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Utilizing Sodium Hydrogen Sulphide to Enhance Renal Secretory Functions: A Promising Approach

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Abstract:
Left Ventricular Hypertrophy (LVH) is the enlargement of cardiomyocytes which will progress to cardiomegaly and heart failure. During the progression of LVH, renal reabsorption and excretory capabilities are also altered. Hydrogen sulphide (H2S) donors have been reported not only arrest the progression of LVH but also has improved the responsiveness of alpha-adrenergic receptors in the rats with LVH. Present study was set out with the hypothesis that chronic administration of H2S can improve the functions of the kidney by improving renal reabsorption and excretory capabilities by modulating oxidative stress and inhibition of collagen deposition in the kidney of Wistar Kyoto rats with LVH. LVH was induced by administration of isoprenaline (5 mg/kg, sub-cutaneous) and caffeine (62 mg/L in drinking water) for 14 days. Sodium hydrogen sulphide, a donor of H2S was administered at the dose of 56μM intraperitoneally for 35 days. Renal functions parameters were measured by taking the urine samples of the rats by placing them in metabolic cages for 24 hours on days 0 and 35. On day 35, rats were euthanized, kidney and heart indices were calculated. Kidney tissues were further processed in 5% formalin for histopathology to measure the collagen content in the tissue by using PicroSirus red staining. Plasma samples were used for the measurement of oxidative stress markers like malondialdehyde (MDA) and superoxide dismutase (SOD). Treatment with H2S donors to LVH group has significantly reversed (all $P<0.05$) the elevated levels of Fractional excretions of sodium (FENa) and potassium (FEK) when compared to LVH-WKY group. Chronic administration of H2S donors to LVH group has significantly increased ($P<0.05$) the plasma levels of H2S in LVH- H2S group which ultimately reduced the kidney indices of LVH-H2S group when compared to LVH-WKY group. Chronic administration of H2S donors to LVH group has significantly increased ($P<0.05$) the plasma levels of SOD and significantly decreased ($P<0.05$) that of MDA in LVH-H2S group when compared to LVH-WKY group. Increased H2S and SOD levels in the plasma of LVH- H2S group has reduced the collagen content in the kidney tissue when compared to LVH-WKY group. In summary, Sodium hydrogen sulphide chronic administrations to Wistar Kyoto rats with left ventricular hypertrophy improved renal reabsorption and excretory capabilities by modulating oxidative stress and inhibition of collagen deposition in the kidney of rats with LVH.

Keywords: Left ventricular hypertrophy; Hydrogen sulphide; Malondialdehyde; Superoxide dismutase; Oxidative stress.

Introduction

Left Ventricular Hypertrophy (LVH) is induced by elevated blood pressure due to sympathetic stimulation while control the blood pressure resulted in the regression of the LVH [1]. Renal sympathetic denervation not attenuate cardiac dysfunctions in heart failure [2] but also modulate kidney functions by acting on pressure-natriuresis relationship[3]. Previously reported studies have shown that onset of LVH decreased the responsiveness of alpha-adrenergic receptors of the kidney [4]. Alpha adrenergic receptors are reported to be involved in the renal hemodynamic in fructose fed Sprague-Dawley rats [5]. It has been reported that down regulation of cystathionine γ lyase (CSE) resulted in reduced responsiveness of α_1A adrenergic receptors in LVH [6]. Chronic administration of H_2S improved the responsiveness of α_1A adrenergic receptors in LVH by the upregulation of CSE/ H_2S pathway [4].

H_2S is known endothelial derived relaxing factor which is produced by three enzymes cystathionine γ lyase (CSE), Cystathionine Beta Synthetase (CBS) and 3-mercaptopyruvate sulphur transferase (3-MST) [4]. Reported data suggested all three H2S producing enzymes including CSE, CBS and 3-MST have been reported to express in the kidney [7]. H_2S has been reported to play multifactorial role in the kidney by interactions with other gaseous transmitter like nitric oxide which are surrogate producer [8]. H_2S along with tempol has been reported to improve the renal excretory functions and lowers the blood pressure in Spontaneously Hypertensive Rats (SHR) [9]. Chronic administration of H2S donor in SHR has been reported to altered kidney excretory function by its diuretic action [10].

Exogenous administration of H_2S is reported to alleviate diabetic nephropathy by reducing oxidative stress and inflammation [11]. Oxidative stress and inflammation in the kidney altered the architecture of the kidney which lead to changes in mesangial cells, podocytes and tubulointerstitial cells which are consequences of DN [12]. Collagen deposition in the kidney will altered the Elastin to Collagen (EC) ratio in Extracellular Membrane (ECM) and collagen III and IV are the biomarkers of renal fibrosis[13]. Collagen marker matrix metalloproteinase 2 and 9 (MMP-2 and MMP-9) are also biomarker of albuminuria in the kidney [14]. Collagen gene variants COL4A4 and podocyte cytoskeleton genes lead to the nephropathy in human [15]. H_2S is known as key regulator of MMP-9 in kidney dysfunction [16].

Based on these evidence that down regulation of cystathionine γ lyase resulted in reduced responsiveness of α_1A adrenergic receptors which are involved in renal functions, present study was set out with the hypothesis that chronic administration of H_2S can improve the functions of the kidney by improving renal reabsorption and excretory capabilities by modulating oxidative stress and inhibition of collagen deposition in the kidney of *Wistar Kyoto* rats with LVH.

