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# ANTIHYPERTENSIVE EFFICACY OF *ROUWOLFIA TETRAPHYLLA* - ROOT OF THE PLANT ON UNINEPHRECTOMIZED DOCA - SALT HYPERTENSIVE RATS

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**Keywords:**

Antihypertensive activity, *Rouwolfia tetraphylla*, Uninephrectomy, DOCA - Salt, Biochemical assays

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**ABSTRACT:** The plant *Rouwolfia tetraphylla* has been used traditionally as antihypertensive and has been proven scientifically to possess high antioxidant and hepato-protective activity. This study was designed to check the potential of medicinal plants in treatment of hypertension which will be helpful to reduce the side and adverse effect of allopathic antihypertensive drugs. During the experimental period, 1% sodium chloride solution was administered orally with drinking water for four weeks and DOCA - salt (20 mg/kg body weight) was injected subcutaneously to elevate the systolic, diastolic and mean arterial blood pressure. The rats were then treated with the methanolic extract of *Rouwolfia tetraphylla* and a significant decrease in the systolic pressure was recorded. Biochemical assays including serum urea, serum creatinine, triglycerides, cholesterol, blood glucose and serum protein were also performed to assist the hypothesis. The study thus, concludes the anti-hypertensive activity of *Rouwolfia tetraphylla* in the DOCA - salt hypertensive wister rats.

**INTRODUCTION:** Cardiovascular disease (CVD) is group of disease that includes all the diseases of the heart and circulation including coronary heart disease, angina, heart attack, congenital heart disease and stroke. It is major health problem and leading cause of premature death. Major risk factors of CVDs are diabetes, dyslipidemia, hyperlipidaemia and hypertension. As estimated, 17.5 million people died from CVDs in 2012, representing 31% of all global deaths (WHO, 2013).

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High blood pressure is also known as "the silent killer". Hypertension is defined as a systolic blood pressure of 140mmHg or a diastolic pressure of 90 mmHg [1]. Hypertension may be primary, which may develop as a result of environmental or genetic causes or secondary, which has multiple etiologies, including renal, vascular and endocrine causes. Primary or essential hypertension accounts for 90 - 95 % of adult cases and secondary hypertension accounts for 2 - 10 % of cases [2 - 3].

Hypertension is the most common cardiovascular diseases and constitutes a major factor for several cardiovascular pathologies including atherosclerosis, coronary artery disease, myocardial infract heart failure, renal insuffiency, stroke and dissecting aneurysm of aorta. There are several anti-hypertensive drugs are thiazide diuretics, an

angiotensin - converting enzyme (ACE) inhibitor/ angiotension receptor blocker (ARB), or calcium channel blocker (CCB) [4]. Due to the associated morbidity and mortality and cost to society, preventing and treating hypertension is an important public health challenge. Globally, an estimated 26 % of the world's population (972 million people) has hypertension and the prevalence is expected to increase to 29 % by 2025, driven largely by increases in economically developing nations. Hypertension is responsible for around 16.5 % annual death worldwide and is main cause of morbidity and mortality [5]. It is important to note that 17th may of every year has been designated world hypertension day by the International Society of Hypertension (ISH).

Blood pressure is asymptomatic, identifiable and usually treatable disease but if left untreated the consequences of that could be fatal. Hypertension is the most common risk factor for acute myocardial infarction, stroke, peripheral vascular disease and is the major known factor for cardiovascular disease and its mortality [6 - 8].

The major concerns that often delay treatment allude to higher costs of antihypertensive drugs, their availability and accessibility, the undesired side effects of antihypertensive drugs and the reduced patient compliance to consume more than a pill per day. Alternative approach for hypertension is herbal remedies. It is safe, easy and cost effective treatment. *Rauwolfia tetraphylla* Linn. a small tree shrub that will attain 6 feet (~ 2 meters) in heights [9]. *Rauwolfia tetraphylla* belongs to family apocynaceae is also known as *Rauwolfia canescens, Rauwolfia hirsute*, and commonly known as papataku, balachandrika [10]. The different species of *Rauwolfia tetraphylla* contains recinnamine, deserpidine, reserpine, ajmalcine, rauwolcine, ajmaline, serpantine, rautine and yohimbine, tetraphyllacine [11].

It is a frequently available species of Rauwolfia which is prevalently used in Ayurvedic and Unani system of medicines and also a part of folk remedies of most Asian countries [12]. From medicinal point of view, *Rauwolfia tetraphylla* is significant in the treatment of cardiovascular diseases, hypertension and a variety of psychiatric diseases [13].

