

Original Article

EFFECT OF *SALIX TETRASPERMARIOXBUGH* ON FRUCTOSE INDUCED HYPERTENSION IN RATS

J. H. VIRUPAKSHA

Acharya Nagarjuna University, Nagarjuna Nagar, Guntur  
Email: jhvirupaksha@gmail.com

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ABSTRACT

**Objective:** The present study was designed to evaluate the effect of the ethanolic and aqueous extract of *Salix tetrasperma* Roxburgh on blood pressure by fructose induced hypertensive rats.

**Methods:** The *Salix tetrasperma* Roxburgh leaves were evaluated for antihypertensive potential by using fructose-induced hypertension model in Wister albino rats. The test animals were given high fructose (10%) diet for 21st days to induced hypertension. Subsequently, the 200 and 400 mg/kg/day (p. o.) doses of ethanolic and aqueous extracts of *Salix tetrasperma* leaves were administered to the different groups of hypertensive and normal animals. The hypertensive condition of the experimental animals was confirmed on 21<sup>st</sup> day by measuring systolic, diastolic and mean arterial pressure (SBP, DBP, MAP) using noninvasive BP (NIBP) system for rodents. The SBP, DBP, MAP were again recorded on day 0 d, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day of administration standard and test extracts. The normal control group of animals were given normal diet and administrated normal saline throughout the experiment.

**Results:** The both test extracts significantly reduced SBP, DBP and MAP significantly ( $P < 0.05$ ) lowered blood pressure effect in 0 d, 7<sup>th</sup> day at the dose of 200 and 400 mg/kg in fructose induced hypertensive rats. On 14<sup>th</sup> day the test extracts at the doses of 400 mg/kg significantly reduce only DBP and MAP. However, the treatment is continued for 21 d but no significant activity observed. The test extracts reveals that antihypertensive of *Salix tetrasperma* Roxburgh in dose dependent manner in hypertensive control rats.

**Conclusion:** These observations established the traditional claim and thus *Salix tetrasperma* Roxburgh could be a potent antihypertensive agent for use in future. The phyto constituents present in the test samples may be responsible for the hypotensive effect. However, further investigation is required to identify the active principles responsible for antihypertensive activity.

**Keywords:** NIBP, Antihypertensive, Fructose, Glibenclamide, *Salix tetrasperma* Roxburgh

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INTRODUCTION

Hypertension (HT) is a most common cardiovascular disease in developed and developing countries. Due to changes in the man behaviour and life style currently one of the major risk factors for coronary artery disease, cardiac failure, insulin resistance, obesity, strokes, atherosclerosis and renal insufficiency. Many studies have reported diets high in carbohydrates, particularly sugars and even more particularly sucrose and fructose increase the risk of cardiovascular diseases including high blood pressure [1]. A number of scientific reports tend to support the fact some metabolic abnormalities such as hyperinsulinemia, insulin resistance and hypertriglyceridemia as well as hyperactivity of the sympathetic nervous system and oxidative stress were frequently associated with the pathogenesis of sucrose induced hypertension [2]. It is well known that high blood pressure can often lead to dangerous complications if left untreated [3, 4]. In developed countries, 10% or more of the total health budget is spent on the management of hypertension, diabetes and its complications.

Many synthetic drugs due to their side effects, people largely use herbal medicine because of easily availability, cost effectiveness to prevent and cure illness [5] for curiosity and also the idea that combining it with conventional treatment would help [6]. A holistic or a spiritual health concern and the belief that herbal drugs are natural (and thus safe) also seem to be associated with the use of alternative medicine [7]. Traditionally, many of the folk remedies of plant origin have long been used for the treatment of various ailments unscientifically exploited or improperly used. Therefore, there is an urgent need to develop new and effective drugs for the treatment of hypertension, can be used as a single plant or in polyherbal formulations.

The genus *Salix* comprises about 500 species that mainly distributed in temperate region worldwide and also in higher altitudes of tropics. The species are rich in phenolic constituents such as

salicylates, flavonoids and tannins that have many pharmacological activities and medicinal uses. Information provided by traditional medical practitioners in the Western Ghats indicates that the leaf of *Salix tetrasperma* Roxburgh is used to treat diabetic and hypertension. However, there is a lack of sufficient scientific data justifying the traditional use of *Salix tetrasperma* Roxburgh in the treatment of hypertension. Hence, there is an increasing need of new natural antihypertensive agent with less adverse effects; safe and easily available can be used alone or in polyherbal formulations. So the present investigations was undertaken to determine the effects of the ethanolic and aqueous extract resulting in antihypertensive activity in fructose induced hypertension in rats.