Materials and Methods

4.1. Study group animals and methodology

A total number of 28 *Wistar Kyoto* rats of 200±10 gm was recruited in this study and were divided into four groups consisting of Control WKY, Control-H_2S, LVH-WKY and LVH-H_2S while each group having equal number of animals (n: 7). These animals were kept in the transit animal room with controlled environment of temperature and food. Continuous supply of food and water was made accessible to animals.

4.1.1. Induction of left ventricular hypertrophy: LVH was induced by a reported method [17] which was later modified [4] which consists of 5 sub-cutaneous injections of isoprenaline (5 mg/kg) on days 1,4,7,10 and 14. Caffeine was administered in drinking water (62 mg/L) for 2 weeks as reported by same laboratory [18]. Control WKY group followed same pattern of days of injection by the administration of normal saline. The NaHS (Sigma-Aldrich), a donor of H_2S, was administered 56µM intraperitoneal dose for 5 weeks [19] which started 3 weeks earlier than the induction of LVH model.

4.1.2. Collection of urine and plasma in the metabolic cages: After acclimatization of the animals in the animal transition room, day 0 and 35 urine collection of rats was done by using custom made stainless steel metabolic cages (Nalgene®, Thermo Scientific, Beaumont, PA, USA) with free access to water and food. Water intake was taken by subtraction of water supplied to the animals and water available in the bottle after 24 hours. Plasma sample was taken on day 0 was taken by tail vein while plasma sample was taken on day 35 on terminal day by cardiac puncture method and was stored in Eppendorf tube (Eppendorf®, USA) which were previously rinsed with heparin (Heparin 5000 i.u./ml, Leo Pharmaceuticals, Denmark). The collected blood was centrifuged at 5000 rpm for 3 minutes and then plasma was separated. This plasma was later stored at -20^{0}C for further analysis.

4.1.3. Renal function parameters on days 0 and 35: Creatinine in the plasma and urine was measured by using a colorimetric method as reported[20]. Readings were taken in 96 well plate reader (Epoch™ Microplate Spectrophotometer, BioTek Instruments, Einooski, Vermont, USA) at wavelength 520 nm by using a formula.
Urine creatinine (mg/dl): (absorbance of the sample/absorbance of the standard) x 2
Urine creatinine (mg/dl): (absorbance of the sample/absorbance of the standard) x100
Creatinine clearance was measured by using creatinine in the plasma and urine. Plasma and urine samples were estimated for sodium and potassium by using flame photometer (Jenway Ltd. Felsted, Essex, UK).

$$\text{Creatinine clearance (ml/min/100g BW)} = \frac{\text{Abs. creatinine clearance (ml/min)}}{\text{Wt. of animal (gm)}} \times 100$$

Estimation of sodium, potassium in plasma and sodium in urine required a dilution 1:200 with distilled water while potassium estimation required 1:1000 dilutions with distilled water. All the samples were prepared in duplicate before estimation. Fractional excretions of sodium or potassium is the measure of percentage of sodium or potassium excreted in the urine versus sodium or potassium reabsorbed by the kidney [21]. Measurement of FE_{Na+} and FE_{K+} requires the plasma and urine concentrations of sodium and potassium. FE_{Na+} and FE_{K+} can be calculated by using following equation;

$$\text{FE X (\%)} = \frac{U_x(\text{mmol/L}) \times P_{cr}\ (\text{mg/dl})}{P_x(\text{mmol/L}) \times U_{cr}\ (\text{mg/dl})}$$

4.2. Estimation of hydrogen sulphide in the plasma
Estimation of the H_2S in the plasma and urine was performed by reported method [19] which involves 4 sequential steps including trapping of H_2S, processing of samples, precipitation of the protein and centrifugation of the samples.

4.2.1. Estimation of oxidative stress marker: Plasma levels of oxidative stress markers like superoxide dismutase (SOD) and Malondialdehyde (MDA) on day 35 were measured by using a commercial kit and followed the instruction of manufacturer (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China).

4.2.3. Heart and kidney indices measurement: On day 35 after euthanasia animals, heart and kidney tissues were extracted, dried and weighed. Heat and kidney indices were measured by dividing the weight of kidney/heart by total body weight of the animals multiplied by 100 as reported [22].

4.2.4. Histopathology of kidney tissue by using PicroSirius Red staining: After extractions of the kidneys from the animals, tissues were placed in 5% formalin for further process of embedding, trimming, sectioning, and staining with PicroSirius red staining as reported. Later, kidney tissues underwent PicroSirius Red staining (Polyscience, Inc. Germany). Procedure involves the use of 3 solutions, solution A, solution B and Solution C. First of all, the slides were dipped in solution A for 2 minutes then rinse well in distilled water. The slides were placed in solution B for 60 minutes and then followed by solution C for 2 minutes. In last step, slides were immersed in the 70% ethanol for 45 seconds. The slides were dehydrated, cleared and then mounted. Collagen on the tissue gave red colour.

