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Evaluation of anti-hypertensive activity of *Ulmus wallichiana* extract and fraction in SHR, DOCA-salt- and L-NAME-induced hypertensive rats

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ABSTRACT

Ethnopharmacological relevance: Ulmus wallichiana Planchon (Himalayan Elm), a traditional medicinal plant, used in fracture healing in folk tradition of Uttarakhand, Himalaya, India. It is also used as diuretic. *U. rhynchophylla*, native to China, known as Gou Teng in Chinese medicine, is used for hypertension (WHO). *U. macrocarpa* has antihypertensive and vasorelaxant activity. However, no detailed studies related to hypertension have been reported previously, so we have explored the antihypertensive activity of *U. wallichiana*.

Aim of the study: To investigate the pharmacological effect of ethanolic extract (EE) and butanolic fraction (BF) of *U. wallichiana* in hypertensive rats.

Materials and methods: SHR, DOCA-salt- and L-NAME-induced hypertension models were used. Treatment was performed by oral administration of EE and BF of *U. wallichiana* (500 mg/kg/day and 50 mg/kg/day) for 14 days. Then blood pressure was measured by non-invasive blood pressure (NIBP) measurement technique. Invasive blood pressure (IBP) was also reported to support the NIBP data. Concentrations of plasma renin, angiotensin II (Ang II), nitrate/nitrite (NO), cGMP were estimated. Angiotensin-converting enzyme (ACE) activity and ROS activity were also estimated.

Results: Blood pressure was significantly higher in SHR as compared to normotensive wistar group (170.59 ± 0.83 mmHg vs 121.54 ± 1.24 mmHg, respectively). SBP was increased in DOCA-salt induced group compared to their control (132.77 ± 3.90 mmHg vs 107.85 ± 5.95 mmHg, respectively) and L-NAME-induced group compared to their control (168.55 ± 5.07 mmHg vs 113.03 ± 4.13 mmHg, respectively). The treatment of extract and fraction of *U. wallichiana* significantly decreased the blood pressure in SHR+EE (151.26 ± 1.85 mmHg, $p < 0.001$), SHR+BF (140.44 ± 1.16 mmHg, $p < 0.001$); DOCA+EE (113.43 ± 5.44 mmHg, $p < 0.05$), DOCA+BF (105.09 ± 5.12 mmHg, $p < 0.05$) and L-NAME+EE (119.76 ± 4.39 mmHg, $p < 0.001$), L-NAME+BF (117.50 ± 7.27 mmHg, $p < 0.001$) compared to their respective diseased control groups. The plasma renin, Ang II and ACE activity were also significantly decreased and augmented the NO and cGMP levels. It also down regulated the expression of Renin, ACE, NOS3 and TGF-β1 at mRNA levels.

Conclusions: The EE and BF probably reducing the BP via Renin-angiotensin-aldosterone system and NO/cGMP signaling pathway. The decrease in blood pressure may be due to presence of quercetin analogue flavonoids (2S,3S)-(+)−3′,4′,5,7-tetrahydroxydihydroflavonol-6-C-β-D-glucopyranoside; 6-Glucopyranosyl-3,3′,4′,5,7-pentahydroxyflavone; 6-Glucopyranosyl-4′,5,7-trihydroxyflavanone and (2 S,3 S)-(+)−4′,5,7-trihydroxydihydroflavonol-6-C-β-D-glucopyranoside, may be due to its antioxidant activity. Thus EE and BF of *U. wallichiana* found to have the potential ability to be used as herbal medicament to treat hypertension.

