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Therapeutic evaluation of rutin in two-kidney one-clip model of renovascular hypertension in rat

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ABSTRACT

Aim: The current investigation, designed to investigate the role of rutin in two-kidney one-clip (2K1C) induced renovascular dysfunction associated with hypertension in rat.
Main methods: The renovascular hypertension was developed by the application of vascular clip on left renal artery in rats; the right kidney was kept as such throughout the experimental protocol. The rutin (200 and 300 mg/kg; p.o.) and aliskiren (50 mg/kg; p.o.) were administered for 9 consecutive days. The battery of pathophysiological tests i.e., systolic pressure, diastolic pressure and heart rate were performed to assess the anti-hypertensive effect of rutin. In addition, changes of kidney weight/body weight (KW/BW) ratio along with plasma renin content and renal tissue biomarkers i.e., thiobarbituric acid reactive substance (TBAR) and reduced glutathione (GSH) levels were estimated.
Key findings: The administration of rutin significantly ($P < 0.05$) attenuated the 2K1C of left kidney induced elevated systolic and diastolic pressure in a dose dependent manner. In addition, it also reduces the ratio of KW/BW along with a decrease in plasma renin content, tissue TBARS and increase the GSH levels. There were no significant changes observed in heart rate. Similar results were observed in aliskiren treated group.
Significance: The anti-hypertensive effect of rutin may be a useful herbal medicine for the management of hypertension due to its potential free radical scavenging, inhibition of lipid peroxidation and plasma renin inhibitory action.

1. Introduction

Hypertension is a major leading cause of death in the world. It is also known as silent killers because it has no warning signs or symptoms. Also, many people do not know they are having hypertension [7,65]. Usually, hypertension does not show any symptoms in acute stage. But, in chronic stage it will affect the heart, kidney, brain and peripheral vascular systems leading to the potential damage on the major target organs, associated disorders like hypertensive heart disease, coronary artery disease, chronic kidney disease, stroke, aortic aneurysm and peripheral artery disease [41,55].

The elevated blood pressure is the key event of the renal artery stenosis (narrowing) due to activation of renin–angiotensin–aldosterone system (RAAS) [61]. The progress of renovascular hypertension is due to the ischemic environment on the kidney, which occurs in the animal due to the application of a ligature or clipping on the renal artery [6,20] and it clinically mimics the renal artery stenosis [24,58]. The prevention of renal artery stenosis associated hypertension has great challenges due to involvement of multiple RAAS components [10,34]. Anti-hypertensive therapy has been documented to produce therapeutic effect by blocking action of RAAS; even though, most of the patients require combination therapy [42,47]. In high risk patients with renovascular stenosis and renovascular hypertension is directly referred to intra-arterial renal artery angiography [45].

The molecular mechanism of renovascular hypertension is due to the over activation of RAAS [5]; adrenergic neuron and their receptors [52,57]; alteration of endothelial derived relaxing factor i.e., nitric oxide, prostacyclin and acetyl choline; and endothelial derived contractility factors i.e., endothelin, prostacyclin-1α, PGF-2α and thromboxane B_2 [25,29,67]; and genetic factors like angiotensin-converting enzyme gene and glucose 6 phosphatase catalytic-3 (G6PC3) gene [2,37,50]. The various conventional medicines are documented to produce the anti-hypertensive effects like thiazide diuretics, angiotensin receptor (ATR) blocker, angiotensin converting enzyme (ACE) inhibitors, beta blockers and calcium channel blockers [9,64]. However, the administration of these agents either monotherapy or combinations are limited in various clinical setup of hypertension. In addition, the treatment of these anti-hypertensive drugs results in producing various unwanted side effects like flushing, palpitation, dizziness, leg edema and visceral angioedema [23,30,59]. The direct renin inhibitor i.e., aliskiren is one of the potent newer anti-hypertensive agent. However, the chronic treatment of aliskiren is known to produce the angioedema and nasopharyngitis [51,62]. The various animal models are established

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for the evaluation of anti-hypertensive drug effects. Two-kidney one-clip (2K1C) model is one of the common models in the induction of renovascular hypertension in rodents and it specifically deals with the RAAS components associated with hypertension ([36]; Pliquett et al. 2014).

Rutin is one of the major phenolic phytoconstituents and it is a glycoside between the flavonol quercetin and the disaccharide of rutinose known as α-L-rhamnopyranosyl-(1 → 6))-β-D-glucopyranose. It has various pharmacological properties like anti-oxidant, anti-inflammatory action and enhancement of vascular integrity [16,28,40]. Furthermore, rutin has been documented to produce the anti-hypertensive action against N(omega)-nitro-L-arginine methyl ester (L-NAME) induced renovascular hypertension in rat (Ranga Lakshmi Naidu et al. [46]). The RAAS component plays an important key role in the pathogenesis of L-NAME induced renovascular hypertension ([63]; Ranga Lakshmi Naidu et al. [46]). However, the role of rutin in renin dependent renovascular hypertension is remaining to be explored. Therefore, the present study designed to investigate the role of rutin in 2K1C of renal artery induced renovascular hypertension in rats.

2. Materials and methods

2.1. Drugs and chemicals

Rutin was procured from SDFCL, Mumbai. 5,5′-dithiobis (2-nitro benzoic acid) (DTNB) was obtained from SD Fine Chemicals Ltd., Mumbai, India. Thiobarbituric acid (TBA), reduced glutathione (GSH), and bovine serum albumin (BSA) were procured from CDH Pvt. Ltd., New Delhi, India. Folin–Ciocalteu's phenol reagent (Merck Limited, Mumbai), 1,1,3,3-tetramethoxy propane (TMP) was procured from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. All the other chemicals were procured as analytical grade from SD Fine Chemicals Ltd., Mumbai, India.

