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 21 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 21st 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, 7th, 14th and 21st 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, 7th day at the dose of 200 and 400 mg/kg in fructose induced hypertensive rats. On 14th 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, Gilbenclamide, *Salix tetrasperma* Roxburgh

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 rich 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. Hence, 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 hypertensive rats.

Plant material

The leaves of *Salix tetrasperma* Roxburgh were collected from the Bommalapura 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. 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 soxlet extractor as per standard procedure using petroleum ether (40-60 °C) and ethanol at their boiling point for 48 h. The marc

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

J. H. VIRUPAKSHA

Acharya Nagarjuna University, Nagarjuna Nagar, Guntur

Email: jhvirupaksha@gmail.com

Received: 01 Mar 2017 Revised and Accepted: 22 Jul 2017

ISSN- 0975-1491                 Vol 9, Issue 9, 2017
International Journal of Pharmacy and Pharmaceutical Sciences

© 2017 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/)

DOI: http://dx.doi.org/10.22159/ijpps.2017v9i9.18232
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**

Wistar albino rats of either sex, weighing around 200-250 g, were used in the present experimental study. They were obtained from 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; 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 manmal 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.

**Statistical analysis**

The results are expressed as mean±standard error mean (SEM). The results were analyzed by one way ANOVA followed by Tukey's multiple comparison test when compared with normal control, P<0.05 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     | 5            | 354.2±1.52 | 179.6±1.20  | 112.6±1.02  | 128±2.66    |
| III    | Glibenclamide    | 25+          | 319.8±1.56 | 167.4±1.20  | 107.6±1.12  | 126±0.74    |
|        | +Propranolol     | 10           |            |             |             |             |
| IV     | STEOH            | 200          | 344.2±0.7  | 175.6±0.97  | 110.8±1.15  | 128±0.96    |
| V      | STEOH            | 400          | 335.4±1.93 | 170.8±0.82  | 111±1.22    | 127.2±0.86  |
| VI     | STAQ             | 400          | 343.6±2.50 | 176.6±0.87  | 111.2±1.12  | 129±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 th day**

| Groups | Treatment        | Dose (mg/kg) | GLU(mg/dL) | SBP (mm/Hg) | DBP (mm/Hg) | MAP (mm/Hg) |
|--------|------------------|--------------|------------|-------------|-------------|-------------|
| I      | Control          | 2 ml         | 91.4±3.32  | 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  | 150.6±0.97  |
| III    | Glibenclamide    | 25+          | 287.4±1.69 | 162±1.14    | 97.2±1.01   | 120.8±0.96  |
|        | +Propranolol     | 10           |            |             |             |             |
| IV     | STEOH            | 200          | 319.2±1.24 | 180±0.81*   | 110.8±0.96  | 127.6±0.81  |
| V      | STEOH            | 400          | 311.2±1.74 | 167.4±0.87* | 94.2±1.02*  | 123.2±1.01  |
| VI     | STAQ             | 400          | 323.6±1.93 | 173.8±0.66* | 111.4±1.12  | 128±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.

**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 21st 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 21st 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 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 (STAG) 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, 21st days [10].

Statistical analysis

P<0.05 significant.
Antihypertensive effect in fructose induced hypertension

In the present investigation, the heart rate and blood pressure in hypertensive rats was significantly (p<0.05) decreased compared with fructose induced hypertensive control. 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 means usually contributes to increase in heart rate and blood pressure. In the present investigation, the test extracts at the doses of 400 mg/kg significantly reduced only DBP and MAP (p<0.05) and groups (table 1 and table 2). On 14th day at the dose of 200 and 400 mg/kg when compared with fructose induced hypertensive control SBP and MAP in the hypertensive rats. The decrease in blood pressure by an ethanolic and aqueous extract of Salix tetrasperma Roxburgh on blood pressure in fructose induced hypertensive rats on 14th day at the dose of 200 and 400 mg/kg showed a significantly (P<0.05) decrease in the SBP, DBP and MAP in fructose induced hypertensive rats. Therefore 2000 mg/kg dose was considered as ALD and 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.

