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Research Article
Acacetin Improves Renovascular Hypertension Via Inhibition of the Renin-Angiotensin Pathway in Experimental Rats

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Abstract

Background and Objective: The kidney is an important organ during blood pressure regulation, however, renal artery occlusion resulted in Renovascular Hypertension (RVH). Researchers have well documented the potential of acacetin against various hypertensive conditions. This study evaluates the putative mechanism of action of acacetin against two-kidney one-clip (2K1C)-induced RVH in experimental rats. **Materials and Methods:** RVH was induced in male Sprague-Dawley rats by the 2K1C method and then they were treated with vehicle (1% DMSO, 10 mg kg^{-1}) or captopril (30 mg kg^{-1}) or acacetin (10, 25 or 50 mg kg^{-1}) orally for 4 weeks. **Results:** Occlusion of a renal artery by using 2K1C resulted in marked ($p<0.05$) alterations in kidney weight, hemodynamic and left ventricular functions in rats which were ameliorated by acacetin (25 or 50 mg kg^{-1}) treatment. Acacetin also significantly ($p<0.05$) inhibited 2K1C-induced elevated renal oxido-nitrosative stress and Angiotensin-Converting Enzyme (ACE) activity. Furthermore, acacetin effectively ($p<0.05$) down-regulated 2K1C-induced up-regulated mRNA expressions of renal KIM-1, NGAL and renin improving renal Ho-1 mRNA expression. In addition, histopathology alteration produced by 2K1C in rat kidneys was ameliorated by acacetin treatment. **Conclusion:** Administration of acacetin significantly ameliorated elevated renal ACE and renin expression, thus inhibiting renin-angiotensin pathway induced renovascular hypertension in the 2K1C model in rats.

Key words: Acacetin, angiotensin-converting enzyme, renin, renovascular hypertension, two-kidney one-clip

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Renovascular hypertension (RVH) is an obstruction of blood flow to the kidney that results from occlusion or stenosis of the renal artery[1]. RVH is associated with modulation of the cardiac and renal system, thus, it is a major healthcare concern in clinical nephrology[1]. Furthermore, it is an independent risk factor for chronic kidney damage, contributing to End-Stage Renal Disease (ESRD) and Chronic Kidney Disease (CKD)[2]. RVH accounted for 5-10% of hypertensive cases and is believed to be an important cause of mortality in cardiovascular disease[3]. According to Kidney Early Evaluation Program study report, the prevalence of hypertension is approximately 86.2% in Chinese patients with CKD[4]. Recently researchers reported that the annual medical costs for treatment of ESDR are ranged between US$ 12.840-15.066[5], thus, it imposes a significant economic burden on the patients with RVH. Additionally, the Chinese Chronic Kidney Disease Cohort Study report suggested that health-related quality of life is poor in patients with CKD[6].

Therapeutic moieties from plant origin contain multi-target potential gained a significant attraction of researchers for the prevention and treatment of RVH. During the development of such therapeutic moieties, experimental animal models play a vital role. The 2 kidneys 1 clip (2K1C) model is one of such animal models developed to evaluate the potential of various agents against RVH[7-9]. In the 2K1C-induced RVH model, activation of RAAS via constriction of renal arteries caused peripheral resistance and thus caused induction of hypertension. Thus, the 2K1C model is a convenient, reliable and reproducible tool that mimics clinical RVH[7-9]. The present investigation employed the 2K1C model to determine the potential of apigenin.

Acacetin is a naturally occurring plant flavonoid that has been reported for its wide range of pharmacological activities, including antianxiety, antiarthritic, anti-inflammatory, anti-apoptotic, anti-hyperglycaemic, antihypertensive, anti-obesity, antioxidant, vasorelaxant, neuroprotective and nephroprotective potential[10]. It inhibited cisplatin-induced renal toxicity via inhibition of TNF-α, Blood Urea Nitrogen (BUN) and creatinine[11]. Additionally, administration of acacetin in spontaneously hypertensive rats improved vasodilatory function via modulation of estrogen signalling pathway and endothelial nitric oxide synthase (eNOS)[12]. Recently, Shiravi *et al.*[13] reported that administration of acacetin offered protection against renal ischemia-reperfusion injury via improving the total antioxidant capacity and inhibition of apoptosis. Furthermore, its antioxidant and anti-inflammatory potential contributed to the amelioration of Renal Ischemia-Reperfusion induced hepatic damage[14]. Although various researchers have evaluated the anti hypertensive potential of acacetin, its possible mechanism of action against RVH is yet to elucidate. Thus, the present study was aimed to evaluate the putative mechanism of action of acacetin against 2K1C-induced RVH in experimental rats.

MATERIALS AND METHODS

Study area: The experiment was performed in the Pharmacology laboratory of the Department of Hypertension, Fifth Affiliated Hospital of Xinjiang Medical University, China, from 12th Jan, to 27th March, 2021. All the experiments were carried out between 08:00 and 17:00 hrs in a quiet laboratory environment.

Experimental animals and research protocol approval: Adult male Sprague-Dawley rats (180-200 g) were purchased from the Fifth Affiliated Hospital of Xinjiang Medical University. They were maintained at 24±1°C, 12:12 hrs dark-light cycle, with standard pellet feed and filtered water. The experimental protocol was approved by the Fifth Affiliated Hospital of Xinjiang Medical University (ethical approval number: 20200925001) and performed following the Guide for Care and Use of Laboratory Animals, National Institute of Health.

Drugs and chemicals: Acacetin (≥97.0%, Sigma Chemical Co., St Louis, MO, USA), Crystalline beef liver catalase, 1,1',3,3'-Tetraethoxypropane, 5,5'-dithiobis (2-nitrobenzoic acid) and reduced glutathione (SD Fine Chemicals, Mumbai, India), One-step RT-PCR and Total RNA Extraction kit (MP Biomedicals India Private Limited, India) were procured from respective suppliers.

