Cardiovascular Pharmacology

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

Thangarasu Silambarasan, Boobalan Raja *

Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India

A R T I C L E   I N F O

Article history:
Received 30 June 2011
Received in revised form 21 December 2011
Accepted 28 December 2011
Available online 12 January 2012

Keywords:
Diosmin
Antioxidant
DOCA
Nitric oxide

A B S T R A C T

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.

© 2012 Elsevier B.V. All rights reserved.

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 (Adroguie 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, antioxidant and antimutagenic properties (Camarda et al., 2007). No sufficient work has been done to study its antihypertensive activity. Therefore present study...
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 intraperitoneal injection of ketamine (75 mg/kg body weight), kidney was visualized by 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% dimethylsulfoxide. 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 (Burris 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 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 (1986), respectively.
2.7. Histopathological examination

Excised heart and kidney samples were cleared of blood and immediately fixed in a neutral buffered solution of 10% formalin for 24 h. 5 μm thick tissue sections from heart and kidney of each animal were prepared from processed paraffin-embedded samples. Sections were stained with Hematoxylin and Eosin for light microscopic examination for evidence of hypertensive tissues changes. The cross-sectional area of heart was evaluated from photographs of whole tissue sections taken at 40× magnification and scanned, digitized and analyzed by computer, using the Adobe Photoshop Imaging program (Adobe System Incorporation).

2.8. Statistical analysis

Statistical analysis was performed by one-way analysis of variance followed by Duncan’s multiple range test using statistical package for the social science (SPSS) software version 11.5. Results were expressed as mean ± S.D. for six rats in each group. A value of P < 0.05 was considered to be statistically significant.

3. Results

3.1. Effects of diosmin on blood pressure, body weight and water intake

Figs. 2–5 show the effects of diosmin at three different concentrations (25, 50 and 100 mg/kg) on systolic, diastolic blood pressure, body weight and water intake, respectively in uninephrectomized control and DOCA-salt hypertensive rats for 6 weeks. The blood pressure and water intake increased and body weight decreased significantly (P < 0.05) in DOCA-salt hypertensive rats. Treatment with diosmin (25, 50 and 100 mg/kg) lowered the systolic, diastolic blood pressure, and water intake and elevated body weight significantly (P < 0.05).

3.2. Effects of diosmin on organ weight and electrolytes

Table 1 shows the effects of diosmin on the weight of kidney, heart, and levels of serum electrolytes such as sodium, potassium and chloride in uninephrectomized control and DOCA-salt hypertensive rats. DOCA-salt rats had significantly (P < 0.05) increased kidney, heart weight and sodium, chloride levels and decreased levels of potassium. Treatment with diosmin at the doses of (25, 50 and 100 mg/kg) significantly (P < 0.05) brought back these values toward near to normal.

3.3. Effects of diosmin on nitric oxide metabolites

Fig. 6 depicts the levels of nitric oxide metabolites (nitrite and nitrate) in plasma of uninephrectomized control and DOCA-salt hypertensive rats. DOCA-salt hypertensive rats had significantly (P < 0.05) decreased levels of total nitrate and nitrate in plasma and treatment with diosmin significantly (P < 0.05) elevated the levels of nitric oxide metabolites with a maximum effect at 50 mg/kg.

3.4. Effects of diosmin on lipid peroxides

Table 2 portrays the levels of thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes, respectively, in the plasma and tissues (liver, kidney, heart and aorta) of uninephrectomized control and DOCA-salt hypertensive rats. DOCA-salt hypertensive rats had significantly (P < 0.05) elevated levels of lipid peroxidation products in the plasma and tissues. Oral administration of diosmin at the doses of 25, 50 and 100 mg/kg significantly (P < 0.05) reduced the levels of lipid peroxidation products in DOCA-salt hypertensive rats.