### 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 to 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, 4, 7th day at the dose of 200 and 400 mg/kg when compared with fructose induced hypertensive control groups (table 1 and table 2). On 14th day the test extracts at the doses of 400 mg/kg significantly reduces only DBP and MAP (p<0.05) and on 21st 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 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 contribute to the increase in blood pressure. 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 means 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. It is also well-known fact that high quantity of sugar consumption is associated to increased tissue production of reactive forms of oxygen. Moreover, in hypertensive patients; lower concentration of antioxidants have been documented. 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. 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.

### 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 phytoconstituenct and validate its exact mechanism of antihypertensive effect.

### ACKNOWLEDGEMENT

The authors are thankful to Principal and Management of Chalapathi Institute of Pharmaceutical Sciences, LAM, Guntur, Andhra Pradesh for providing the facilities to carry out research work.

### CONFLICT OF INTERESTS

Declared none

### REFERENCES

1. Parks EJ, Hellerstein MK. Carbohydrate-induced hyper triacyl-glycerolomeria: historical perspective and review of biological mechanisms. Am J Clin Nutr 2000;71:412-53.
2. Fried SK, Rao SP. Sugars, hypertriglyceridemia and cardiovascular disease. Am J Clin Nutr 2003;78:873-80.
3. Badyal DK, Lata H, Dadhich AP. Animal models of hypertension and effect of drugs. Indian J Pharmacol 2003;35:349-62.
4. Deep Raj Paul, Kartika Paul. Diabetes mellitus and its complications: a review. Int J Curr Pharm Res 2012;4:12-7.
5. Noumi E, Houngue F, Lontsi D. Traditional medicines in primary health care: plants used for the treatment of hypertension in Bafia, Cameroon. Fitoterapia 1999;70:134-9.
6. Barnes PM, Powell-Griner E, McFann K, Nahin RL. Complementary and alternative medicine use among adults: United States, 2002. Seminars Integrative Med 2004;2:54-71.
7. Bishop FL, Yardley L, Lewith GT. A systematic review of beliefs involved in the use of complementary and alternative medicine. J Health Psychol 2007;12:851-67.
8. OECD Guidelines for the Testing of Chemicals, Acute oral Toxicity-Up-and-Down-Procedure (UDP). OECD/OCDE 425; 2008. Available from: http://ntp.niehs.nih.gov/?objectid=62883FD5-99D2-26AC-F2ED0896915682B. [Last accessed on 10 Oct 2016].
9. Kulkarni SK. Handbook of Experimental Pharmacology. 3rd Ed. New Delhi: Vallabh Prakashan; 2002.
10. Alamgeer. Antihypertensive activity of aqueous methanol extract of Berberis Orthobotrys Bien ex. aitch in rats. Trop J Pharm Res 2013;12:393-9.
11. Ika Purwidayaningrum, Eln Yulmah Sukandar, Irda Fikrianny. Antihypertensive activity of extract and fractions of Matoa (pometia pinnata j. R and g forts) leaves. Asian Pharm Clin Res 2017;10:323-8.
12. Hwang IS, HHO, BB Hoffman, GM Reaven. Fructose-induced insulin resistance and hypertension in rats. Hypertension 1987;10:512–6.
13. Tomiyama. Close relationship of abnormal glucose tolerance with endothelial dysfunction in hypertension. Hypertension 2000;36:245–9.
14. Cai H, DG Harrison. Endothelial dysfunction in cardiovascular diseases-the role of oxidant stress. Circ Res 2000;87:940–4.
15. Midaoui ELA, JD Champlain. Prevention of hypertension, insulin resistance and oxidative stress by lipoic acid. Hypertension 2002;39:303-7.
16. Rodrigo R. Relationship between oxidative stress and essential hypertension. Hypertens Res 2007;30:1159-67.
17. Stoclet JE. Vascular protection by dietary polyphenols. Eur J Pharmacol 2004;500:299-313.
18. Eman A. Identification of the chemical composition of the methanolic extracts of Salix tetrasperma Rob using LC-MS and evaluation of its potential as an antioxidant agent. Pharm Chem 2015;7:168–77.
19. Modal Sumanta, Hechhu Ramana, Suresh P, Chhetree Rishi Raj. Studies on diuretic and laxative activity of the Salix tetrasperma roxburgh. Int Res J Pharm 2010;1:145-9.

How to cite this article
• J H Virupaksha. Effect of Salix tetraspermaroxbugh on fructose induced hypertension in rats. Int J Pharm Pharm Sci 2017;9(3):243-246.