Renovascular hypertensive models: The renovascular hypertensive models (two-kidney one-clip, 2K1C) were produced[14]. In short, the rats were anaesthetized by peritoneal injection of pentobarbital sodium (60 mg kg^{-1}) i.p., A retroperitoneal flank incision was made to expose the right renal artery and a U-shaped silver clip of 0.2-mm internal diameter was used to partly occlude the right renal artery under sterile techniques. A separate group of rats have received surgery but without clipping (Sham). Animals were divided randomly into the following groups (n =12 each) to receive respective treatments:

Group 1: Sham: Rats were exposed to the right renal artery but did not receive any occlusion. They were treated with Dimethyl sulfoxide (DMSO 1%, 10 mg kg^{-1})

Table 1: Primer sequences for KIM-1, NGAL, HO-1, Renin and β-actin

Gene	Sequence		Size (bp)
	Forward primer	Reverse primer	
KIM-1	ACTCCTGCAGACTGGAATGG	CAAAGCTCAGAGAGCCCATC	212
NGAL	GATGTTGTTATCCTTGAGGCCC	CACTGACTCACGACCAGTTTGCC	230
HO-1	TTGTAACAGACTTGCCAGAG	CACTCACTGGTTGTATGCG	202
Renin	CAGTACTATGGTAGATCGGCT	ACTCCATCAACAGCCTGAGC	362

Group 2: 2K1C-control: Rats were exposed to the right renal artery and occluded. They were treated with DMSO (1%, 10 mg kg^{-1})

Group 3: Captopril (30): Rats were exposed to the right renal artery and occluded. They have received captopril (30 mg kg^{-1}, p.o.) for 4 weeks

Group 4: Acacetin (10): Rats were exposed to the right renal artery and occluded. They were received Acacetin (10 mg kg^{-1}, p.o.) for 4 weeks

Group 5: Acacetin (25): Rats were exposed to the right renal artery and occluded. They were received Acacetin (25 mg kg^{-1}, p.o.) for 4 weeks

Group 6: Acacetin (50): Rats were exposed to the right renal artery and occluded. They were received Acacetin (50 mg kg^{-1}, p.o.) for 4 weeks

The doses of Acacetin (10, 20 and 40 mg kg^{-1}, p.o.) were selected based on a previously reported study[15,16], whereas the dose of captopril (30 mg kg^{-1}, p.o.) was according to the previously reported method[14].

Invasive measurement of hemodynamic changes: A polyethylene cannula (PE 50) connected with a transducer bio amplifier (for signal amplification) was used to determined hemodynamic changes such as HR (Heart Rate), SBP (Systolic Blood Pressure), DBP (Diastolic Blood Pressure), MABP (Mean Arterial Blood Pressure) and left ventricular functions. In addition, Millar micro-tip transducer catheter was utilized to assess Left Ventricular Systolic Pressure (LVESP) whereas Left Ventricular End-diastolic Pressure (LVEDP), dp/dt_{max} and dp/dt_{min} estimated by using AD Instrument data acquisition system (LabChart 7.3, AD Instrument Pvt. Ltd.)[17,18].

Biochemical estimation: On the last day of the study, rats were anaesthetized with ether and retro-orbital plexus was used to withdraw blood. After blood withdrawal, rats were sacrificed by cervical dislocation. The immediately right kidney was isolated and stored at -70°C. Levels of BUN (Blood Urea Nitrogen, creatinine and LDH (Lactic dehydrogenase) were measured in serum, whereas albumin levels were estimated in urine using, respective reagent kits (Accurex Biomedical Pvt. Ltd., Mumbai, India).

Antioxidant and ACE activity determination in kidney: Kidney tissue homogenates were prepared with 0.1 M tris-HCl buffer (pH 7.4) and supernatant of homogenate was employed to estimate superoxide dismutase (SOD), reduced glutathione (GSH), lipid peroxidation (MDA content) as described previously[19]. The commercially available kit (Beijing Equation Biological Science and Technology Co., Ltd., Beijing, China) was used to the determined activity of ACE.

KIM-1, NGAL, HO-1 and Renin mRNA expression determination in kidney: The mRNA expressions of Kidney Injury Molecule-1 (KIM-1), Neutrophil gelatinase-associated lipocalin (NGAL), Tumor necrosis factor-α (TNF-α), Interleukins (ILs), Heme oxygenase-1 (HO-1) and Renin by using quantitative Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR)[20]. The primer sequence was selected based on the previous study and presented in Table 1.

Histopathological analysis of kidney: Right kidney tissue was stored in 10% formalin for 24 hrs. The specimen was dehydrated and placed in xylene for 1 hr (3 times) and later in ethyl alcohol (70, 90 and 100%) for 2 hrs resp. The paraffin wax treatment (twice, 1 hr each) was used to assess infiltration and impregnation. Kidney specimens were cut into 3-5 µm thickness sections and stained with hematoxylin and eosin (H and E). The specimen was mounted on a slide using Distrene Phthalate Xylene (DPX) as a mounting medium. Sections were examined under a light microscope to obtain a general impression of the histopathology features of the specimen and infiltration of cells. The various changes in histological features were graded as Grade 0 (not present), Grade 1 (slight/minimal), Grade 2 (mild), Grade 3 (moderate) or Grade 4 (severe)[21].

Statistical analysis: Data are articulated as Mean±Standard Error Mean (SEM). GraphPad Prism 5.0 software (GraphPad, San Diego, CA) was used to achieve data analysis. Data of hemodynamic changes were analyzed using a two-way analysis of variance (ANOVA) followed by Tukey's multiple range *post hoc* analysis. In contrast, data of biochemical parameters were analyzed using One-Way ANOVA followed by Tukey's multiple range *post hoc* analysis. A $p<0.05$ was measured to be statistically significant.