Result

5.1. Physiological data

5.1.1. Body weight: Body weight was measured taken twice in whole study which consisted of 35 days. On day 0, body weight was measured (gm) 191 ± 5, 191 ± 3, 185 ± 4 and 190 ± 6 in Control WKY, Control- H_2S, LVH-WKY and LVH-H_2S respectively. On day 35, body weight was measured 298 ± 5, 288 ± 7, 273 ± 3 and 275 ± 23 in Control WKY, Control- H_2S, LVH-WKY and LVH-H_2S respectively. A significant reduction ($P<0.05$) in body weight was observed in LVH-WKY when compared to Control WKY at day 35 as shown in Table 1.

5.1.2. Renal function parameters: Urine output was measured taken twice in whole study which consisted of 35 days. On day 0, Urine output was measured (ml/24 hours) 13 ± 1, 12 ± 1, 13 ± 1 and 14 ± 1 in Control WKY, Control- H_2S, LVH-WKY and LVH-H_2S respectively. On day 35 Urine output was measured 12 ± 1, 18 ± 1, 16 ± 1 and 24 ± 1 in Control WKY, Control- H_2S, LVH-WKY and LVH-H_2S respectively. A significant increase ($P<0.05$) in water intake were observed in LVH-WKY and LVH-H_2S when compared to Control WKY at day 35 as shown in Table 1.

FeNa (%) was measured taken twice in whole study which consisted of 35 days. On day 0, FeNa (%) was measured 1.6 ± 0.07, 1.6 ± 0.03, 1.6 ± 0.07 and 1.6 ± 0.07 in Control WKY, Control- H_2S, LVH-WKY and LVH-H_2S respectively. On day 35, FeNa (%) was measured 1.9 ± 0.15, 1.7 ± 0.20, 3.3 ± 0.06 and 1.7 ± 0.05 in Control WKY, Control- H_2S, LVH-WKY and LVH-H_2S respectively. A significant increase ($P<0.05$) in FeNa (%) were observed in LVH-WKY when compared to Control WKY at day 35 while a significant reduction ($P<0.05$) in FeNa (%) was calculated in LVH-H_2S when compared to LVH-WKY as shown in Table 1.

FeK (%) was measured taken twice in whole study which consisted of 35 days. On day 0, FeK (%) was measured 14 ± 2, 14 ± 1.8, 13 ± 0.9 and 13 ± 1.0 in Control WKY, Control- H_2S, LVH-WKY and LVH-H_2S respectively. On day 35, FeK (%) was measured 21 ± 1.2, 42 ± 4.8, 81 ± 0.5 and 46 ± 2.4 in Control WKY, Control- H_2S, LVH-WKY and LVH-H_2S respectively. A significant increase ($P<0.05$) in FeK (%) were observed in LVH-WKY when compared to Control WKY at day 35 while a significant reduction ($P<0.05$) in FeK (%) was calculated in LVH-H_2S when compared to LVH-WKY as shown in Table 1.

Urinary NA: K was measured taken twice in whole study which consisted of 35 days. On day 0, Urinary NA: K was measured 3 ± 0.2, 3 ± 0.2, 3 ± 0.2 and 3 ± 0.2 in Control WKY, Control- H_2S, LVH-WKY and LVH-H_2S respectively. On day 35, Urinary NA: K was measured 2 ± 0.1, 1 ± 0.1, 1 ± 0.04 and 1 ± 0.03 in Control WKY, Control- H_2S, LVH-WKY and LVH-H_2S respectively. A significant decrease ($P<0.05$) in Urinary NA: K were observed in LVH-WKY when compared to Control WKY at day 35 while no significant change in Urinary NA: K was calculated in LVH-H_2S when compared to LVH-WKY as shown in Table 1.

Creatinine clearance (ml/min) was measured taken twice in whole study which consisted of 35 days. On day 0, Creatinine clearance (ml/min) was measured 0.40 ± 0.02, 0.40 ± 0.02, 0.43 ± 0.02 and 0.42 ± 0.02 in Control WKY, Control- H_2S, LVH-WKY and LVH-H_2S respectively. On day 35, Creatinine clearance (ml/min) was measured 0.33 ± 0.02, 0.66 ± 0.06, 0.36 ± 0.009 and 0.85 ± 0.07 in Control WKY, Control- H_2S, LVH-WKY and LVH-H_2S respectively. There was no significant change in Creatinine clearance (ml/min) were observed in LVH-WKY when compared to Control WKY at day 35 while a significant increase ($P<0.05$) in Creatinine clearance was calculated in LVH-H_2S when compared to LVH-WKY and control WKY as shown in Table 1.

Table 1: Urine output, fractional excretion of sodium and potassium, urinary sodium to potassium ratio and creatinine clearance of Control groups of WKY and LVH treated with H_2S on days 0 and 35. The values are mean ± SEM (n= 6) $P<0.05$. Statistical analysis was done by repeated measure one-way analysis of variance (ANOVA) followed by Bonferroni *post hoc* test for all the groups on days 0 and 35. * $P<0.05$ vs. Control WKY and $^{\#}$ $P<0.05$ vs. LVH-WKY on D-35.

