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Diosmin, a bioflavonoid reverses alterations in blood pressure, nitric oxide, lipid peroxides and antioxidant status in DOCA-salt induced hypertensive rats

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ABSTRACT

The present study was aimed to evaluate the antihypertensive effect of diosmin in deoxycorticosterone acetate (DOCA)-salt induced hypertension in male Wistar rats. Hypertension was induced in uninephrectomized rats by weekly twice subcutaneous injection of DOCA (25 mg/kg body weight) and 1% NaCl in the drinking water for six consecutive weeks. The important pathological events that occurred in DOCA-salt treated rats were significant increase in systolic, diastolic blood pressure, sodium and chloride in serum and lipid peroxidation products (thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes) in plasma and tissues (liver, kidney, heart and aorta) and significant decrease in serum potassium, total nitrite and nitrate levels in plasma. The activities of hepatic aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and gamma-glutamyl transpeptidase and the levels of renal urea, uric acid, creatinine in serum, water intake, and organ weight (kidney and heart) were significantly increased in DOCA-salt hypertensive rats. DOCA-salt treated rats also showed a significant decrease in body weight, activities of superoxide dismutase, catalase and glutathione peroxidase in erythrocyte and tissues and the levels of reduced glutathione, vitamin C and vitamin E in plasma and tissues. Treatment with diosmin (25, 50 and 100 mg/kg body weight) brings back all the above parameters to near normal level, in which 50 mg/kg body weight showed the highest effect than that of other two doses. Histopathology of heart and kidney also confirmed the protective effect of diosmin. Thus the experiment clearly showed that diosmin acts as an antihypertensive agent against DOCA-salt induced hypertension.

1. Introduction

Cardiovascular disease accounts for considerable mortality and morbidity in developed countries. Most of the common forms of cardiovascular disease, such as atherosclerosis and hypertension, are caused by functional and structural changes in the blood vessel wall (Luscher, 1994). Hypertension affects approximately 25% of the adult population worldwide, and its prevalence is predicted to increase by 60% by 2025 (Kearney et al., 2005). Various genetic and environmental factors are known to be involved in the pathogenesis of primary hypertension, among which excess sodium intake has long been regarded as the pivotal environmental factor for this disorder (Adrogue and Madias, 2007).

In animal models, such as the deoxycorticosterone acetate (DOCA)-salt rat, hypertension develops as a result of increased concentrations of aldosterone leading to increased reabsorption of sodium ions and water in the distal nephron of the kidney, thereby influencing blood pressure levels (Tomaschitz et al., 2010). Excessive production of reactive oxygen species is hallmark of cardiovascular diseases, including hypertension. Mineralocorticoid induced hypertension is associated with increased oxidative stress which is caused by increased NADPH oxidase and is responsible for increased superoxide production and possibly contributes to the increased blood pressure in the DOCA-salt hypertensive rat (Beswick et al., 2001). Recent studies have demonstrated that high blood pressure is accompanied by oxidative stress and impaired renal function in salt-sensitive hypertension (Seifi et al., 2010).

Citrus juices are among the richest dietary sources of flavonoids (Benavente-Garcia and Castillo, 2008). The lemon has many important natural chemical components, including citric acid, ascorbic acid, minerals and flavonoids (Elangovan et al., 1994). Flavonoids are a group of plant polyphenols that are generally found in vegetables, fruits, herbs, tea, and wine as secondary metabolites and have received much attention due to their anti-inflammatory and antioxidant activities (Beecher, 2003).

Diosmin is a flavonoid found in citrus fruits and its structure (3′,5,7-trihydroxy-4′-methoxyflavone-7-ramnoglucoside) was shown in Fig. 1. As a flavonoid, it possesses a multitude of biological activities including antihyperglycemic (Smith, 1999), anti-lipid peroxidation (Berqvist et al., 1981), anti-inflammatory, antioxidant, and antimutagenic properties (Camarda et al., 2007). No sufficient work has been done to study its antihypertensive activity. Therefore present study

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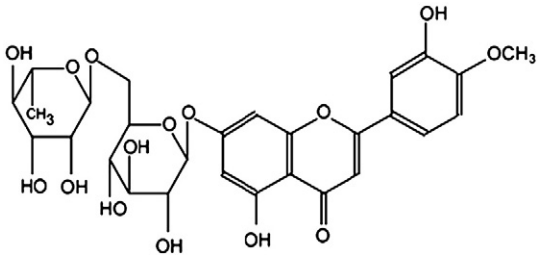

Fig. 1. Structure of diosmin (3′,5,7-trihydroxy-4′-methoxyflavone-7-ramnoglucoside).

was designed to determine the dose-dependent effect of chronic administration of diosmin on DOCA-salt induced hypertension in albino Wistar rats.

2. Materials and methods

2.1. Animals

Male albino Wistar rats (10–12 week old) were obtained from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, Tamil Nadu, India. They were housed in polypropylene cages (47×34×20 cm) lined with husk, renewed every 24 h under a 12:12 h light/dark cycle at around 22 °C and had free access to tap water and food. The rats were fed on a standard pellet diet (Kamadhenu Agencies, Bangalore, India). The whole experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India and approved by the Animal Ethical Committee of Annamalai University (Reg. no: 160/1999/CPCSEA, Approval no: 680).