Table 2: Effect of acacetin on 2K1C induced alteration in kidney weight and left ventricular functions in rats

Parameters	Sham	2K1C-control	C (30)	AC (10)	AC (25)	AC (50)
Right kidney relative weight ($\times 10^{-3}$)	2.66±0.10	0.88±0.05$^{\#}$	2.28±0.10$^{*,\$}$	1.07±0.07	1.49±0.08$^{*,\$}$	2.06±0.09$^{*,\$}$
Left kidney relative weight ($\times 10^{-3}$)	2.67±0.07	4.06±0.08$^{\#}$	3.03±0.08$^{*,\$}$	3.87±0.04	3.59±0.10$^{*,\$}$	3.28±0.11$^{*,\$}$
LVEDP (mmHg)	3.86±0.22	7.20±0.25$^{\#}$	4.86±0.25$^{*,\$}$	6.70±0.23	5.85±0.26$^{*,\$}$	5.21±0.24$^{*,\$}$
$Max_{dp/dt}$ (mmHg s^{-1})	902.40±5.22	766.10±4.38$^{\#}$	866.40±4.03$^{*,\$}$	781.70±5.74	831.50±6.07$^{*,\$}$	863.40±5.58$^{*,\$}$
$Min_{dp/dt}$ (mmHg s^{-1})	-581.50±6.42	-205.00±7.19$^{\#}$	-510.90±4.83$^{*,\$}$	-227.60±5.45	-310.70±5.86$^{*,\$}$	-468.70±6.98$^{*,\$}$
Contractility index (s^{-1})	16.85±0.71	9.56±0.62$^{\#}$	14.82±0.38$^{*,\$}$	9.27±0.45	12.36±0.71$^{*,\$}$	12.85±0.86$^{*,\$}$
Tau (ms)	20.10±0.85	21.30±0.84	21.85±0.64	22.38±0.34	20.66±0.65	20.56±0.94
Pressure time index	4.28±0.42	12.28±0.61$^{\#}$	5.97±0.40$^{*,\$}$	11.07±0.57	7.89±0.25$^{*,\$}$	6.56±0.36$^{*,\$}$

Values in parentheses indicate a dose in mg kg^{-1} (n = 6). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. For comparison with 2K1C-control group: *p<0.05, comparison with sham group: $^{\#}$p<0.05 and comparison with one another. LVEDP: Left ventricular end-diastolic pressure, 2K1C: Two-kidney-one-clip, C (30): Captopril (30 mg kg^{-1}) treated, AC (10): Acacetin (10 mg kg^{-1}) treated, AC (25): Acacetin (25 mg kg^{-1}) treated and AC (50): Acacetin (50 mg kg^{-1}) treated

RESULTS

Effect of acacetin on 2K1C induced alteration in relative kidney weight in rats: There was a significant (p<0.05) decrease in the right kidney relative weight (0.88±0.05), whereas an increase in left kidney weight (4.06±0.08) in 2K1C-control rats compared to sham rats (2.66±0.10 and 2.67±0.07). Administration of captopril effectively (p<0.05) inhibited 2K1C induced alteration in right and left kidney weights (2.28±0.10 and 3.03±0.08) compared to 2K1C-control rats. Treatment of acacetin (25 and 50 mg kg^{-1}) also noticeably (p<0.05) increased right kidney weight (1.49±0.08 and 2.06±0.09) and decreased left kidney weight (3.59±0.10 and 3.28±0.11) as compared to 2K1C-control rats. However, these ameliorations were more prominent (p<0.05) in captopril treatment than Acacetin in Table 2.

Effect of acacetin on 2K1C induced alteration in hemodynamic and left ventricular functions in rats: Figure 1(a-c) depicted the effect of acacetin on 2K1C-induced alteration in hemodynamic parameters including SBP, DBP and MABP which were gradually (170.76±2.24, 99.00±1.03 and 122.72±1.29 mmHg, respectively, p<0.05) increased whereas HR was effectively (290.00±8.78 BPM, p<0.05) decreased in 2K1C-control rats in Fig. 1d compared to sham rats (108.00±4.30, 72.16±2.65 and 84.11±2.32 mmHg, 354.00±8.77 BPM). However, administration of captopril markedly (p<0.05) attenuated 2K1C-induced alterations in SBP (118.00±5.05 mmHg), DBP (73.33±1.67 mmHg), MABP (88.22±1.86 mmHg) and HR (338.00±8.78 BPM) compared to 2K1C-control rats. Acacetin (25 and 50 mg kg^{-1}) treatment also effectively (p<0.05) restored these SBP (149.17±4.66 and 128.00±4.94 mm Hg), DBP (86.00±1.03 and 77.00±1.03 mm Hg), MABP (107.06±1.16 and 94.00±1.76 mm Hg) and HR (320.00±8.78 and 330.00±8.78 BPM) when compared with 2K1C-control rats.

The left ventricular functions (LVEDP, $Max_{dp/dt}$, $Min_{dp/dt}$, Contractility Index and Pressure Time Index) were significantly (p<0.05) varied in 2K1C-control rats (7.20±0.25 mmHg, 766.10±4.38 mmHg s^{-1}, -205.00±7.19 mmHg s^{-1}, 9.56±0.62 s^{-1} and 12.28±0.61, respectively) compared to sham rats (3.86±0.22 mmHg, 902.40±5.22 mmHg, -581.50±6.42 mmHg s^{-1}, 16.85±0.71 s^{-1} and 4.28±0.42, respectively). Treatment with captopril effectively (p<0.05) decreased LVEDP (4.86±0.25 mmHg) and Pressure Time Index (5.97±0.40) whereas increase $Max_{dp/dt}$ (866.40±4.03 mmHg s^{-1}), $Min_{dp/dt}$ (-510.90±4.83 mmHg s^{-1}) and Contractility Index (14.82±0.38 s^{-1}) compared to 2K1C-control rats. Administration of acacetin (25 and 50 mg kg^{-1}) markedly (p<0.05) attenuated 2K1C induced alterations in LVEDP (5.85±0.26 and 5.21±0.24 mmHg), $Max_{dp/dt}$ (831.50±6.07 and 863.40±5.58 mmHg s^{-1}), $Min_{dp/dt}$ (-310.70±5.86 and -468.70±6.98 mmHg s^{-1}), Contractility Index (12.36±0.71 and 12.85±0.86 s^{-1}) and Pressure Time (7.89±0.25 and 6.5±0.36) compared to 2K1C-control rats. The levels of Tau did not differ significantly in the sham (20.10±0.85 ms), 2K1C-control (21.30±0.84 ms), captopril (21.85±0.64 ms) and acacetin (25 and 50 mg kg^{-1}, 20.66±0.65 and 20.56±0.94 ms) groups (Table 2).