MATERIALS AND METHODS

Hypertension was induced in rats by feeding them a fructose (10%) diet for 21 d instead of tap water. Antihypertensive effect of *Salix tetrasperma* Roxburgh was evaluated by observing the degree of lowering of blood pressure in fructose induced in hypertensive rats.

Plant material

The leaves of *Salix tetrasperma* Roxburgh were collected from the Bommalapur village of Koppa taluk, Chikmagalur district of Karnataka and were authenticated by Dr. E. Kumara Swamy Udupa, Professor and H. O. D Dept. of Botany S. J. C. B. M. College Sringeri, Karnataka. A voucher specimen (NCP/15/2010-11) has been deposited at Pharmacognosy department for further reference.

Preparation of the extracts

The leaves of *Salix tetrasperma* Roxburgh were cleaned and dried under shade at room temperature for several days and coarsely powdered. Dried powder was subjected to successive extracted in soxhlet extractor as per standard procedure using petroleum ether (40-60 °C) and ethanol at their boiling point for 48 h. The marc

obtained from ethanol extraction was further utilized for aqueous extraction by maceration for 48 hr. The extracts were concentrated under reduced pressure and stored in a refrigerator for further use.

### Animals

Wister albino rats of either sex, weighing around 200-250 g. were used in the present experimental study. They were obtained from in house breed animals of Chalapathi Institute of Pharmaceutical Sciences, Guntur. The rats were provided standard laboratory feed and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Chalapathi Institute of Pharmaceutical Sciences, Guntur, A. P (Approval No. 27/IAEC/CIPS/2014-15; dt 21/02/2015) and care of the animals was taken as per guidelines of the CPCSEA.

### Drugs and reagents

Fructose (Hi media), Streptozotocin was purchased from Spectrochem, Mumbai. All the chemicals and drugs used in the experiments were of standard quality.

### Acute toxicity test

Toxicity test studies conducted as per internationally accepted protocol drawn under OECD guidelines. 425. (OECD guidelines. 425 modified, adopted Oct 3, 2008) in Swiss albino mice [8].

### Phytochemical analysis

Preliminary phytochemical studies of both the extracts were performed for steroids, glycosides, flavonoids, triterpenoids, tannins and phenolic compounds using standard procedures [9].

### Instrument

The instrument used for recording blood pressure non-invasive BP instrument for rodents (NIBP System, Panlab Harvard apparatus), Dr. Morepens gluco one BG03 blood sugar monitoring system. The entire equipment set up includes magnetic animal holders connected to the manual scanner, pulse amplifier and dual channel recorder.

### Principle of B. P recording

The animal was placed in the NIBP restrainer and an appropriate cuff with sensor was then mounted on its tail and warmed to about 33-35 °C. The tail cuff was inflated to a pressure well above the expected systolic blood pressure i.e. 250 mm Hg and slowly released during which the pulse was recorded by using Power Lab data acquisition system and computer. Systolic blood pressures (SBP), diastolic and mean arterial pressure (MAP) were measured for each rat. Mean arterial pressure is the average pressure in arteries during one cardiac cycle and it is considered as a better indicator to perfusion of coronary arteries, brain and kidneys.

Mean arterial pressure can be calculated by  $MAP = SBP + 2(DBP)$

### Fructose induced diabetic hypertensive model

Dietary fructose is a monosaccharide which can lead to metabolic disorders namely hyperinsulinemia increased levels of insulin in the blood. This condition associated with glucose intolerance, obesity, hypertension and dyslipidaemias, increased risk of heart diseases and coronary artery diseases. The increased intake of either sucrose or glucose was shown to enhance the development of either spontaneous hypertension or salt hypertension in rats.

The wistar albino rats of either sex were allowed free access to regular laboratory rat standard pellet food and water before the study. In addition, rats were acclimatized to the procedure of blood pressure measurement at 2 P. M daily for 1 w. Thereafter, the rats were divided into six groups containing 6 animals in each. Normal control (+ve control) rats were maintained on standard pellet food and in a hypertensive control (-ve control) group or other test groups, rats were fed with high-fructose diet (fructose 10% solution) for 21 d. Blood glucose levels of group 2-6 were measured on 21<sup>st</sup> day in order to the confirmed diabetic condition of the animals [10]. The experimental animals exhibited elevated blood glucose levels above 300 mg/dL were selected for the antihypertensive study. [11] (Blood glucose level data not included in this manuscript). High-fructose food was removed on the 21<sup>st</sup> day and animals were allowed free access to regular laboratory rat standard pellet food and water before initiated the antihypertensive treatment [10].

