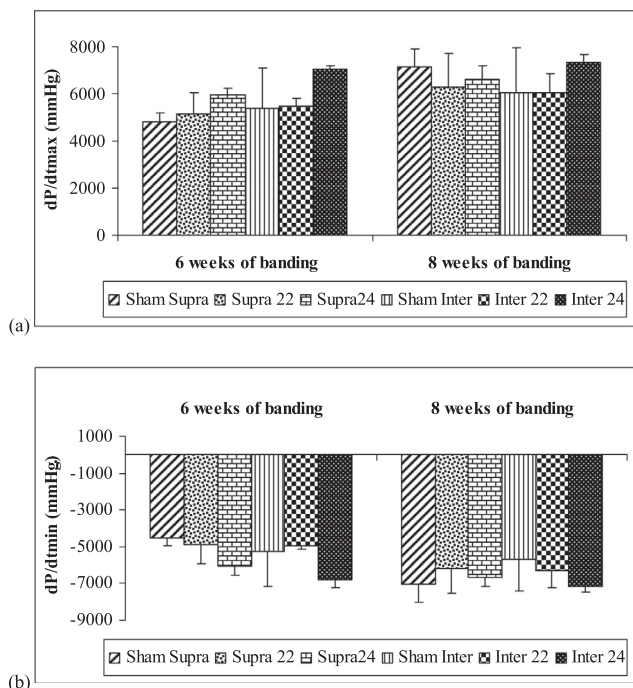
Fig. 8. (a) Average dP/dt max and (b) Average dP/dt min after 6 and 8 weeks of aortic banding.
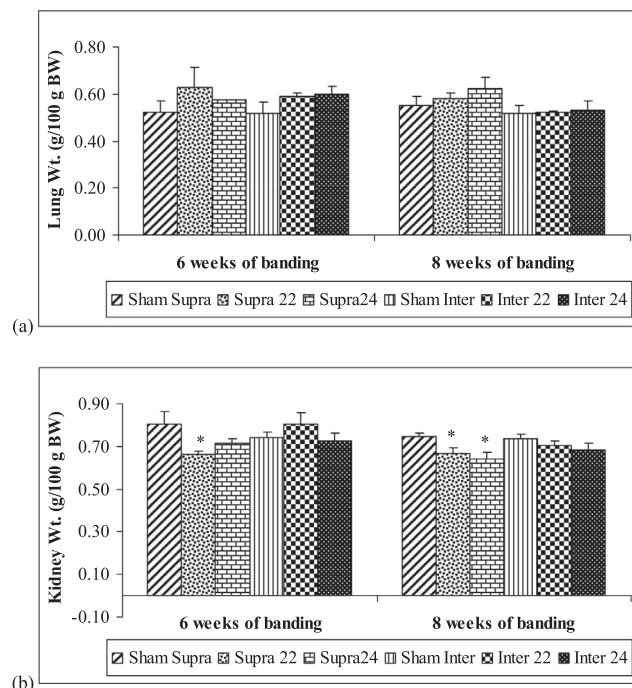

Fig. 9. (a) Average QRS Interval and (b) Average QT Interval after 6 and 8 weeks of aortic banding.

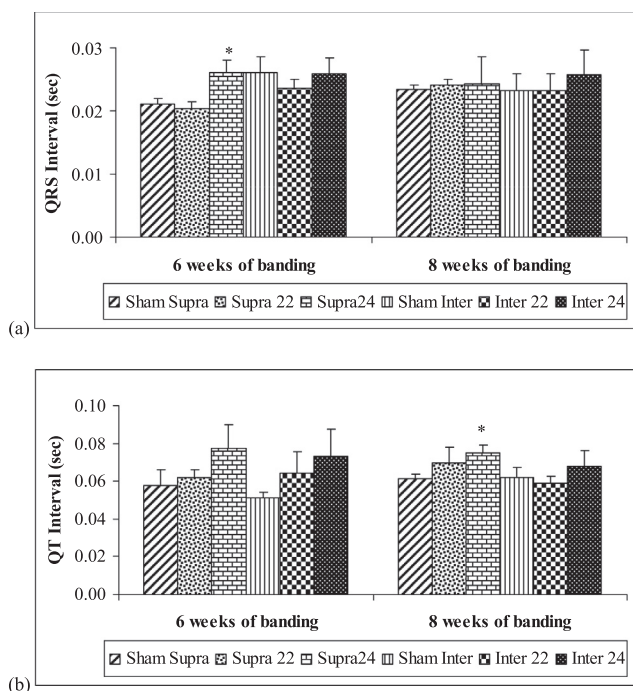**Fig. 10.** (a) Average lung weight and (b) Average kidney weight after 6 and 8 weeks of aortic banding.
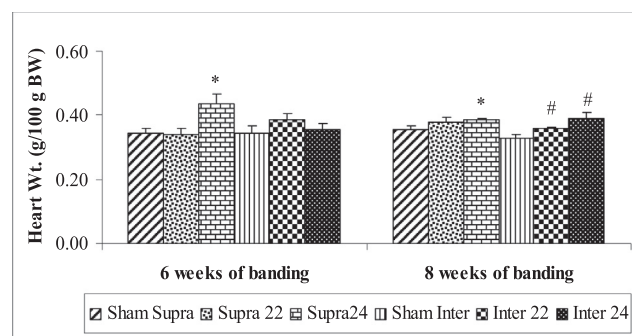