Effect of acacetin on 2K1C induced alteration in serum BUN, creatinine, LDH and urinary albumin in rats: The levels of serum BUN (42.33±0.77 mg dL^{-1}), creatinine (2.27±0.04 mg dL^{-1}), LDH (2161.00±66.94 mg dL^{-1}) and urinary albumin (40.30±1.67 µg day^{-1}) were noticeably (p<0.05) increased in 2K1C-control rats when compared with sham rats (25.04±1.09, 0.48±0.07, 1148.00±49.89 mg dL^{-1} and 143.20±2.25 µg day^{-1}). Captopril treatment effectively (p<0.05) reduced serum BUN (29.33±1.15 mg dL^{-1}), creatinine (1.04±0.05 mg $^{-1}$), LDH (1302.00±65.24 mg dL^{-1}) and urinary albumin (58.99±2.37 µg day^{-1}) compared to 2K1C-control rats. Acacetin (25 and 50 mg kg^{-1}) treatment also prominently (p<0.05) lessened serum BUN

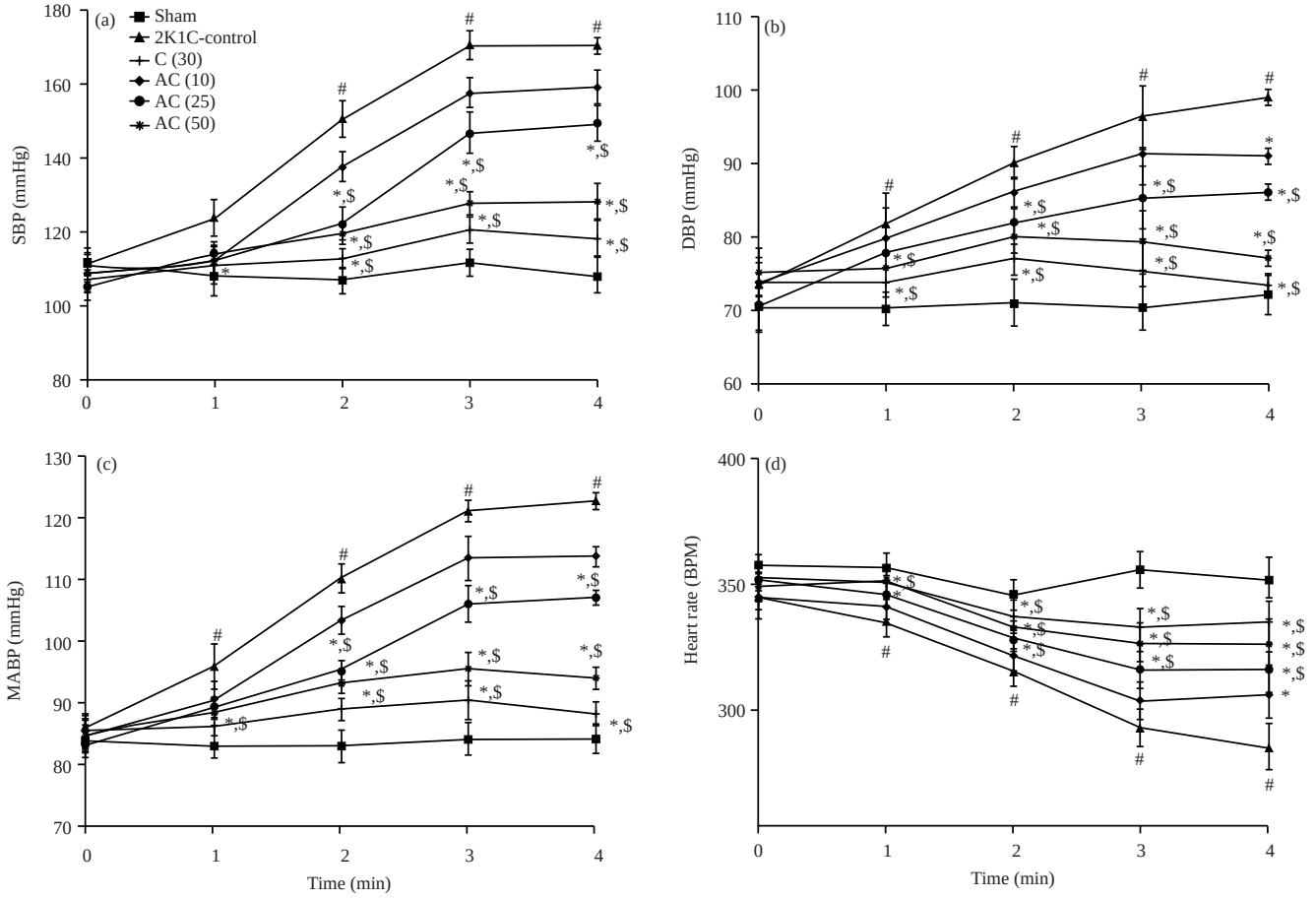

Fig. 1(a-d): Effect of acacetin on 2K1C induced alteration in hemodynamic parameters (a) SBP, (b) DBP and (c) MABP Heart rate in 2K1C-induced hypertensive rats