### Study of antihypertensive effect of *Salix tetrasperma* roxburgh leaf extracts

The experimental diabetic animals on high fructose diet exhibited elevated systolic, diastolic and mean arterial blood pressure (SBP, DBP, MAP) were divided into five groups of 6 animals each as mention above, apart from a normal control (+ve control) group maintained on standard pellet food. The rats were treated either with normal saline; 5 ml/kg (+ve and -ve Control) or glibenclamide (25 mg/kg and Propranolol 10 mg/kg) p. o. (reference) or 200 mg/kg and 400 mg/kg (p. o.) of ethanolic (STEtOH) and aqueous (STAQ) extracts of *Salix tetrasperma* leaves in respective groups for 21 d. The animals SBP, DBP and MAP were measured using the tail cuff method by using noninvasive BP (NIBP) system for rodents on day 0 d 7th, 14th, 21<sup>st</sup> days [10].

### Statistical analysis

The results are expressed as mean ± standard error mean (SEM). The results were analysed by one way ANOVA followed by Tukey's multiple range test using graph pad Prism 3.0 version values < 0.05 were considered as statistically significant.

**Table 1: Effect of *Salix tetrasperma* Roxburgh on blood pressure in fructose induced hypertensive rats on 0 d**

Groups	Treatment	Dose (mg/kg)	GLU(mg/dL)	SBP (mm/Hg)	DBP (mm/Hg)	MAP (mm/Hg)
I	Normal Control	5	89.2±1.15	120.6±1.53	91.4±1.50	101.6±1.02
II	Hypertensive control	5	354.2±1.52	179.6±1.20	112.6±1.02	128.2±0.66
III	Glibenclamide +Propranolol	25+ 10	319.8±1.56	167.4±1.20	107.6±1.12	126.4±0.74
IV	STEtOH	200	344±2.07	175.6±0.97*	110.8±1.15*	128.8±0.96*
V	STEtOH	400	335.4±1.93	170±0.83*	111±1.22*	127.2±0.86*
VI	STAQ	400	343.6±2.50	177.6±0.87*	111.2±1.12*	129.8±0.73*

SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MAP: Mean Arterial blood pressure. Data are expressed as mean±SEM; n=6. One way ANOVA followed by Tukey's multiple comparison test when compared with normal control, \*P<0.05 significant

**Table 2: Effect of *Salix tetrasperma* Roxburgh on blood pressure in fructose induced hypertensive rats on 7<sup>th</sup> day**

Groups	Treatment	Dose (mg/kg)	GLU(mg/dL)	SBP (mm/Hg)	DBP (mm/Hg)	MAP (mm/Hg)
I	Control	2 ml	91.4±1.32	120.6±0.81	94.2±1.35	105.2±1.71
II	Positive control	10 %	371±0.89	187±0.89	113.2±1.01	130.6±0.97
III	Glibenclamide +Propranolol	25+ 10	287.4±1.69	162±1.14	97.2±1.01	120.8±0.96
IV	STEtOH	200	319.2±1.24	180.6±0.81*	110.8±0.96*	127.6±0.81*
V	STEtOH	400	311.2±1.74	167.4±0.87*	94.6±1.02*	123.2±1.01*
VI	STAQ	400	323.6±1.93	173.8±0.66*	111.4±1.12*	128.2±0.86*

Data are expressed as mean±SEM; n=6. One way ANOVA followed by Tukey's multiple comparison test when compared with normal control, \*P<0.05 significant

**Table 3: Effect of *Salix tetrasperma* Roxburgh on blood pressure in fructose induced hypertensive rats on 14<sup>th</sup> day**

Groups	Treatment	Dose (mg/kg)	GLU(mg/dL)	SBP (mm/Hg)	DBP (mm/Hg)	MAP (mm/Hg)
I	Control	2 ml	90.8±1.35	122.2±1.49	92.6±1.16	104.2±1.28
II	Positive Control	10%	382.6±1.88	192.4±0.97	116.4±1.12	130.2±1.11
III	Glibenclamide +Propranolol	25+ 10	259±1.22	153±1.14	93.2±0.73	125.8±0.66
IV	STEtOH	200	292.8±2.15	174.8±1.06	97.8±0.66*	129±0.99*
V	STEtOH	400	267.4±2.20	161.4±0.97	94.2±0.66*	125±0.70*
VI	STAQ	400	290±2.16	167.2±101	94.8±0.96*	128±0.83*

Data are expressed as mean±SEM; n=6. One way ANOVA followed by Tukey's multiple comparison test when compared with normal control, \*P<0.05 significant.