Fig. 11. Average heart weight after 6 and 8 weeks of aortic banding.

ure (CHF) and its associated complications [4,7,8]. In present study, effect of various techniques of aortic banding in the development of CHF by measuring myocardial functional and structural parameters. Abdominal aortic banding model of CHF in rat is associated with about 20% mortality [6]. Zhang et al. [9] reported up to 33% mortality in the first 48 h after abdominal aortic banding. In present study overall mortality of 19.5% in set of animals was found coinciding with the reports. Wistar strain of rat was selected for standardization of aortic banding model as this strain has been extensively used in the study of Congestive heart failure [6,10–13]. Males show early response to pressure overload, including the transition to heart failure with aortic banding than females [14] hence, male rats were used in our study. They suggested that the possible cause of sex differences in response to pressure overload may be due to the cardio protective action of female hormones (mainly estrogen). According to them, estrogen may be a transcriptional regulator of genes implicated in hypertrophy, including myosin heavy chain isoforms, structural matrix proteins, angiotensin mRNA levels and ACE activity [14]. In abdominal aortic banding, commonly used locations of banding of aorta are above both renal arteries and between renal arteries [6,15]. Moreover, many researchers have tried different extent of aortic constriction using 21, 22, 25, or 26 G needles for periods ranging from 1, 3, 4, to 10 weeks to monitor progression of disease [4,16,17]. Therefore, in an effort to compare, evaluate and optimize better technique in terms of extent and duration of abdominal aortic constriction, abdominal aortic banding at two different locations of abdominal aorta i.e. above both renal arteries and between renal arteries were performed. Extent of aortic constriction was varied using needles of 22 G and 24 G. Thus, four aortic banded groups were included
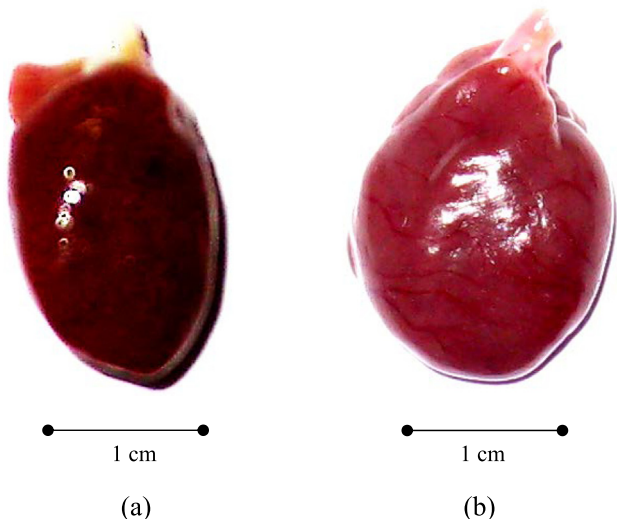
Fig. 12. Representative photographs of hearts. (a) Control heart from sham supra group at 8 weeks of surgery and (b) Hypertrophied heart from supra 24 group at 8 weeks of aortic banding. Scale in millimeters.
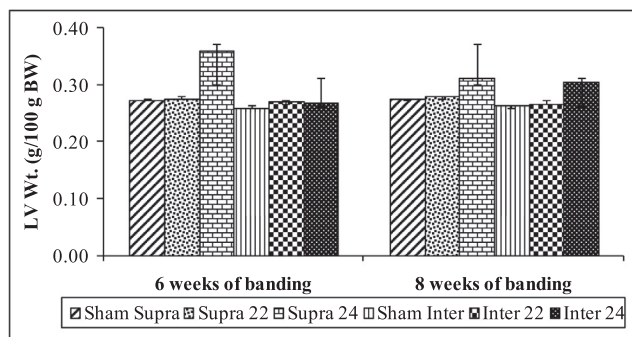