Values in parentheses indicate a dose in mg kg^{-1} (n = 6). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. For comparison with 2K1C-control group: *p<0.05, comparison with sham group: #p<0.05 and comparison with one another: $^{\$}$p<0.05, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MABP: Mean arterial blood pressure, 2K1C: Two-kidney-one-clip, C (30): Captopril (30 mg kg^{-1}) treated, AC (10): Acacetin (10 mg kg^{-1}) treated, AC (25): Acacetin (25 mg kg^{-1}) treated and AC (50): Acacetin (50 mg kg^{-1}) treated

Table 3: Effect of acacetin on 2K1C induced alteration in serum BUN, creatinine, LDH and urinary albumin in rats

Parameters	Sham	2K1C-control	C (30)	AC (10)	AC (25)	AC (50)
BUN (mg dL^{-1})	25.04±1.09	42.33±0.77$^{\#}$	29.33±1.15$^{*,\$}$	41.22±0.57	36.35±0.84$^{*,\$}$	33.49±0.80$^{*,\$}$
Creatinine (mg dL^{-1})	0.48±0.07	2.27±0.04$^{\#}$	1.04±0.05$^{*,\$}$	1.86±0.05*	1.63±0.05$^{*,\$}$	1.21±0.06$^{*,\$}$
LDH (mg dL^{-1})	1148.00±49.89	2161.00±66.94$^{\#}$	1302.00±65.24$^{*,\$}$	2080.00±75.9	1674.00±37.45$^{*,\$}$	1428.00±51.37$^{*,\$}$
24 hrs urinary albumin excretion (µg day^{-1})	40.30±1.67	143.20±2.25$^{\#}$	58.99±2.37$^{*,\$}$	132.20±2.6	103.70±1.16$^{*,\$}$	70.61±1.56$^{*,\$}$

Values in parentheses indicate a dose in mg kg^{-1} (n = 6). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. For comparison with the 2K1C-control group: *p<0.05, comparison with the sham group: #p<0.05 and comparison with one another. BUN: Blood urea nitrogen, LDH: Lactic dehydrogenase, 2K1C: Two-kidney-one-clip, C (30): Captopril (30 mg kg^{-1}) treated, AC (10): Acacetin (10 mg kg^{-1}) treated, AC (25): Acacetin (25 mg kg^{-1}) treated and AC (50): Acacetin (50 mg kg^{-1}) treated

(36.35±0.84 and 33.49±0.80 mg dL^{-1}), creatinine (1.63±0.05 and 1.21±0.06 mg dL^{-1}), LDH (1674.00±37.45 and 1428.00±51.37 mg dL^{-1}) and urinary albumin (103.70±1.16 and 70.61±1.56 µg day^{-1}) levels compared to 2K1C-control rats. However, captopril treatment more strikingly (p<0.05) repressed serum BUN, creatinine, LDH and urinary albumin compared to Acacetin in Table 3.

Effect of acacetin on 2K1C induced alteration in renal oxido-nitrosative stress and ACE activity in rats: Partial occlusion of the right renal artery caused significant (p<0.05) induction of oxido-nitrosative stress reflected by decreased SOD (11.85±0.76 U mg^{-1} of protein) and GSH (20.67±0.48 µg mg^{-1} protein) as well as increased MDA (12.27±0.47 nmol mg^{-1} of protein) and NO (11.31±0.50 µg mg^{-1} of protein) levels in

Table 4: Effect of acacetin on 2K1C induced alteration in renal oxido-nitrosative stress and ACE activity in rats

Parameters	Sham	2K1C-control	C (30)	AC (10)	AC (25)	AC (50)
SOD (U mg^{-1} of protein)	11.85±0.76	6.85±0.88$^{\#}$	11.52±0.69$^{*,\$}$	6.75±0.70	9.14±0.82$^{*,\$}$	11.16±0.51$^{*,\$}$
GSH (μg mg^{-1} protein)	31.72±0.98	20.67±0.48$^{\#}$	28.34±0.61$^{*,\$}$	23.02±1.12	25.30±0.78*	26.73±1.00$^{*,\$}$
MDA (nmol mg^{-1} of protein)	4.33±0.35	12.27±0.47$^{\#}$	4.86±0.40$^{*,\$}$	10.48±0.52*	8.61±0.44$^{*,\$}$	5.93±0.40$^{*,\$}$
NO (μg mg^{-1} of protein)	5.76±0.65	11.31±0.50$^{\#}$	6.36±0.77$^{*,\$}$	11.71±0.38	8.78±0.31$^{*,\$}$	7.74±0.68$^{*,\$}$
ACE activity (U mg^{-1} protein)	33.58±1.90	99.59±1.23$^{\#}$	41.07±2.36$^{*,\$}$	90.23±2.17	70.51±1.56$^{*,\$}$	50.47±2.69$^{*,\$}$

Values in parentheses indicate a dose in mg kg^{-1} (n = 6). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. For comparison with the 2K1C-control group: *p<0.05, comparison with the sham group: $^{\#}$p<0.05 and comparison with one another. SOD: Superoxide dismutase, GSH: Glutathione peroxidase, MDA: Malondialdehyde, NO: Nitric oxide, ACE: Angiotensin I converting enzyme, 2K1C: Two-kidney-one-clip, Captopril (30 mg kg^{-1}) treated, AC (10): Acacetin (10 mg kg^{-1}) treated, AC (25): Acacetin (25 mg kg^{-1}) treated and AC (50): Acacetin (50 mg kg^{-1}) treated

renal tissue of 2K1C-control rats compared to sham rats (6.85±0.88 U mg^{-1} of protein, 31.72±0.98 μg mg^{-1} protein, 4.33±0.35 nmol mg^{-1} of protein and 5.76±0.65 μg mg^{-1} of protein, respectively). Nevertheless, treatment with captopril notably (p<0.05) increased renal SOD (11.52±0.69 U mg^{-1} of protein) and GSH (28.34±0.61 μg mg^{-1} protein) levels whereas decreased MDA (4.86±0.40 nmol mg^{-1} of protein) and NO (6.36±0.77 μg mg^{-1} of protein) levels compared to 2K1C-control rats. Acacetin (25 and 50 mg kg^{-1}) administration was also associated with effective (p<0.05) reduction in MDA (8.61±0.44 and 5.93±0.40 nmol mg^{-1} of protein) and NO (8.78±0.31 and 7.74±0.68 μg mg^{-1} of protein) levels and increase in SOD (9.14±0.82 and 11.16±0.51 U mg^{-1} of protein) and GSH (25.30±0.78 and 26.73±1.00 μg mg^{-1} protein) levels compared to 2K1C-control rats in Table 4.

