



## EVALUATION OF ANTI-HYPERTENSIVE POTENTIAL OF SIDDHA FORMULATION RATHA AZHUTHA NIVARANI CHOORANAM ON RENAL ARTERY LIGATION INDUCED HYPERTENSIVE RATS

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### ABSTRACT

Hypertension is one of the most popular risk factor that prevailing cardiovascular disease. Blood pressure (BP) sustaining high systolic pressure  $\geq 140$  mmHg or diastolic pressure  $\geq 90$  mmHg may be categorized as hypertension. According to epidemiology research, the occurrence of hypertension is persistent and shows an increasing trend. The global population suffering from hypertension is predicted to reach 1.56 billion by 2025. Conventional allopathic drugs currently used to lower blood pressure have some adverse effects including orthostatic hypotension, hypercholesterolemia, depression and impotency. Hence there is dire need to search for drugs derived from herbal derivatives nature that are more potent but have less or no side effects. Recently, traditional preparations are being used for the treatment of a variety of disorders including cardiovascular diseases because of their safety, efficacy, cultural acceptability and lesser side effects. The main objective of the present study is to investigate the anti-hypertensive potential of the siddha formulation ratha azhutha nivarani chooranam (RANC) on renal artery ligation induced hypertension in wistar rat model. Results of the study clearly indicates that there was a significant increase in mean arterial pressure in ligated rats belongs to disease control group. Treatment with RANC at the dose of 250 and 500 mg/kg has shown dose dependent decrease in mean arterial pressure of rats belongs to treatment group when compared with arterial ligation group. Further treatment with RANC at both the dose level have shown higher level of protection in kidney tissues of treated rats.

**KEY WORDS:** Hypertension, Allopathic drugs, Adverse effects Siddha formulation, Ratha azhutha nivarani chooranam Paruthi, Anti-hypertensive activity

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## 1. Introduction

Hypertension is one of the most critical concerns for human health that nearly influences 40% of people in the world [1]. The prevalence of hypertension rises with advancing age and more than half of people aged 60 to 69 years are affected by this disease [2]. Elevated arterial pressure causes pathological changes in the vasculature and is a major risk factor for life-threatening cardiovascular diseases such as myocardial infarction, stroke, heart and renal failure [3]. Several mechanisms are known to participate in the pathogenesis of this disease including disruption of the autonomic nervous system, activation of the renin-angiotensin-aldosterone system, oxidative stress, inflammation, immune system disorder, endothelial dysfunction and imbalance between vasoconstrictor and dilator factors [4-7].

As antihypertensive drugs have some side effects, many studies have been conducted to find more suitable anti-hypertensives from natural sources, such as herbal medicine or components derived from food. Numerous epidemiologic studies have indicated that high intake of fruits and vegetables reduce the risk of cardiovascular diseases [8]. The use of herbal therapies for treatment and management of cardiovascular diseases (CVDs) is increasing. Plants contain a bounty of phytochemicals that have proven to be protective by reducing the risk of various ailments and diseases [9].

Approximately two-thirds of the world's plant species are widely used in medicines, and almost all of these exhibit excellent antioxidant potential [10]. The antioxidant potential of plants has received a great amount of attention for increased oxidative stress has been identified as a major causative factor of CVD. Approximately 80% of the world population uses herbal medicines due to their low toxicity and better acceptability by the human body [11,12].

Siddha formulation RANC comprises of herbal components such as Cuminum cyminum, Elattaria cardaomum, Borneo camphor, Zingiber officinale, Piper nigrum, Piper longum, Coriandrum sativum and Withania somnifera. Together with the versatile combination of phytotherapeutics the formulation RANC may possess numerous pharmacological activity as prescribed in the literature. The main aim of the present investigation is to evaluate the anti-

hypertensive potential of the ratha azhutha nivarani chooranam (RANC) on renal artery ligation induced hypertension in wistar rat model.

## 2. Materials and Methods

### 2.1. Animals

Healthy adult Wistar albino rats weighing between 250-270 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit. A 12 light / dark cycle were maintained. Room temperature was maintained between  $22 \pm 2$ °C and relative humidity 50–65%. They were provided with food (Sai feeds, Bangalore, India) and water ad libitum. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama Institute of science and technology, Chennai, Tamil Nadu, India with the protocol number SU/CLATR/IAEC/X/094/2018.

### 2.2. Experimental Groups

The animals were grouped into four groups of 6 animals each. Group I (Sham Operated group) – Surgically exposed renal artery without ligation. Group II – Rats underwent surgical renal artery ligation served as hypertension control. Group III – Rats underwent surgical renal artery ligation prior treatment with low dose of RANC 250 mg/kg.(p.o). Group IV – Rats underwent surgical renal artery ligation prior treated with high dose of RANC 500 mg/kg (p.o).