## MATERIALS AND METHODS:

**Collection of Plant Sample:** Root of the plant, *Rouvolfia tetraphylla* was collected from Botanical Garden at Shri Bapalal Vaidya Botanical Research Centre of Biosciences, Department of Biosciences, Veer Narmad South Gujarat University, Surat, Gujarat, India. The plant was authenticated and voucher specimen no BVBRC/3003 of the plant was deposited in the herbarium of the University. The root was washed under running tap water followed by distilled water and dried at 40 °C in the oven for 3 days. The dried root was then pulverized into fine powder that passed through a 30-mesh sieve and stored for the future use [14].

**Preparation of Extract:** The ground plant material was subsequently extracted with methanol using soxhlet apparatus. The resulting crude methanolic extract was filtered by passing through a whatmann number 3 filter paper followed by concentrating in vacuum at 40 °C using a rotary evaporator and freeze drying. The freeze dried sample was suspended in 0.2% agar solution and mixed thoroughly [15, 16].

**Experimental Protocol:** Albino Wister rats (Young animal wt 150-200 gm) were obtained from the Animal House, Department of Pharmacology and Toxicology, B.V. Patel Perd Centre, at Ahmadabad, India. Animals were housed (3 rats/cage) in polypropylene cages lined with husk, renewed every 24hr under a 14:10 hr of light/dark regime and had free access to tap water and food. The rats were fed on a standard pellet diet. The experimental protocol was approved by the Institutional Animal House Ethics Committee, (IEAC) PERD/IAEC/005 constituted by the Ministry of Social Justice and Empowerment, Government of India, prior to the initiation of the experiment.

**Induction of Hypertension (Uninephrectomy):** Left uninephrectomy was performed on all the rats by anaesthetizing with intramuscular injection of ketamine (20 mg/kg). The kidney was visualized by a left lateral abdominal incision, and the left renal artery and ureter were ligated by silk thread, followed by the removal of left kidney. The muscle and skin layer (incision site) were sutured with highly sterile suture needles. After uninephrectomy, rats were allowed to drink tap water *ad libitum*,

with no further treatment. All uninephrectomized animals were given 1% NaCl in the drinking water with weekly twice subcutaneous injection of DOCA - Salt (20 mg/kg body weight in olive oil) for four consecutive weeks (DOCA-salt hypertensive rat). The rats were then, randomly divided into four groups each comprising of four rats including 2 males and 2 females (**Table 1**).

**Experimental Work Out on DOCA - Salt Hypertensive Rats:** The four groups of the hypertensive rats were divided according to the **Table 1** and categorized into normal control, disease control, positive control and test group. The animals in the group 1 were not given any surgery or treatment at all, while the group 2 animals were undergone for only surgery and no treatment was done. The animals in group 3 were undergone for surgery and treated with standard drug, ramiprill at 1 mg/kg of the total body weight. The animals in the group 4 were undergone for surgery and treated with the plant extract at 500 mg/kg of the total body weight (**Table 1**).

**TABLE 1: GROUP DISTRIBUTION AND TREATMENT FORM**

| | | |
|---|---|---|
| Group 1<br>Normal Control | Without surgery<br>and treatment | - |
| Group 2<br>Disease control | Uninephroctimized rat without<br>any treatment | - |
| Group 3<br>Positive Control | Uninephroctimized rat treated with standard<br>positive control | Ramiprill, 1mg/kg<br>body weight |
| Group 4<br>Test group | Uninephroctimized rat treated with plant<br>extract | Methanolic extract, 500mg/kg<br>body weight |

The test animals were treated with the stated dose of plant extract at every 24 hr interval, consecutively for 14 days. Systolic and diastolic blood pressures were recorded every week during the entire period of the study by tail cuff method (IITC, Non-Invasive Blood Pressure Instrument) [17]. All the recordings and data analyses were done using computerized data acquisition system and software. At the end of treatment, all the rats were anesthetized with intramuscular injection of ketamine and sacrificed in $CO_2$ incubator for biochemical assays.

**Biochemical Assays:** At the end of the treatment, after a 12 h of fast but *via* access to deionised water, the animals in groups I - IV were sacrificed. Blood samples were collected from each of the animal by retina puncture into plain sterile tubes. Each blood sample was allowed to clot and tubes were subsequently centrifuged at 2000 rpm for 5 min to obtain sera which was transferred into new tubes and kept at -200 $^{\circ}$C until used for bioassays. From each of the sample sera, serum urea, serum creatinine, triglycerides, cholesterol, glucose and protein, were measured by biochemical analyser, Erba 360 - fully automated clinical chemistry analyzer [17, 18].