The activity of ACE was elevated effectively (p<0.05) in renal tissue of 2K1C-control rats (99.59±1.23 U mg^{-1} protein) compared to sham rats (33.58±1.90 U mg^{-1} protein). Nevertheless, treatment with captopril notably (p<0.05) decreased ACE levels (41.07±2.36 U mg^{-1} protein) compared to 2K1C-control rats. Acacetin (25 and 50 mg kg^{-1}) administration was also associated with effective (p<0.05) reduction in ACE levels (70.51±1.56 and 50.47±2.69 U mg^{-1} protein) levels compared to 2K1C-control rats. However, captopril treatment showed more prominent inhibition of 2K1C-induced elevated oxido-nitrosative stress and ACE activity than acacetin (Table 4).

Effect of acacetin on 2K1C induced alteration in renal mRNA expressions of KIM-1, NGAL, HO-1 and renin in rats: Figure 2a represented the potential of acacetin on amelioration of 2K1C-induced alteration in mRNA expressions of KIM-1, NGAL, HO-1 and Renin in renal tissue. The mRNA expressions of renal KIM-1 (1.25±0.06) in Fig. 2b, NGAL (1.56±0.03) in Fig. 2c and renin (1.59±0.04) in Fig. 2e were markedly (p<0.05) up-regulated in 2K1C-control rats after partial occlusion of the right renal artery compared to sham rats (0.67±0.02, 1.01±0.06 and 0.80±0.04). Captopril noticeably (p<0.05) down-regulated 2K1C-induced elevated renal KIM-1 (0.72±0.03), NGAL (1.12±0.05) and renin (0.96±0.04) mRNA expressions compared to 2K1C-control rats. It also significantly (p<0.05) up-regulated renal HO-1 mRNA expression (1.11±0.03) as compared to 2K1C-control rats (0.36±0.06) in Fig. 2d. Acacetin (25 and 50 mg kg^{-1}) also showed a marked (p<0.05) amelioration in 2K1C-induced elevated renal KIM-1 (0.93±0.04 and 0.83±0.03), NGAL (1.29±0.03 and 1.21±0.04) and Renin (1.33±0.04 and 1.08±0.02) mRNA expressions compared to 2K1C-control rats. Renal HO-1 mRNA expressions were effectively up-regulated by acacetin (25 and 50 mg kg^{-1}, 0.65±0.05 and 1.25±0.04) treatment compared to 2K1C-control rats.

Effect of acacetin on 2K1C induced histopathology alteration in rat kidney: Partial occlusion of the right renal artery caused aberration in the right kidney of 2K1C-control rats reflected by significant (p<0.05) necrosis (4.00±2.11), tubular hyperplasia (3.67±2.11) and inflammatory infiltration (3.67±2.11) in Fig. 3b compared to sham rats (0.33±1.87, 0.00±1.87 and 0.00±1.87, respectively). Renal tissue from sham rats showed a normal histological arrangement of Bowman's capsule and space, proximal tubule, granular cortex devoid of any infiltration of inflammatory cells and hyperplasia in Fig. 3a. Treatment with captopril effectively (p<0.05) reduced 2K1C-induced histological aberration of renal tissue reflected by decreased necrosis (0.67±2.25), tubular hyperplasia (0.67±2.25) and inflammatory infiltration (0.67±2.25) in Fig. 3c compared to 2K1C-control rats. Administration of acacetin (25 and 50 mg kg^{-1}) also showed effective (p<0.05) amelioration of 2K1C-induced histological aberration (necrosis (2.33±2.70 and 0.67±1.43), tubular hyperplasia (2.00±2.70 and 0.33±1.43) and inflammatory infiltration (2.00±2.70 and 0.67±1.43) of renal tissue in Fig. 3d compared to 2K1C-control rats in Fig. 3e.