Abbreviations: ACE, angiotensin converting enzyme; Ang II, angiotensin II; BF, butanolic fraction; BMD, bone mineral density; cGMP, cyclic guanosine monophosphate; DBP, diastolic blood pressure; DCFDA, 2′,7′-Dichlorodihydrofluorescein diacetate; DOCA, deoxycorticosterone acetate; EE, ethanolic extract; FAPGG, N-[3-(2-Furyl) acryloyl]-L-phenylalanyl-glycyl-glycine; GTDF, 6-C-β-D-glucopyranosyl-(2S,3S)-(+)−3′,4′,5,7-tetrahydroxyflavonol; HR, heart rate; L-NAME, Nω-Nitro-L-arginine methyl ester; L, litre; MBP, mean blood pressure; mRNA, messenger ribonucleic acid; NaCl, sodium chloride; NIBP, non-invasive blood pressure; NO, nitric oxide; NOS, nitric oxide synthase; p.o, per oral; qPCR, quantitative PCR; RAAS, Renin-angiotensin-aldosterone system; ROS, reactive oxygen species; SBP, systolic blood pressure; s.c., subcutaneous; SD rat, Sprague Dawley rat; SHR, spontaneously hypertensive rat

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1. Introduction

Hypertension is a common progressive disorder leading to numerous chronic diseases such as cardiovascular disease, stroke, renal disease, diabetes and accounts for about 50% of cardiovascular disease worldwide (Joffres et al., 2013; Kearney et al., 2005; Rao et al., 2012). It is estimated that nearly 1 billion people are affected by hypertension worldwide, and is predicted to increase to 1.5 billion by 2025 according to World Health Organization (WHO), reasons for more than seven million death yearly (Guilbert, 2003). In India, the cardiovascular disease accounts for 2.3 million deaths in the year 1990 and is predicted to double by 2020 (Gupta, 2004). As per 2002 world health report, hypertension is one of the most central cause among five non-communicable disease universally (Rao et al., 2012). Hypertension often is part of metabolic syndrome. The other components of metabolic syndrome are diabetes mellitus, hyperlipidemia, obesity and this co-occurrence will increase the risk of cardiovascular disease and cardiovascular events (Nava, 2013; Ratto et al., 2006).

Since antihypertensive drugs are extensively used for the treatment of hypertension and related cardiovascular diseases. Due to increased adverse side effects of these synthetic drugs, the preferred alternative therapy is herbal drugs, which have least side effects as compared to synthetic drugs available (Maurya et al., 2014).

Herbal medicines are very prevalent, being used in the developing countries for the primary health care (Khare, 2008). The natural herbal products are considered because of better cultural acceptability, safety, potent, inexpensive and lesser side effects, due to presence of antioxidants (Khare, 2008; Maurya et al., 2014). Medicinal plants have been reported to be useful in hypertension worldwide and have been used empirically as antihypertensive (Oh et al., 2008b; Prahalathan et al., 2012), antidiabetic (Galindo et al., 2012; Maurya et al., 2014) and antihyperlipidemic (Maurya et al., 2014; Sharan et al., 2010). Antihypertensive effects of plants are attributed to their ability to restore the function of RAAS pathway by causing a decrease in cardiac output. Further most of plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that are frequently implicated as having antihypertensive effect. Plants having antihypertensive effect include *Tropaeolum majus* (Junior et al., 2011), *Allium cepa* (Sakai et al., 2003), *Allium sativum* (Al-Qattan et al., 1999), *Zingiber officinale* (Akinyemi et al., 2013), *Terminalia superba (*Tom et al., 2011*)*, *Tribulus terrestris* (Phillips et al., 2006) and *Nigella sativa* (Dehkordi and Kamkhah, 2008). Flavonoids act as ACE inhibitors (Actis-Goretta et al., 2003; Guerrero et al., 2012; Loizzo et al., 2007) and increases NO/cGMP content providing vasorelaxant effect (Benito et al., 2002; Hernández-Abreu et al., 2009; Kuhlmann et al., 2005). Flavonoids are well known for its antihypertensive activity. The vasodilation action of flavonoids are involved in the mechanism of antihypertensive effect (Duarte et al., 1993). Flavonoids also causes decrease in plasma concentration of Ang II (vasoconstrictor) (Li et al., 2005).