### 2.3. Experimental Procedure [13-17]

All the experimental animal belongs to group III and IV were treated with 250 and 500 mg/kg of RANC orally for the period of four weeks followed by this on the 29th day of experiment the renal artery was occluded for 6 h (ischemia) following the surgery; the animals than anesthetized by intraperitoneal injection of 30–40 mg/kg pentobarbital sodium. After 6 h the renal arterial ligation was removed (reperfusion). This leads to a rise in blood pressure as a consequence of elevated plasma renin level. Within 15 min a stable hypertension is achieved. Kidney being placed back in its original position. All rats were observed for 4 h following surgery, then individually housed for 24 h and allowed access to standard rat chow and water ad libitum. The test substance RANC was then

administered at doses of 250 mg/kg and 500 mg/kg to the animal belongs to treatment group. Hemodynamic change in the Blood pressure were monitored using non-invasive blood pressure monitoring instrument with Powerlab data acquisition system. After measurement of blood pressure, the left kidney was removed and stores at 10% formalin saline for histopathological analysis.

**2.4. Histopathological Analysis [18]**

Sample obtained were immersed in 10% formalin for 24 h-48h for histopathological examination. After standard processing, the cut tissue was embedded in paraffin (Leica TP1020 tissue processor) and cut into 5 µm thick sections in a rotary microtome (Leica RM2255 - Fully Automated Rotary Microtome). The sections were stained with haematoxylin-eosin (Merck). Histological measurement and photographs were taken with Olympus CX31, Trinocular Biological Microscope (magnification 10x & 40 x).

**2.5. Statistical Method**

The statistical analysis was carried by one-way analysis of variance ANOVA (GRAPH PAD PRISM 5 computer program). Results are expressed as ±SEM. The data were statistically analyzed by ONE WAY ANOVA followed by Dunnett’s multiple comparison test. Probability P values < 0.05 were considered as significant.

**3.Results**

**3.1. Effect of RANC on mean arterial and systolic pressure of renal artery ligation induced hypertensive rats**

Result analysis of the study on estimation of mean arterial and systolic pressure clearly reflects that treatment with RANC at the dose of 250 and 500 mg/kg has shown dose dependent decrease in mean arterial pressure of rats belongs to group III and IV. There was significant increase in systolic blood pressure observed in rats belongs to group II. Treatment with RANC at the dose of 250 and 500 mg/kg have shown marked decrease in systolic BP of rats belongs to group III and IV. As shown in Table 1.

**Table 1. Effect of RANC on mean arterial and systolic pressure of renal artery ligation induced hypertensive rats**

Group	Mean arterial pressure in (mm Hg)	Systolic BP (mm Hg)
Control	96.98 ± 5.015	119.3 ± 1.116
Renal artery ligation	162 ± 3.351*	138.7 ± 1.764*
Renal artery ligation+ RANC 250 mg/kg,(p.o)	132.3 ± 3.174*	134.7 ± 2.348
Renal artery ligation+ RANC 500 mg/kg,(p.o)	127 ± 2.037*	125.7 ± 1.406*

Values represent mean ± SEM of 6 experimental animals. \* P< 0.05; \*\* P< 0.01; \*\*\* P < 0.001.

**3.2. Effect of RANC on Heart rate of renal artery ligation induced hypertensive rats**

Result analysis of the study on enumeration of heart rate signifies elevated heart rate in group II ligated rats when compare to that of normal control rats. Treatment with RANC at the dose of 250 and 500 mg/kg have shown significant decrease in heart rate of rats belongs to group III and IV. As shown in Table 2.

**Table 2. Effect of RANC on Heart rate of renal artery ligation induced hypertensive rats**

Group	Heart Rate (Beats /Min)
Control	423.7 ± 2.011
Renal artery ligation	457.8 ± 3.25**
Renal artery ligation+ RANC 250 mg/kg,(p.o)	445.8 ± 1.922
Renal artery ligation+ RANC 500 mg/kg,(p.o)	437.7 ± 1.647*

Values represent mean ± SEM of 6 experimental animals. \* P< 0.05; \*\* P< 0.01; \*\*\* P < 0.001.