		Days of observation	
Parameters	**Groups**	**Day 0**	**Day 35**
Body weight (gm)	Control WKY	191 ± 5	298 ± 5
	Control- H_2S	202 ± 3	288 ± 7
	LVH-WKY	185 ± 4	273 ± 3*
	LVH-H_2S	190 ± 6	275 ± 23
Urine output (ml/24 hrs)	Control WKY	13 ± 1	12 ± 1
	Control- H_2S	12 ± 1	18 ± 1*
	LVH-WKY	13 ± 1	16 ± 1*
	LVH-H_2S	14 ± 1	24 ± 1 *#
FeNa (%)	Control WKY	1.6 ± 0.07	1.9 ± 0.15
	Control- H_2S	1.6 ± 0.03	1.7 ± 0.20
	LVH-WKY	1.6 ± 0.07	3.3 ± 0.06 *
	LVH-H_2S	1.6 ± 0.07	1.7 ± 0.05 #
FeK (%)	Control WKY	14 ± 2	21 ± 1.2
	Control- H_2S	14 ± 1.8	42 ± 4.8 *
	LVH-WKY	13 ± 0.9	81 ± 0.5 *
	LVH-H_2S	13 ± 1.0	46 ± 2.4 *#
Urinary NA: K	Control WKY	3 ± 0.2	2 ± 0.1
	Control- H_2S	3 ± 0.2	1 ± 0.1 *
	LVH-WKY	3 ± 0.2	1 ± 0.04 *
	LVH-H_2S	3 ± 0.2	1 ± 0.03 *#
Creatinine clearance (ml/min)	Control WKY	0.40 ± 0.02	0.33 ± 0.02
	Control- H_2S	0.39 ± 0.02	0.66 ± 0.06 *
	LVH-WKY	0.43 ± 0.02	0.36 ± 0.009
	LVH-H_2S	0.42 ± 0.03	0.85 ± 0.07 * #

5.1.3. Plasma and urine concentration of hydrogen sulphide: H_2S concentrations (μM) were measured in plasma and urine on day 35. There was a significant decrease ($P<0.05$) in concentration of H_2S in the plasma of

LVH-WKY group when compared to Control WKY (40±1 vs. 15±1). Treatment with H_2S in LVH group resulted in significant increase (P<0.05) in plasma concentrations when compared to LVH-WKY group (65±2 vs. 15±1) as shown in Table 2.

Table 2: Hydrogen sulphide (H_2S) in plasma and H_2S in urine of Control groups of WKY and LVH treated with H_2Son days 35. The values are mean ± SEM (n= 6). P<0.05. Statistical analysis was done by one-way analysis of variance followed by Bonferroni *post hoc* test for all the groups. * P<0.05 vs. Control WKY and # P<0.05 vs. LVH-WKY on D-35.

	Parameters	
Groups	H_2S in plasma (µM)	H_2S in urine (µM)
Control WKY	40 ± 1	16 ± 1
Control- H_2S	54 ± 1 *	45 ± 5 *
LVH-WKY	15 ± 1 *	28 ± 1 *
LVH-H_2S	65 ± 2 * #	47 ± 2 * #

There was a significant increase (P<0.05) in concentration of H_2S in the urine of LVH-WKY group when compared to Control WKY (28 ± 1 vs. 16 ± 1). Treatment with H_2S in LVH group resulted in significant increase (P<0.05) in urine concentrations when compared to LVH-WKY group (47 ± 2 vs. 16 ± 1) as shown in Table 2.

5.1.4. Superoxide dismutase (SOD) and malondialdehyde (MDA) measurements in the plasma: SOD levels in the plasma of LVH-WKY were significantly reduced (P<0.05) when compared to that of Control WKY (6 ± 1 vs. 3 ± 1) while treatment with H_2S significantly increased (P<0.05) the plasma levels of SOD in LVH-H_2S group when compared to LVH-WKY (7 ± 1 vs. 3 ± 1) as shown in Figure 1-A.

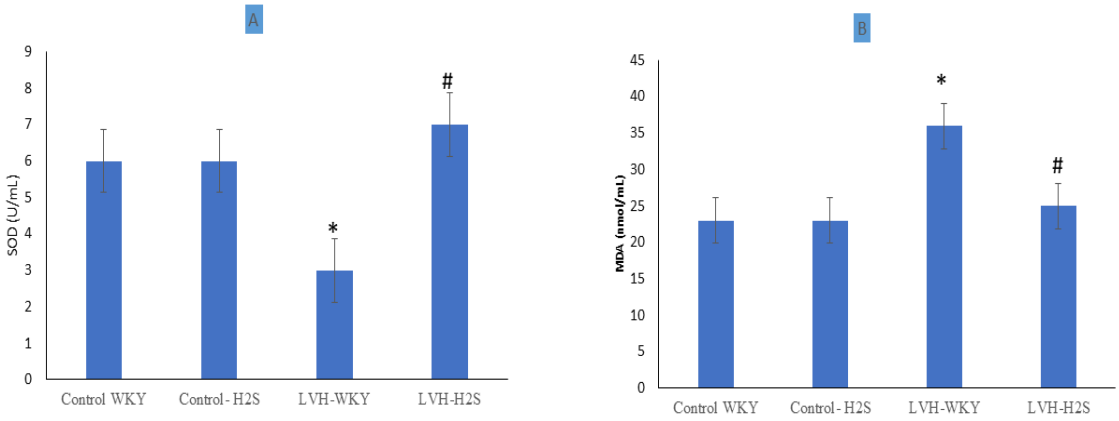

Figure 1: Superoxide dismutase (SOD) and malondialdehyde (MDA) of Control groups of WKY and LVH treated with H_2S on days 35. The values are mean ± SEM (n= 6). P<0.05. Statistical analysis was done by one-way analysis of variance followed by Bonferroni *post hoc* test for all the groups.* P<0.05 vs. Control WKY and # P<0.05 vs. LVH-WKY on D-35.