2.2. Chemicals

Diosmin, deoxycorticosterone acetate (DOCA) and dimethyl formamide (DMF) were purchased from Sigma-Aldrich Chemical Company, St. Louis, Missouri, USA. All other chemicals used in this study were of highest analytical grade obtained from Sisco Research Laboratories and Himedia, Mumbai, India.

2.3. Experimental induction of hypertension in rats

Left uninephrectomy was performed on all rats. Rats were anesthetized with intraperitonial injection of ketamine (75 mg/kg body weight), kidney was visualized by a left lateral abdominal incision, and the left renal artery and ureter were ligated by silk thread, and then the left kidney was removed and weighed. The muscle and skin layer (incision site) were sutured with highly sterile suture needles.

Uninephrectomized rats were given 1% NaCl in the drinking water with weekly twice subcutaneous injection of DOCA [(25 mg/kg body weight) in 0.4 mL of dimethyl formamide (vehicle) with mild heating (Fenning et al., 2005)] for six consecutive weeks (DOCA-salt hypertensive rats).

2.4. Experimental design

The rats were randomly divided into six groups each comprising ten rats. 25, 50 and 100 mg/kg of diosmin were dissolved in vehicle solution of 0.5% dimethylsulfoxide and administered to rats orally using an intragastric tube daily for a period of six consecutive weeks.

Group I — Uninephrectomized control
Group II — Uninephrectomized control + diosmin (100 mg/kg body weight)
Group III — DOCA-salt control (25 mg/kg body weight)
Group IV — DOCA-salt + diosmin (25 mg/kg body weight)
Group V — DOCA-salt + diosmin (50 mg/kg body weight)
Group VI — DOCA-salt + diosmin (100 mg/kg body weight)

Uninephrectomized control and DOCA-salt control rats were also received 0.5% dimethylsulphoxide. At the end of 6th week, all the rats were anesthetized with intramuscular injection of ketamine and sacrificed by cervical dislocation. Blood was collected from orbital sinus with great care using a dry test tube and allowed to coagulate at ambient temperature for 40 min. Serum was separated by centrifugation at 224 ×*g* for 10 min. The blood, collected in a heparinized centrifuge tube was centrifuged at 224 ×*g* for 10 min and the plasma was separated by aspiration. After the separation of plasma, the buffy coat, enriched in white cells, was removed and the remaining erythrocytes were washed three times with physiological saline. Erythrocytes were lysed with hypotonic phosphate buffer at pH 7.4. The hemolysate was separated by centrifugation at 350 ×*g* for 10 min and 0.5 mL of supernatant was used for the estimation of enzymatic antioxidants. 250 mg of heart, liver, and kidney and 90 mg of aorta tissues were sliced into pieces and homogenized in appropriate buffer in cold condition (pH 7.0) to give 20% homogenate (w/v). The homogenate was centrifuged at 56 ×*g* for 10 min at 0 °C in refrigerated centrifuge. The supernatant was separated and used for various biochemical estimations.

2.5. Measurement of blood pressure

Before commencement of the experiment, animals were trained with instrument for measuring blood pressure. Systolic and diastolic blood pressures were recorded every week during the entire period of the study by tail-cuff method (IITC, model 31, Woodland Hills, CA, USA). The animals were placed in heated chamber at an ambient temperature of 30–34 °C for 15 min and from each animal; 1–9 blood pressure values were recorded. The lowest three readings averaged to obtain a mean blood pressure. All the recordings and data analyses were done using a computerized data acquisition system and software (IITC Inc./Life Science Instruments, USA).

2.6. Biochemical estimations

The electrolytes such as Na^+, K^+ and Cl^- were analyzed by AVL 9180 Electrolyte analyzer (ROCHE-USSR). Methodology is based on the ion-selective electrode measurement principle to precisely determine the measurement values (Burtis and Ashwood, 1994). Nitrite and nitrate [stable nitric oxide metabolites] in the plasma samples were measured based on the Griess reaction (Green et al., 1982). The levels of thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes in plasma and tissues (liver, kidney, heart and aorta) were estimated by the method of Niehaus and Samuelsson (1968), Jiang et al. (1992) and Rao and Recknagel (1968), respectively. The activities of enzymatic antioxidants superoxide dismutase, catalase and glutathione peroxidase were estimated by the method of Kakkar et al. (1984), Sinha (1972) and Rotruck et al. (1973), respectively. The non-enzymatic antioxidants reduced glutathione, vitamin C and vitamin E and were estimated by the method of Ellman (1959), Roe and Kuether (1943) and Baker et al. (1980), respectively. The activities of serum aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase and the level of total proteins were estimated by using commercially available kit (Fisher scientific, Kerala). The activity of gamma glutamyl transferase was measured by the method of Rosalki and Rau (1972). The serum urea, uric acid and creatinine were estimated by using the diagnostic kit based on the method of Fawcett and Scott (1960), Caraway (1955) and Jaffe (1886), respectively.