1.1. Ulmus wallichiana planch

Himalayan Elm is a traditional Indian medicinal plant used in bone fracture (Bartunek et al., 2000). It is also used as astringent, demulcent, emollient, expectorant and diuretic (Khare, 2008). A related species *Ulmus rhynchophylla*, native to China, known as Gou Teng in Chinese medicine, is used for eclampsia, headache, dizziness, convulsions, high fever and hypertension (WHO). *Ulmus macrocarpa* has antihypertensive and vasorelaxant activity (Oh et al., 2008a). The EE and its BF contains known concentrations of flavonoids with known chemical structures. It contains quercetin analogue flavonoids (2S,3S)-(+)−3′,4′,5,7-tetrahydroxydihydroflavonol-6-C-β-D-glucopyranoside; 6-Glucopyranosyl-3,3′,4′,5,7-pentahydroxyflavone; 6-Glucopyranosyl-4′,5,7-trihydroxyflavanone and (2S,3S)-(+)−4′,5,7-trihydroxydihydroflavonol-6-C-β-D-glucopyranoside (Maurya et al., 2014; Rawat et al., 2009; Sharan et al., 2010). The plant bark also contains 0.76% tannins (Khare, 2008), β-sitosterol, Ursolic acid and lupeol (Arya et al., 2013). Quercetin (a polyphenolic flavonoid) and its major metabolites modulate NO/cGMP dependent vasorelaxation and regulates blood pressure (Suri et al., 2010). *U. wallichiana* contains flavonoids which are potent for anabolic effect on osteoporotic bone and Quercetin analogue GTDF has potent osteoanabolic compound that induced proliferation, differentiation of cultured primary osteoblasts (Sharan et al., 2011) and lower blood glucose level in diabetic rats (Rawat et al., 2011). Quercetin analogues from *U. wallichiana* stimulates osteoblast growth and differentiation (Sharan et al., 2011). In growing rats, *U. wallichiana* extract increased bone strength, BMD and enhanced rate of bone formation (Sharan et al., 2010). In streptozotocin induced diabetic rats, phenolic C-glycosides isolated from *U. wallichiana* showed low blood glucose level (Rawat et al., 2011).

In this present work, we have investigated the antihypertensive activity of EE and BF of *U. wallichiana,* a novel natural analogue of the dietary flavonoid quercetin, or other naturally occurring quercetin analogs. Further we have reported the detailed characterization of EE and BF of *U. wallichiana* as antihypertensive in different hypertensive rat models.

2. Materials and methods

2.1. Chemicals and reagents

Deoxycorticosterone acetate, Nω-Nitro-L-arginine methyl ester hydrochloride, Olive oil, Gum arabic, N-[3-(2-Furyl) acryloyl]-L-phenylalanyl-glycyl-glycine (FAPGG), 2′, 7′-Dichlorofluorescein diacetate and TRIzol reagent were procured from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. Sodium Chloride was procured from Merck Specialities Private Limited, Mumbai, India. Tris (Hydroxymethyl) Aminomethane was procured from SRL Pvt. Ltd., Mumbai, India. High capacity RNA to cDNA Reverse Transcriptase kit was procured from Applied Biosystems, USA. 2X SYBR green Premix Ex Taq (Takara Bio, Japan), List of primers for Real-Time (RT) qPCR (Eurofins Scientific, Luxembourg) as shown in Supplementary Table 1, Renin ELISA kit (Shanghai, China), Human/Mouse/Rat Angiotensin II Enzyme Immunoassay kit (RayBiotech, Inc.), Nitrate/Nitrite Colorimetric Assay Kit (Cayman Chemical, Michigan, USA), cGMP assay kit (Cayman Chemical, Michigan, USA) were purchased. EE and BF of *U. wallichiana* were obtained from Medicinal and Process Chemistry division of CSIR-Central Drug Research Institute (CSIR-CDRI), Lucknow.

2.2. Plant materials

The stem bark of *Ulmus wallichiana* was collected from Uttarakhand Himalaya, Nainital (1800M). Samples were identified by the Scientists of Botany Division, CSIR-CDRI, Govt. of India, Lucknow and compared with flora of The District Garhwal, North West Himalaya. Herbal specimen were deposited in the Herbarium of Botany Division, CSIR-CDRI, Lucknow, vide voucher specimen number KRA 24432.

2.3. Extraction and fractionation

The extraction from the powdered stem bark (EE and BF of *U.*