MDA levels in the plasma of LVH-WKY were significantly increased (P<0.05) when compared to that of Control WKY (37 ± 3 vs. 23 ± 3) while treatment with H_2S significantly decreased (P<0.05) the plasma levels of MDA in LVH-H_2S group when compared to LVH-WKY (37 ± 3 vs. 25 ± 4) as shown in Figure 1-B.

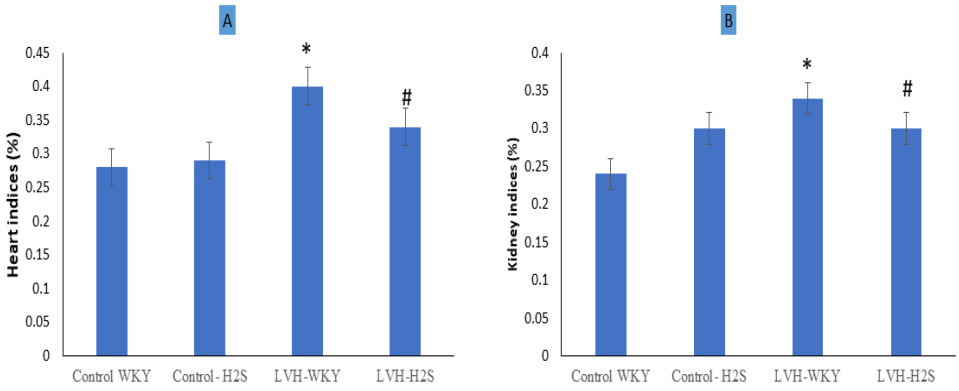

Figure 2: Heart and kidney indices of Control groups of WKY and LVH treated with H_2S on days 35. The values are mean ± SEM (n= 6). P<0.05. Statistical analysis was done by one-way analysis of variance followed by Bonferroni *post hoc* test for all the groups.* P<0.05 vs. Control WKY and # P<0.05 vs. LVH-WKY on D-35.

5.1.5. Heart and kidney indices: On day 35, heart and kidney indices were significantly increased (all P<0.05) in LVH-WKY group when compared to Control WKY while treatment with H_2S has significantly reduced (all P<0.05) the heart and kidney indices when compared to LVH-WKY as shown in Figure 2 (A and B).

5.1.6. Histopathology of kidney tissues: Development of LVH in WKY group resulted in deposition of thick bands of collagen which can be seen in Figure 3-B which are obvious when compared to Control WKY (Figure 3-A). Treatment with H_2S has diffused these bands of collagen deposition around the glomerulus and changed to thin thread of collagen as shown in Figure 3-D which is comparable to Control WKY (Figure 3-B).

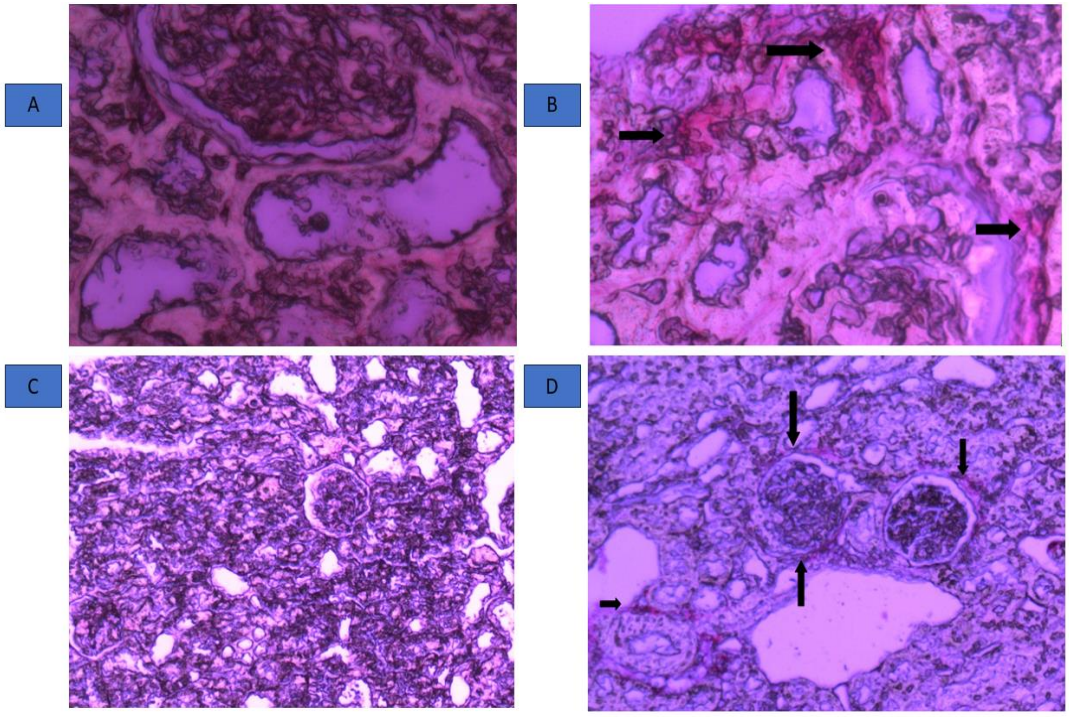

Figure 3: Histopathology of kidney tissues of Control WKY (A), Control-H2S (B). LVH-WKY (C) and LVH-WKY (D) on days 35 by using PicroSirius Red Stain with magnification 400X)