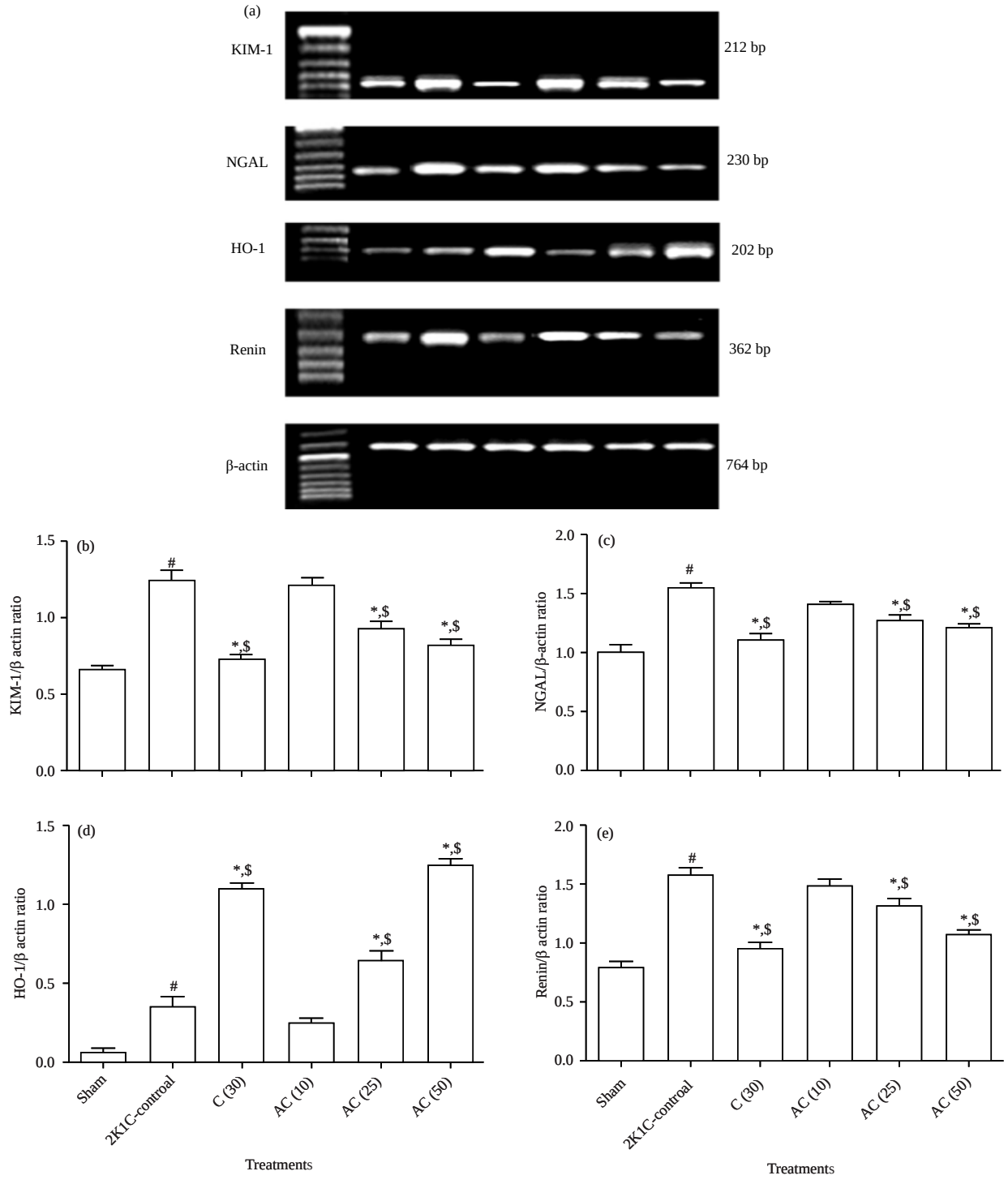

Fig. 2(a-e): (a) Effects of acacetin on 2K1C induced alteration in mRNA expressions of KIM-1, NGAL, HO-1 and renin in renal tissue by reverse transcriptase-polymerase chain reaction analysis, (b) Quantitative representation of mRNA expression of KIM-1, (c) Quantitative representation of mRNA expression of NGAL, (d) Quantitative representation of mRNA expression of HO-1 and (e) Quantitative representation of mRNA expression of Renin in renal tissues

Data are expressed as Mean±SEM (n = 6) and analyzed by one-way ANOVA followed by Tukey's multiple range test. For comparison with the 2K1C-control group: *p<0.05, comparison with the sham group: #p<0.05 and comparison with one another. HO-1: Heme oxygenase-1, KIM-1: Kidney injury molecule-1, NGAL: neutrophil gelatinase-associated lipocalin, 2K1C: Two-kidney-one-clip, Captopril (30 mg kg^{-1}) treated, AC (10): Acacetin (10 mg kg^{-1}) treated, AC (25): Acacetin (25 mg kg^{-1}) treated and AC (50): Acacetin (50 mg kg^{-1}) treated

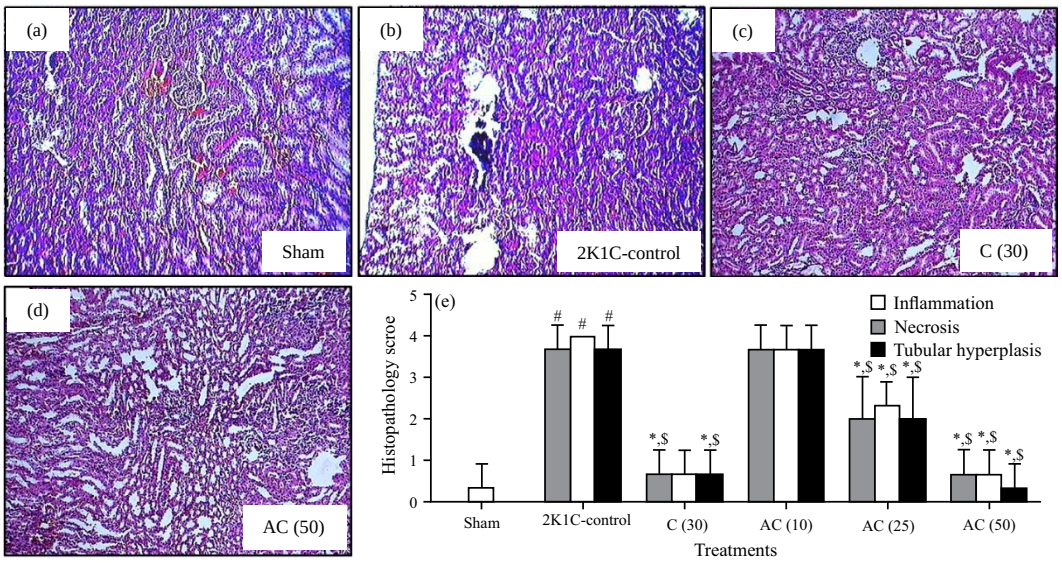

Fig. 3(a-e): Representative histological images of kidney from (a) Sham, (b) 2K1C-control rats, (c) captopril (30 mg kg^{-1}), (d) Acacetin (50 mg kg^{-1}) and (e) Effect of acacetin on histopathology alteration produced by 2K1C-in rat kidney

Images stained with H and E (X 100). The quantitative representation of histological score (E). Data were expressed as Mean±SEM (n = 3) and one-way ANOVA followed by the kruskal-wallis test was applied for *post hoc* analysis. For comparison with the 2K1C-control group: *p<0.05, comparison with the sham group: #p<0.05 and comparison with one another