**Statistical Analysis:** The statistical approaches for the data generated were evaluated by the SPSS - 15 statistical software. Analysis includes the expression of data in deviation of mean values (SD). Differences in mean values of biochemical parameters investigated between the treatment groups, the control and the involving treatment period were analyzed using chi-square test. The P value was considered to be and the outcomes with P value below - were considered to be significant.

**RESULT AND DISCUSSION:** Kidney plays a central role in the regulation of the balance of the body salt and water and then disordered regulation of renal functions is responsible for the altered balance of salt and water in pathophysiological states including some experimental models of hypertension [19]. The systolic and diastolic blood pressure were considerably ($P<0.01$) increased in DOCA - salt hypertensive rats compared to normal control.

Oral administration of plant extract 500 mg/kg total body weight) for a period of two weeks considerably ($P<0.01$) decreased systolic pressure in DOCA- salt treated rats (about 21 %), where as in positive control with remiprill the reduction was about 13 % (**Table 2**, **Fig. 1**). Oral administrations of methanolic extracts of plant for two week considerably decrease systolic pressure in DOCA - salt treated rats. Selected plant showed anti-hypertensive activity against DOCA-salt hypertensive

rats. *R. tetraphylla* had shown 5 % reduction in first week of treatment and 21.06 % reduction at the end of treatment. This considerable reduction in the blood pressure by *Rowvolfia tetraphylla* might be due to the ACE inhibition property of the plant, mimicking the structure of its substrate, like ramiprill which is an ACE inhibitor. ACE inhibitors directly block the formation of angiotensin-II, and increasing bradykinin level simultaneously. The net results are reduced vasoconstriction, reduced sodium and water retention and increased vasodilation (through bradykinin). The increase in bradykinin level is due to less inactivation done by ACE enzyme. *Rouwolfia tetraphylla* has also been reported for diuretic activity [20].

**TABLE 2: EFFECT OF PLANT EXTRACT ON DOCA - SALT HYPERTENSIVE RATS**

| Group | 0 day (mmHg) | 7th day (mmHg) | 14th day (mmHg) |
|---|---|---|---|
| Normal control | 129.96 ± 1.24 | 129.96 ± 1.24 | 129.73 ± 1.24 |
| Disease control | 169.57 ± 6.77 | 163.87 ± 1.19# | 163.87 ± 2.10 # |
| Positive control | 177.17 ± 3.32 | 146.3 ± 6.34#* (17.43) | 144.15 ± 1.98 #* (17.43) |
| Test group | 174.8 ± 2.54 | 166.06 ± 0.76 (5) | 138 ± 0.98* (21.06) |

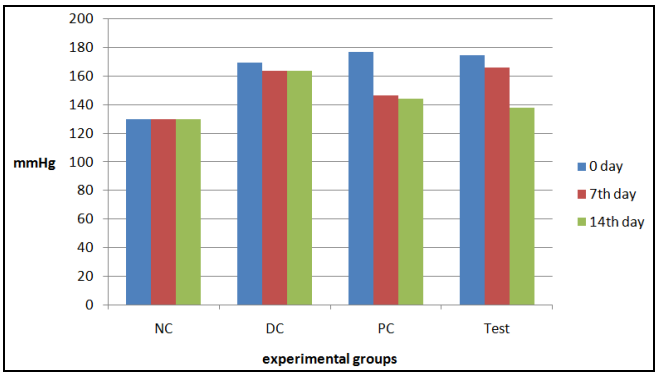


**FIG 1: ANTIHYPERTENSIVE EFFECT OF METHANOLIC EXTRACT ON DOCA - SALT INDUCED HYPERTENSIVE RATS NC - NORMAL CONTROL; DC - DISEASE CONTROL; PC- POSITIVE CONTROL; TEST**

The biochemical parameters including serum urea, creatinine, triglycerides, cholesterol, glucose and protein did not show any significant variation (data not shown) as reported by Prahalathan for morin in DOCA salt induced hypertension, where a significant variation in serum creatinine and urea is reported. However, further studies are needed to know the exact mechanism of antihypertensive action of plant. An increased concentration of aldosterone leads to increased re-absorption of sodium ions and water from epithelial cells in the distal nephron of kidney, thereby influencing the blood pressure levels [21]. In addition, increased aldosterone concentrations may activate oxidative stress in the DOCA - salt model [22]. In agreement with previous reports [23], we also observed that systolic and diastolic blood pressures were considerably increased in DOCA-salt hypertensive rats, which might be due to increased oxidative stress and decreasing the bioavailability of nitric oxide. Daily oral administration of plant extract resulted in a remarkable reduction in systolic blood pressure which is due to antihypertensive property of this plant.

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