Discussion

Present study was set out with the hypothesis that chronic administration of H_2S can improve the functions of the kidney by improving renal reabsorption and excretory capabilities by modulating oxidative stress and inhibition of collagen deposition in the kidney of *Wistar Kyoto* rats with LVH. Present study came up with some novel findings that chronic administrations of Sodium hydrogen sulphide to *Wistar Kyoto* rats with left ventricular hypertrophy improved renal reabsorption and excretory capabilities by modulating oxidative stress and inhibition of collagen deposition in the kidney of rats with LVH.

Induction of LVH in WKY did not change the urinary output but treatment with H_2S has significantly increased ($P<0.05$) the urine output in LVH-H_2S group which indicates that exogenous administration of H_2S in rats with LVH has diuretic effects. Diuretic effects of H_2S are in coordination with study reported on spontaneously hypertensive diabetic rats [10]. Apparently it looks onset of LVH did not change the excretory abilities of the kidney in LVH as urine output remains unchanged in Control WKY and LVH-WKY but it seems tubular function of the kidney is changed which can be observed by measuring the Fractional excretions of sodium (FENa) and potassium (FEK) in LVH-WKY and Control WKY as shown in Table 1.Treatment with H_2S has reversed the changes in tubular function of the kidney by bringing the value lowers than LVH-WKY and closer to Control WKY as shown in Table 1. It can be deduced that onset of the LVH damages the kidney function by altering the tubular reabsorption and excretion capabilities of the kidney as kidney regulate the fluid and electrolyte balance through controlling the composition and volume of urine [23] while chronic administrations of the H_2S restores the kidney function by acting on the proximal tubules and loop of Henle of the nephron by increasing sodium and potassium reabsorption as 90% of sodium, potassium and calcium are reabsorbed by proximal tubules and loop of Henle [23]. Recent data also support our findings where they reported that H2S results in inhibition of Na-K-ATPase in renal tubular epithelial cells by regulating the EGFR/GAB1/PI3K/Akt pathway, thus reducing sodium and potassium ion exchange of renal tubular epithelial cells, and promoting sodium excretion [24]. In present study, creatinine clearance was increased in LVH-H_2S which shows that H_2S has preglomerular and postglomerular vasodilation by its ACE inhibition activity [25,26] while ACE inhibitors are reported to have increase in GFR actions [27] . Since creatinine clearance is marker of Glomerular Filtration Rate (GFR) [27] so it can be safely assumed that chronic administration of H_2S donor increases GFR in rats with LVH.

Improved renal functions by chronic administration of H_2S donor is multifactorial including glomerular vasodilation by its ACE inhibition activities, increase in responsiveness of alpha-adrenergic receptors[4], tubular reabsorption and excretion capabilities, increasing antioxidant and reducing prooxidant markers levels in present study (Figure 1 (A-B). antioxidant action of the H_2S donor in present study is in line with previously reported data [28,29] where administration of antioxidants has improved renal functions in animal study. This improved function of the kidney was reported due to improved function of tubular function of the kidney by SOD mimetic antioxidants like avasopasem manganese[29] and preventing SOD 2 degradation[30]. Recent studies have shown that upregulation of CSE/ H_2S pathway in diabetic kidney has improved kidney functions when compared to H_2S inhibitor group [31]. This antioxidant role of H_2S donor has also resulted in reduction in the kidney and heart indices in present study as shown in Figure 2 (A and B).

Chronic administration of H_2S donor has improved renal function by altering the collagen content in the kidney by maintain a balance ratio of elastin to collagen in elastic collagen membrane (ECM) which can be seen in Figure 3 (A-D) in present study. Induction of LVH resulted in the deposition of collagen around the glomerulus which can be seen in Figure 3-B while treatment with H_2S donor has significantly reduced these thick bands of collagen into thin and diffused threads of collagen as shown in Figure 3-D. Collagen deposition mechanism usually initiated in hypoxic condition which is in oxidative stress environment. This hypoxia induced by oxidative stress led to upregulation of MMP-2 and 9 [32,33]. This local oxidative stress in the kidney results in cellular dysfunction and extracellular metabolism around the glomerulus all of which are associated with increased NAD(P)H activity[34]. These MMPs play a role in matrix accumulation which help in the renal scaring [35].These MMPs also contribute renal fibrosis [36]. Treatment with H_2S mitigate the MMP-9 levels [16,37]. This decreased in collagen bands in H_2S donor group in present study is in coordination with previously reported data where treatment with H_2S donor mitigate MMP-9 levels and improved renal function [16]. MMP-2 and MMP-9 disrupt kidney architecture [38] which can be observed in present study (Figure 3-B). Antioxidant mechanism of the H_2S play a multiple purpose role by increasing antioxidant markers levels, tubular reabsorption and excretion capabilities and maintaining the integrity of elastin to collagen ratio in the kidney.