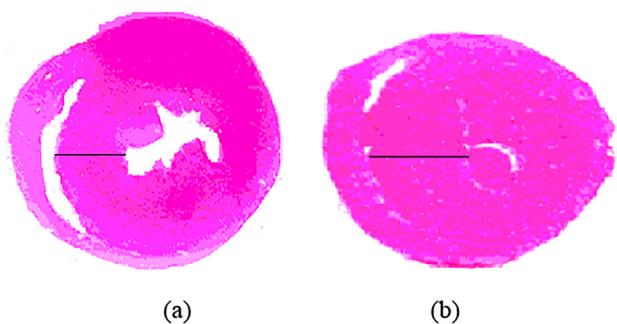Fig. 13. Average LV weight after 6 and 8 weeks of aortic banding.

Fig. 14. Representative sections of ventricles of (a) control heart from supra sham group at 8 weeks and (b) hypertrophied heart from supra 24 group at 8 weeks. Line shows LV wall thickness.

in the study. In addition, the banded animals were evaluated for the progression of the disease by terminating them at either 6 weeks or 8 weeks of aortic banding. Ribeiro et al. [18] reported significant LV hypertrophy with 2 weeks of follow-up. According to their study after 6 weeks, LV weight or LV wall thickness did not increase for which they have suggested that, after aortic stenosis induction, LV hypertrophy occurs precociously and then it becomes stable. Grossman et al. [19] have reported increased LV mass and wall thickness in patients with chronic pressure overloaded left ventricles during compensatory phase of heart failure.
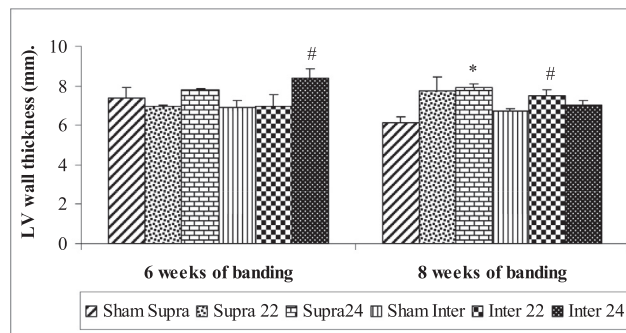

Fig. 15. Average LV wall thickness after 6 and 8 weeks of aortic banding.

In this study, increased heart weight in supra 24 groups after 6 weeks of banding was found, whereas both heart weight and LV wall thickness after 8 weeks of banding was found to be increased in groups other than supra 24. This indicates that the progression of hypertrophy has set in supra 24 group earlier than other groups. A reduction in body weight was observed in animals with the increase in banding duration. The observation is in line with reports by Toussaint et al. [5]. Ribeiro et al. [18] have also shown significant decrease in body weight in supravalvular aortic stenosis group compared to control at the end of 6 weeks period. Maintenance of arterial blood pressure in the presence of reduced cardiac output is an effective compensatory mechanism. The increase in the activity of the sympathetic nervous system, RAAS and endothelins are major mechanisms to maintain blood pressure during compensatory phase. As a result, increased SBP and DBP can be observed during this phase [20]. Similar results were observed with increased SBP and DBP after 6 and 8 weeks of banding which indicates compensatory phase of CHF at the time of termination. Heart failure is known to be associated with a marked reduction in beta receptor density and contractile response to beta adrenergic agonists which may be possible reason behind decreasing HR in failing heart [21]. Observed decrease in HR of our supra 24 group animals at 8 weeks of banding are in line with report of Bristow [21], which indicates that this group is progressing towards decompensatory phase of CHF. The pressure demand of aortic stenosis causes concentric hypertrophy to occur [22]. During compensatory phase of hypertrophy, the increase in wall thickness (Laplace equation) allows the ventricle to maintain normal wall stress [23]. When the pressure overload is severe and prolonged, the hypertrophied myocardium assumes pathologic characteristics, exhibiting systolic or diastolic dysfunction or both [24]. According to present study findings, LVEDV, LVESV, LVEDP and LVESP values were higher in banded groups at the 6 weeks whereas they found to be similar to sham at the 8 weeks. In addition, heart weight and LV wall thickness were increased at this time period but left ventricular systolic and diastolic function was found to be normal which could suggest that the animals were in compensatory phase of hypertrophy when terminated at the 8th week of aortic banding. In a rat model of chronic pressure overload, concentric chamber remodeling with preserved ejection fraction has been reported [25]. Preserved EF of rats with cardiac hypertrophy and remodeling suggesting continuation of compensatory phase of CHF in experimental animals was observed. After 8 weeks of banding, supra 24 group showed significant decrease in CO amongst all groups. In addition to this, a decrease in +dP/dt and −dP/dt was also found in supra 24 group at 8 weeks. These findings could be interpreted to conclude that after 8 weeks of banding the animals in the supra 24 group was progressing towards decompensatory phase of CHF, though not yet exhibiting frank decompensatory heart failure. Cardiac hypertrophy, a risk factor for QT-