**3.3 Effect of RANC on histological changes in Rat Kidney**

Histopathological analysis of rat kidney reveals samples belongs to group I rats showed Glomerular Basement Membrane (GBM) separating between Capillary Space (Cs) and Urinary Space (Us). Foot processes of podocytes are separated from one another by a regular narrow space called Filtration Slit (FS). Vascular, degenerative with inflammatory changes were been observed in the sample belongs to group II. The lining epithelial cells of the renal tubules shown pyknosis of the nuclei Basement membrane of the capillaries are thickened with narrow lumen. Rare appearance of intercapillary sclerosis were observed in the sample belongs to group III.Prominent histology of

glomeruli with mild vascular congestion were observed in the sample belongs to group IV

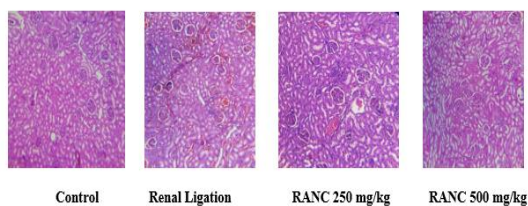


Figure 1: Effect of RANC on histological changes in Rat Kidney (H&E) under Low Power Magnification

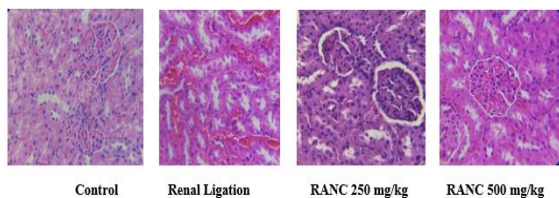


Figure 2: Effect of RANC on histological changes in Rat Kidney (H&E) under high Power Magnification

#### 4. Discussion

Hypertension is considered as the most common chronic illness among all the people in the world [19]. It is not only responsible for morbidity but also mortality. Hypertension causes considerable damage to the blood vessels leading to conditions like end organ failure [20]. In heart, mechanical stress initiates numerous pathways, including ion channels, integrin interaction between cells and matrix, activation of various tyrosine kinases, autocrine production, and release of growth factors [21]. Elevated pressure has been one of the major issues in public health.

Ligation of renal artery activates the sympathetic nervous system are activated [22]. Renin is secreted by the kidneys when sympathetic activity is enhanced. Angiotensinogen is converted to angiotensin-I (Ang I) in the presence of renin. Angiotensin-converting enzyme (ACE) plays a vital role in the regulation of BP via hydrolysis of the inactive form of Ang I to the active form, angiotensin II (Ang II). ACE is mainly located on the surface of the endothelium and epithelium involved in the constriction of blood vessels, subsequently leading to elevation of BP. Ang II is a potent vasoconstrictor and affects cardiovascular homeostasis. Apart from the role in vasoconstriction, Ang II also stimulates the release of aldosterone, further increasing blood volume and BP due to water and salt retention [23].

Siddha formulation RANC comprises of herbal components such as Cuminum cyminum, Elattaria cardaomum, Borneo camphor, Zingiber officinale, Piper nigrum, Piper longum, Coriandrum sativum and Withania somnifera. Together with the versatile combination of phytotherapeutics the formulation RANC may possess numerous pharmacological activity as prescribed in the literature. It was observed from the results of the present investigation that elevated mean arterial pressure in ligated rats belongs to group II signifies the induction of hypertension in the experimental animals. Treatment with RANC at the dose of 250 and 500 mg/kg has shown dose dependent decrease in mean arterial pressure of rats belongs to group III and IV. There was significant increase in systolic blood pressure observed in rats belongs to group II. Treatment with RANC at the dose of 250 and 500 mg/kg have shown marked decrease in systolic BP of rats belongs to group III and IV. The data's obtained from the present investigation indicates the anti-hypertensive potential of the trial drug in the treated rats. Further it was also noticed that profound increase in the heart rate of rats belongs to group II when compare to group I sham operated animals, Treatment with trial drug RANC have shown marginal decrease in the heart rate

Light microscopic observation of samples belongs to group I rats showed normal glomerular basement Membrane (GBM) separating between Capillary Space (Cs) and Urinary Space (Us). Foot processes of podocytes are separated from one another by a regular narrow space called Filtration Slit (FS). Vascular, degenerative with inflammatory changes were been observed in the sample belongs to group II. The lining epithelial cells of the renal tubules shown pyknosis of the nuclei. Basement membrane of the capillaries are thickened with narrow lumen. Rare appearance of interpapillary sclerosis was observed in the sample belongs to group III. Prominent histology of glomeruli with mild vascular congestion were observed in the sample belongs to group IV

#### 5. Conclusion

Several research outcomes are suggesting the growing incidence on mortality due to hypertension throughout the world. Commonly used conventional medicines for hypertension fails to provide adequate benefits due to potential adverse effects upon long-

term usage. The results of the present research work depict that siddha formulation ratha azhutha nivarani chooranam (RANC) proves significant anti-hypertensive activity in the tested animal's, hence clinical recommendation of the formulation in hypertensive patients shall provide measurable clinical benefits.

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