**Table 4: Effect of *Salix tetrasperma* Roxburgh on blood pressure in fructose induced hypertensive rats on 21<sup>st</sup> day**

Groups	Treatment	Dose (mg/kg)	GLU(mg/dL)	SBP (mm/Hg)	DBP (mm/Hg)	MAP (mm/Hg)
I	Normal Control	5	93±1.70	120.6±1.88	91.6±1.20	104.8±0.96
II	Hypertensive control	5	389.6±1.63	194.2±0.66	114.4±1.16	129.6±0.50
III	Glibenclamide +Propranolol	25+ 10	210.4±1.86	145.6±0.81	90.4±1.20	119±0.83
IV	STEtOH	200	222.2±2.19	170.8±1.15	94.6±0.74*	125±0.89
V	STEtOH	400	222.6±1.53	153.2±1.01	91±0.99*	120.6±1.16
VI	STAQ	400	239±2.28	157.6±0.81	93.4±0.81*	120.6±1.12

Data are expressed as mean±SEM; n=6. One way ANOVA followed by Tukey's multiple comparison test when compared with normal control. \*P<0.05 significant

## RESULTS

### Acute toxicity test

In acute toxicity study of ethanolic (STEtOH) and aqueous (STAQ) extracts of *Salix tetrasperma* leaves does not showed mortality at the dose level of 2000 mg/kg. Therefore 2000 mg/kg dose was considered as ALD<sub>50</sub> cut off the dose under GHS 5 (safe dose), as per Globally Harmonised Classification System (GHS) for Chemical Substances and Mixtures described in OECD guideline 423 (Annexure 2b and 3b). Hence, in present study, only 200 mg/kg (low) and 400 mg/kg (high) body weight doses were selected for *in vivo* antihypertensive therapeutic study.

### Study of antihypertensive effect of *Salix tetrasperma* roxburgh leaf extracts

The ethanolic and aqueous extract of *Salix tetrasperma* Roxburgh exhibited a significant (p<0.05) decrease in SBP, DBP and MAP antihypertensive effect in 0 d, 7<sup>th</sup> day at the dose of 200 and 400 mg/kg when compared with fructose induced hypertensive control groups (table 1 and table 2). On 14<sup>th</sup> day the test extracts at the doses of 400 mg/kg significantly reduces only DBP and MAP (p<0.05) and on 21<sup>st</sup> day treatment only decrease in DBP was recorded however the treatment is continued but no significant activity observed for SBP and MAP in the hypertensive rats.

## DISCUSSION

### Antihypertensive effect in fructose induced hypertension

The ethanolic and aqueous extract of *Salix tetrasperma* Roxburgh showed a significantly (P<0.05) decrease in the SBP, DBP and MAP in fructose induced hypertensive rats at the doses of 200 mg/kg and 400 mg/kg in 0 and 7<sup>th</sup> day of treatment. However antihypertensive activity is continued for 14<sup>th</sup> and 21<sup>st</sup> days and found that test extracts decreased all the parameters in dose dependent manner in the hypertensive control group.

The decrease in blood pressure by an ethanolic and aqueous extract of *Salix tetrasperma* Roxburgh in hypertensive rats could be linked to a number of mechanisms. Previous experimental studies demonstrated that glucose and fructose contributes to the increase in blood pressure [12]. It has been clearly stated that one of the reasons for glucose-induced hypertension is an increase in sympathetic activity. Increase in sympathetic activity by any mean usually contributes to increase in heart rate and blood pressure. In the present investigation, the extracts tested were found to significantly show hypotensive effect

could be a strong reason of its antihypertensive effect in hypertensive rats. Endothelial dysfunction and oxidative stress are the important factors which favours hypertension [13, 14]. It is also well-known fact that high quantity of sugar consumption is associated to increased tissue production of reactive forms of oxygen [15]. Moreover, in hypertensive patients; lower concentration of antioxidants have been documented [16]. Furthermore, an increased blood glucose level has also been involved in a reduction in nitric oxide levels ultimately resulting in an increased hypertension.

It has been proved that plants rich with polyphenols having an antioxidant effect which improves endothelial dysfunction through increase NO formation. Decrease LDL formation, increase prostacyclin formation and increase EDHF mediated vasorelaxation and decrease Endothelin-1 production [17]. It has also been reported that *Salix tetrasperma* Roxburgh is rich with flavonoids and polyphenols and contains antioxidant effect [18]. Thus the antihypertensive effect of *Salix tetrasperma* could be due to the antioxidant effect of polyphenols. Besides the diuretic activity of *Salix tetrasperma* has been reported by earlier authors which might in part be responsible for the antihypertensive effect [19].

## CONCLUSION

The outcome of this study provides evidence that the ethanolic and aqueous extract of *Salix tetrasperma* Roxburgh most likely contains certain active principles which exert an antihypertensive effect in experiment animals. Moreover, the present investigation shows that *Salix tetrasperma* Roxburgh is safe for use and these findings justify the folklore claim. However, more research is needed to isolate the phytoconstituents and validate its exact mechanism of antihypertensive effect.

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## CONFLICT OF INTERESTS

Declared none

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