prolongation and cardiac sudden death is an indicator for the time for ventricular depolarization and recovery. Recent studies in human patients and animal models have demonstrated that cardiac hypertrophy cause down regulation of gap junctional proteins (mainly connexin 43) causing a reduction in cell coupling and cardiac conduction properties leading to disturbance in action potential duration and potential malignant arrhythmia and cardiac sudden death [26]. In addition, cardiac hypertrophy resulting from the hypertrophic growth of cardiac myocytes is suggested to cause an unbalanced distribution of Purkinje fibers in the remodeling heart which results in interruption of pacemaker conduction potentials which is also contributing factor for QT prolongation [27]. Significant QT interval prolongation in supra 24 group at 8 weeks period amongst all groups was recorded in present study. However, as reported by others [28,29], increase in QRS interval was not observed which may be because this stage was yet to be achieved by animals during course of progression of CHF. Slight increase in lung weight was observed in banded animals but the difference was not significant which could be due to initial stage of pulmonary congestion. In the decompensatory stage, excessive pulmonary congestion leads to increased pulmonary capillary pressure hence movement of the liquid from the blood to the interstitial space and in some instances to the alveoli, which is the reason of occurrence of pulmonary edema in the development of CHF [20]. Significant decrease in kidney weight at the 8 weeks of banding in supra groups was observed whereas inter groups did not show major difference. The reduction in kidney weight may be due to abdominal aortic constriction along with decreased renal perfusion as a result of decreased cardiac output [30]. The possible reason for difference in results between supra and inter groups could be due to difference in location of aortic banding where supra groups lack sufficient blood supply to both kidneys whereas in inter groups only the left kidney is affected with poor perfusion. Similar decrease in kidney weight was obtained by Toussaint et al. [5] at the 8 weeks of supravalvular aortic banding. Several authors have reported induction of aortic banding in rats promotes early development of concentric left ventricular hypertrophy with cardiac function initially being normal, followed by diastolic dysfunction and finally reduction of systolic ventricular performance from 2 to 21 weeks of banding [31]. According to our findings, cardiac concentric hypertrophy has set in by 6 and 8 weeks of aortic banding with normal LV systolic and diastolic function. This suggests that our banded rats were still in compensatory phase. However, earlier significant increase in HW, QT interval prolongation, fall in CO and reduction in HR observed in our supra 24 banded group indicates that CHF is progressing faster in this group. Although, rate of progression was highest in supra 24 group of animals at the time of termination, it had not yet progressed into frank decompensatory phase of heart failure, so it is necessary to increase number of animals along with longer duration of banding in supra 24 group to cause frank CHF.

5. Conclusions

The findings from the present study proposed that abdominal aortic banding in rats can induce a chronic pressure overload, leading to transition from compensatory left ventricular hypertrophy to heart failure. Abdominal aortic banding is a suitable method to induce pressure overload mediated alteration in myocardial function and structure, which could ultimately lead to CHF. Abdominal aortic constriction above both the renal arteries with 24G needle is a model which show faster rate for progression of CHF in comparison to other abdominal aortic banding techniques. Thus, constricting the abdominal aorta above both the renal arteries with 24 G needle may be better technique to induce experimen-

tal pressure overload heart failure in rats. Rats in none of the banded groups progressed into decompensatory heart failure by end of 8 weeks of abdominal aortic banding. This is the limitation of the present study and it is recommended that the duration of model still needs to be lengthened to observe progression of the disease towards decompensatory phase of heart failure. However, to make firm conclusions it needs to explore the model further with larger number of animals in support of trend observed in present study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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