2.2. Animals

Male Sprague–Dawley (SD) rats weighing 200–250 g (age: 10–14 months) were used in the present study. Animals were purchased from Sanjay Biological Museum, Amritsar, Punjab, India. Rats were fed with standard laboratory diet, procured from Markfed cotton seed processing plant, Gidderbaha, Mukatsar, Punjab India and water was provided with feeding bottle ad libtium. Further animals were maintained to expose in 12 h natural light and dark cycle. The experimental protocol was duly approved by Institutional Animal Ethics Committee (IAEC No.: ATRC/02/14) and care of the animals were taken as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environmental and Forest, Government of India (Reg. No.: 1407/a/11/CPCSEA).

2.3. Induction of renovascular hypertension by two-kidney one-clip (2K1C) method

The hypertension was induced in the rat by application of vascular clip on the left renal artery (2K1C) as described in the method of Goldblatt et al. [15]. Briefly, all SD rats were weighed and anesthetized with chloral hydrate (350 mg/kg; i.p.). The animal was placed on thermo controlled (37 °C) heating pad with supine position for maintaining the body temperature during the surgery. The temperature was monitored with a digital rectal thermometer. A left paracostal celiotomy was performed and the left kidney was exposed. The renal artery, renal vein and renal nerve were carefully isolated by blunt tipped vascular scissors and hooks. A vascular clip was applied to the left renal artery and a nylon suture was applied to the clip and muscular tissue to prevent clip dislodgement [8]. After application of clip on renal artery, the color changes of the kidney (dark brown to yellowish red) were noted. The kidney was placed back into the abdominal cavity in its original position and the abdominal muscle and skin layers were closed separately with silk suture thread (No.: 4). The topical antibiotic powder (Neosprin® consisting of neomycin and polymyxin B sulfate and bacitracin zinc; GlaxoSmithKline, Bangalore, India) was applied once. After that post-operative care of rats were performed for a further 4 h to safe and easy recovery from the surgical stress. Furthermore, rats were kept for 24 h in separate cages and allowed to assess the soft chewable diet and water ad libitum. Furthermore, the home cages are kept in animal storage room with controlled temperature (35 ± 3 °C) and humidity (30–40%) system.

2.4. Assessment of cardiovascular functional changes

The cardiovascular changes are assessed by tail-cuff method as described by Daugherty et al. [11]. Briefly, the cardiovascular functional changes i.e., systolic and diastolic blood pressure; and heart rate were assessed by non-invasive blood pressure (NIBP) method using a digital Biopac device with Data Acquisition System (DAS) software (Biopac software for MP100 model, Biopac® System Inc., Monrovia, CA, USA) on the 9th day. During the assessment, the rapid movement of the rat body was controlled by using the suitable size of the restrainer cage with avoidance of unwanted physical stress on the animal's body. Also, the body temperature was also controlled throughout the experimental period; because these two factors frequently interfere with the accuracy of the readings. The cuff was applied on the tail within 2–3 cm from the starting point of the tail. The readings are directly recorded in the computer system with DAS software.

2.5. Assessment of kidney weight and body weight (KW/BW) ratio

On the 9th day, before sacrificing the animal, the body weight of all the rats was noted. Further, the collection of blood samples was performed by retro-orbital sinus puncture method for estimating the plasma renin content level. After that, animals were sacrificed. The left kidney was isolated and weighed immediately (within a few minutes). Then, the ratio of kidney weight and body weight was calculated.

2.6. Biochemical analysis

All the group of animals were sacrificed after the 9th day of experiments by cervical dislocation and left renal tissue was isolated immediately and kept in a freeze dryer at 4 °C for further tissue analysis. The homogenate (10% w/v) of renal tissue was performed by using a homogenizer (Elvehjem Tissue Homogenizer, Omni International, India) with a phosphate buffer (pH 7.4) with protease (soybean trypsin) inhibitors and it is centrifuged at 3500 rpm for 15 min at 4 °C. The supernatant was used for the estimation of tissue total protein, thio-barbituric acid reactive substance (TBAR) and reduced glutathione (GSH) levels.

2.6.1. Estimation of plasma renin content

The plasma renin content was estimated by using commercially available rat renin enzyme linked immunosorbent assay (ELISA) Kit (Kamiya Biomedical Company, 12779 Gateway Drive, Seattle, Washington, USA). Briefly, the 100 μl of plasma sample was added in the wells of the microtiter plate and incubated for 2 h at room temperature (37 °C). After that, the excess serum samples were removed from the plates and added 100 μl of detection reagent A in the microtiter plate without washing and incubated for further 1 h at room temperature. Plates were washed three times with washing buffer for the removal of excess reagents. Then, 100 μl of detection reagent B was added and incubated for 30 min at 37 °C. Further plates were washed five times with washing buffer and added 90 μl chromogenic substrate (3,3′,5,5′-tetramethylbenzidine, TMB) solution and incubated for 15–25 min at 37 °C with covering of plate sealer to avoid light reaction. The reaction was stopped by the addition of 50 μl stop solution. The changes of blue color chromogen were estimated immediately at 450 nm wavelength by using an ELISA reader (Thermo Fisher Scientific