Conclusion

Chronic administrations of Sodium hydrogen sulphide to *Wistar Kyoto* rats with left ventricular hypertrophy improved renal reabsorption and excretory capabilities by modulating oxidative stress and inhibition of collagen deposition in the kidney of rats with LVH.

References

1. Kim M., J.H. Choi, H.K. Kim, et al. (2024) Effects of intensive blood pressure control on left ventricular hypertrophy in aortic valve disease. *American Heart Journal* 268: 45-52.
2. Doiron J.E., Z. Li, X. Yu, et al. (2024) Early Renal Denervation Attenuates Cardiac Dysfunction in Heart Failure With Preserved Ejection Fraction. *Journal of the American Heart Association* 2024:e032646.
3. Honetschlägerová Z., Z. Husková, S. Kikerlová, et al. (2024) Renal sympathetic denervation improves pressure-natriuresis relationship in cardiorenal syndrome: insight from studies with Ren-2 transgenic hypertensive rats with volume overload induced using aorto-caval fistula. *Hypertension Research* 2024: 1-19.
4. Ahmad A., M.A. Sattar, M. Azam, et al. (2016) Cystathione gamma lyase/hydrogen sulphide pathway up regulation enhances the responsiveness of α1A and α1B-adrenoreceptors in the kidney of rats with left ventricular hypertrophy. *Plos one* 11: e0154995.
5. Abdulla M.H., M.A. Sattar, E.J. Johns, N.A. Abdullah, M.A.H. Khan (2011) Evidence for the role of α 1A-adrenoceptor subtype in the control of renal haemodynamics in fructose-fed Sprague–Dawley rat. *European Journal of Nutrition* 50: 689-697.
6. Ahmad A., M.A. Sattar, H.A. Rathore, S.A. Khan, N.A. Abdullah, E.J. Johns (2016) Downregulation of cystathionine Î³ lyase and endothelial nitric oxide synthase and reduced responsiveness of Î±1A adrenergic receptors in the kidneys of left ventricular hypertrophied Wistar Kyoto rats. *Turkish Journal of Biology* 40: 1129-1139.
7. Snijder P.M, A.R.S. Frenay, A.M. Koning, et al. (2014) Sodium thiosulfate attenuates angiotensin II-induced hypertension, proteinuria and renal damage. *Nitric Oxide* 42: 87-98.
8. Altaany Z., G. Yang, R. Wang (2013) Crosstalk between hydrogen sulfide and nitric oxide in endothelial cells. *Journal of Cellular and Molecular Medicine* 17: 879-888.
9. Ahmad F.U.D, M.A. Sattar, H.A. Rathore et al. (2014) Hydrogen sulphide and tempol treatments improve the blood pressure and renal excretory responses in spontaneously hypertensive rats. *Renal Failure* 36: 598-605.
10. Fiaz-ud-Din-Ahmad Fu.D.A, M. Sattar, H. Rathore, S. Akhter, J.O. Jin OhHui, E. Johns (2014) Diuretic action of exogenous hydrogen sulfide in spontaneously hypertensive diabetic rats. *Tropical Journal of Pharmaceutical Research* 13: 1867-1876.
11. Zhou X., Y. Feng, Z. Zhan, J. Chen (2014) Hydrogen sulfide alleviates diabetic nephropathy in a streptozotocin-induced diabetic rat model. *Journal of Biological Chemistry* 289: 28827-28834.
12. Pourghasem M., H. Shafi, Z. Babazadeh (2015) Histological changes of kidney in diabetic nephropathy. Caspian *Journal of Internal Medicine* 6: 120-127.
13. Genovese F., A. Akhgar, S.S. Lim, et al. (2021) Collagen type III and VI remodeling biomarkers are associated with kidney fibrosis in lupus nephritis. *Kidney* 2: 1473-1481.
14. Cheng Z., M.H. Limbu, Z. Wang et al. (2017) MMP-2 and 9 in chronic kidney disease. *International Journal of Molecular Sciences* 18: 776.
15. Xu H., X. Yu, Y. Li, et al. (2023) Collagen IV and Podocyte-Related Gene Variants in Patients with Concurrent IgA Nephropathy and Thin Basement Membrane Nephropathy. *Nephron* 147: 301-310.
16. Kundu S., S. Pushpakumar, U. Sen (2015) MMP-9-and NMDA receptor-mediated mechanism of diabetic renovascular remodeling and kidney dysfunction: hydrogen sulfide is a key modulator. *Nitric Oxide* 46:172-185.
17. Flanagan E.T., M.M. Buckley, C.M. Aherne, F. Lainis, M. Sattar, E.J. Johns (2008) Impact of cardiac hypertrophy on arterial and cardiopulmonary baroreflex control of renal sympathetic nerve activity in anaesthetized rats. *Experimental Physiology* 93:1058-1064.
18. Ahmad A., M.A. Sattar, H.A. Rathore, et al. (2014) Functional contribution of α1D-adrenoceptors in the renal vasculature of left ventricular hypertrophy induced with isoprenaline and caffeine in Wistar–Kyoto rats. Canadian *Journal of Physiology and Pharmacology* 92: 1029-1035.
19. Yan H., J. Du, C. Tang (2004) The possible role of hydrogen sulfide on the pathogenesis of spontaneous hypertension in rats. *Biochemical and Biophysical Research Communications* 313: 22-27.