DISCUSSION

Renal hypertension is a major cause of morbidity and mortality amongst the patient with a cardiovascular disorder. Additionally, hypertension is an important risk factor for chronic kidney disease and end-stage renal disease[2]. The existing landscape for the management of RVH includes ACE inhibitors (such as enalapril, captopril and ramipril) and angiotensin II receptor blockers (such as losartan, valsartan and olmesartan)[15]. However, their several side effects limit their clinical utility. Evidence suggested that multiple therapeutic targets halted the development and progression of RVH. According to the Chinese Cohort Study of Chronic Kidney Disease report, more than 50% of patients suffering from CKD needed more than two antihypertensive medications to manage the hypertensive condition[16]. Thus, therapeutic moiety with multi-targeted modulatory potential is a need of the hour to manage RVH. In the present investigation, we have evaluated the potential of acacetin against 2K1C-induced RVH in experimental rats. Findings suggested that administration of acacetin significantly ameliorated RVH via inhibition of the renin-angiotensin pathway reflected by decreased expression of renal ACE and Renin in the 2K1C model.

It has been well documented that renal artery occlusion associated with elevated systolic and diastolic blood pressure resulted in cardiac dysfunction[4]. Studies suggested that activating the renin-angiotensin system is an important pathophysiological factor contributing to RVH[1,17]. Elevated renin levels in renal tissue activate circulatory angiotensin II caused increased blood pressure during occlusion[1,17]. A consistent increase in blood pressure every week post renal artery occlusion is a hallmark for a well-established model of 2K1C hypertension[7-9]. The present investigation documented the sustained increase in SBP, DBP and MABP week by week post renal artery occlusion, according to the previous researcher[7-9]. Additionally, left ventricular functions such as dp/dt_{max}, dp/dt_{min} and left ventricular end-diastolic pressure were also markedly affected by renal artery occlusion. Nevertheless, treatment with acacetin effectively ameliorated 2K1C-induced alterations in hemodynamic and left ventricular functions depicting its antihypertensive potential. Wei *et al.*[12] also reported the vasodilatory function of acacetin responsible for its antihypertensive effect in spontaneously hypertensive rats and the results of the present investigation are in line with the findings of this research.

Documented studies show that renal tissue is a highly sensitive system toward elevated oxidative stress[18]. Moreover, occlusion of a renal artery caused a systemic increase in the Reactive Oxygen Species (ROS), which further contributed to elevated oxidative stress[19]. It has been demonstrated that elevated ROS levels cause the peroxidation of membrane

polyunsaturated fatty acids, leading to a decrease in the levels of sulfhydryl and some non-sulfhydryl enzymes[20]. This vicious cycle caused damage to the cellular membrane and eventually leads to cell death. Furthermore, the researcher has shown that elevated levels of lipid peroxidation, i.e., Malondialdehyde (MDA) are associated with decreased antioxidant defences, including superoxide dismutase (SOD) and glutathione peroxidase (GSH) in the renal tissue[21]. The alteration in the renal antioxidant system (SOD and GSH) levels results in tissue toxicity via oxidation of lipids, protein and Deoxyribonucleic acid (DNA)[7]. However, exogenous antioxidant supplementation may reduce lipid peroxidation. Furthermore, intracellular nitric oxide plays a vital role in renal damage via augmenting oxidative stress. Nitric oxide forms peroxynitrite via it interacts with superoxide anion and this peroxynitrite induces nitrosative stress, which further caused tissue damage. Numerous evidence suggested that acacetin is a potent antioxidant with inhibitory activity against elevated MDA and NO levels in several tissues[10-14,22]. In the present study, acacetin treatment replenished SOD and GSH whereas decreased the MDA and NO levels in renal tissue, suggesting its nephroprotective potential under its antioxidant property. Numerous researchers studied the blood pressure regulatory potential of the HO-1 system during various hypertension, including RVH[22,23]. The mechanism behind the blood pressure-lowering effect of HO-1 includes the production of CO, which serves as a vasodilator via inhibition of endothelin-1 that plays a vital role in vasoconstriction[24]. Furthermore, bilirubin formed as a by-product during the conversion of HO-1 to CO[22]. This bilirubin is a potent antioxidant that protected inflammatory cytokines induced vascular injury. Thus, the previous researcher showed a beneficial effect of antioxidant-induced increased production of HO-derived bilirubin in inhibition of ROS during the 2K1C model[9]. In agreement with the previous researcher, the findings of the present study showed a significant increase in HO-1 levels posts acacetin treatment which might inhibit elevated oxidative stress, thus protecting against RVH induced renal damage[22].

FDA has approved captopril as a first-line medication to manage hypertension in patients with renal insufficiency[25]. Clinically it showed a reduction in the development of overt congestive heart failure and associated mortality in hypertensive patients[26]. However, in some patients, long-term treatment of captopril resulted in deterioration of renal function[26]. In this view, researchers have documented the antihypertensive potential of herbal moieties *Allium sativum*, *Camellia sinensis*, *Hibiscus sabdariffa* and *Nigella sativa* during clinical studies[27]. Thus, acacetin can also be considered an important herbal moiety bearing antihypertensive potential for further clinical evaluation during RVH conditions.

CONCLUSION

Taken together, findings of the present investigation suggested that chronic administration of acacetin protected against 2K1C-induced elevated renal oxidative stress, renin and Angiotensin-Converting enzyme, which contributed to the amelioration of renovascular hypertension in experimental rats.

SIGNIFICANCE STATEMENT

This study discovered the putative mechanism of action of acacetin against two-kidney one-clip (2K1C)-induced RVH that can be beneficial for the amelioration of renovascular hypertension. This study will help the researchers to uncover the critical areas of chronic administration of acacetin protected against 2K1C-induced elevated renal oxidative stress, renin and Angiotensin-Converting enzyme that many researchers were not able to explore.

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