20. Hashmi S.F., H.A. Rathore, M.A. Sattar et al. (2021) Hydrogen Sulphide Treatment Prevents Renal Ischemia-Reperfusion Injury by Inhibiting the Expression of ICAM-1 and NF-kB Concentration in Normotensive and Hypertensive Rats. *Biomolecules* 11: 1549.
21. Steiner R.W. (1984) Interpreting the fractional excretion of sodium. *The American Journal of Medicine* 77: 699-702.
22. Ahmad A. (2022) Prophylactic Treatment with Hydrogen Sulphide Can Prevent Renal Ischemia-Reperfusion Injury in L-NAME Induced Hypertensive Rats with Cisplatin-Induced Acute Renal Failure. *Life.* 12: 1819.
23. John A., Pasha T., (2024) Laboratory tests of renal function. *Anaesthesia & Intensive Care Medicine*.
24. Ge S.N., M.M. Zhao, D.D. Wu et al. (2014) Hydrogen sulfide targets EGFR Cys797/Cys798 residues to induce Na+/K+-ATPase endocytosis and inhibition in renal tubular epithelial cells and increase sodium excretion in chronic salt-loaded rats. *Antioxidants & Redox Signaling* 21: 2061-2082.
25. Macabrey D., Deslarzes-Dubuis C., Longchamp A., et al. (2022) Hydrogen sulphide release via the angiotensin converting enzyme inhibitor zofenopril prevents intimal hyperplasia in human vein segments and in a mouse model of carotid artery stenosis. *European Journal of Vascular and Endovascular Surgery* 63: 336-346.
26. Ahmad A., Sattar M.A., Rathore H.A., et al. (2015) A critical review of pharmacological significance of Hydrogen Sulfide in hypertension. *Indian Journal of Pharmacology* 47: 243-247.
27. Digne-Malcolm H., M.C. Frise, K.L. Dorrington (2016) How Do Antihypertensive Drugs Work? Insights from Studies of the Renal Regulation of Arterial Blood Pressure. *Frontiers in Physiology*.
28. Al-Magableh M.R, B.K. Kemp-Harper, J.L. Hart (2015) Hydrogen sulfide treatment reduces blood pressure and oxidative stress in angiotensin II-induced hypertensive mice. *Hypertension Research* 38: 13-20.
29. Mapuskar K.A, C.F. Pulliam, A. Tomanek-Chalkley, et al. (2024) The antioxidant and anti-inflammatory activities of avasopasem manganese in age-associated, cisplatin-induced renal injury. *Redox Biology* 70: 103022.
30. Yang Q., M. Wang, H. Wang, C. Ren, Y. Li (2024) Exogenous Hydrogen Sulfide Prevents SOD2 Degradation to Safeguard Renal Function in Diabetic Kidney Disease. *Biochemistry and Cell Biology*.
31. Dugbartey G.J., K.K. Alornyo, D.E. Diaba, I. Adams (2022) Activation of renal CSE/H(2)S pathway by alpha-lipoic acid protects against histological and functional changes in the diabetic kidney. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 153: 113386.
32. Jing S.W, Y.D. Wang, M. Kuroda, et al. (2012) HIF-1α contributes to hypoxia-induced invasion and metastasis of esophageal carcinoma via inhibiting E-cadherin and promoting MMP-2 expression. *Acta Medica Okayama* 66: 399-407.
33. Pulido-Olmo H., C.F. Garcia-Prieto, G. Álvarez-Llamas, et al. (2016) Role of matrix metalloproteinase-9 in chronic kidney disease: a new biomarker of resistant albuminuria. *Clinical Science* 130: 525-538.
34. Yi F., P.L. Li (2008) Mechanisms of homocysteine-induced glomerular injury and sclerosis. *American Journal of Nephrology* 28: 254-264.
35. Rao V.H., G.E. Lees, C.E. Kashtan, et al. (2003) Increased expression of MMP-2, MMP-9 (type IV collagenases/gelatinases), and MT1-MMP in canine X-linked Alport syndrome (XLAS). *Kidney International* 63: 1736-1748.
36. Zhao H., Y. Dong, X. Tian, et al. (2013) Matrix metalloproteinases contribute to kidney fibrosis in chronic kidney diseases. *World Journal of Nephrology* 2: 84-89.
37. Kumar M., R. Sandhir (2022) Hydrogen sulfide attenuates hyperhomocysteinemia-induced blood-brain barrier permeability by inhibiting MMP-9. *International Journal of Neuroscience* 132: 1061-1071.
38. Sen U., P. Basu, O.A. Abe, et al. (2009) Hydrogen sulfide ameliorates hyperhomocysteinemia-associated chronic renal failure. *American Journal of Physiology-Renal Physiology* 297: F410-F419.