3.5. Effects of diosmin on enzymatic antioxidants

The activities of superoxide dismutase, catalase and glutathione peroxidase in erythrocyte and tissues (liver, kidney, heart and aorta) of uninephrectomized control and DOCA-salt hypertensive rats are presented in Table 3. The activities of these enzymatic antioxidants were significantly (P < 0.05) decreased in DOCA-salt hypertensive rats. Treatment with diosmin significantly (P < 0.05) restored the activity of these enzymatic antioxidants in erythrocyte and tissues with a maximum effect at 50 mg/kg.

3.6. Effects of diosmin on non-enzymatic antioxidants

Table 4 illustrates the levels of non-enzymatic antioxidants such as vitamin C, vitamin E and glutathione in the plasma and tissues (liver, kidney, heart and aorta) of uninephrectomized control and DOCA-salt hypertensive rats. The levels of non-enzymatic antioxidants were significantly (P < 0.05) decreased in DOCA-salt hypertensive rats. Oral administration of diosmin significantly (P < 0.05) improved these parameters toward normalcy with a maximum effect at 50 mg/kg.
3.7. Effects of diosmin on hepatic and renal markers

Table 5 summarizes the effects of diosmin on the activities of hepatic marker enzymes such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma glutamyl transferase, and levels of renal function marker such as urea, uric acid and creatinine in serum of uninephrectomized control and DOCA-salt hypertensive rats. The activities of these pathophysiological marker enzymes were significantly (P < 0.05) elevated in DOCA-salt hypertensive rats. Treatment with diosmin (25, 50 and 100 mg/kg) significantly (P < 0.05) declined these levels toward normal.

3.8. Effects of diosmin on histopathology of kidney

Fig. 7(A–F) shows the effects of diosmin on the histology of kidney in uninephrectomized control and DOCA-salt hypertensive rats. Uninephrectomized control rats showed normal glomerulus without any congestion (Fig. 7A). Uninephrectomized control rats treated with diosmin (100 mg/kg) showed normal glomerulus without any damage (Fig. 7B). However DOCA-salt hypertensive rats showed atrophy of tubular cells with dilated lumen of distal convoluted tubules and congestion of glomerulus (Fig. 7C). Diosmin (25, 50 and 100 mg/kg) treated DOCA-salt rats showed reduced congestion of glomerulus and focal tubular necrosis (Fig. 7D–F).

3.9. Effects of diosmin on histopathology of heart

Fig. 8(A–F) shows the effects of diosmin on the histology of heart in uninephrectomized control and DOCA-salt hypertensive rats. Uninephrectomized control rats showed normal histology of cardiac tissue (Fig. 8A). Uninephrectomized control rats treated with diosmin (100 mg/kg) showed normal cardiac myocytes (Fig. 8B). DOCA-salt hypertensive rats showed distorted cardiac myofibril arrangement and thickened arteriole with hypertrophy (Fig. 8C). Diosmin (25, 50 and 100 mg/kg) treated DOCA-salt rats showed reduced distortion of cardiac myofibril arrangement and hypertrophy (Fig. 8D–F). Diosmin treatment (50 mg/kg) to DOCA-salt hypertensive rats showed the highest effect on all the biochemical parameters when compared to other two doses (25 and 100 mg/kg). However treatment with diosmin (100 mg/kg) to uninephrectomized control rats did not show any significant effect. Diosmin at a dosage of 50 mg/kg also showed better protective effect on the histology of kidney and heart.
4. Discussion

4.1. Blood pressure

Several studies found that the mechanisms by which salt increases blood pressure may result from an alteration in renin–angiotensin and nitric oxide levels, increased oxidative stress and damage to kidneys (Bayorh et al., 2004). In the present study, there is a significant increase in systolic and diastolic blood pressure of DOCA-salt hypertensive rats. In this respect, Li et al. (2003) reported that endothelin-1 enhances vascular superoxide production through NADPH oxidase pathway in DOCA-salt hypertensive rats. Thus, increased superoxide production has been proposed to be an important factor contributing to endothelial dysfunction, tissue damage and hypertension (Hamilton et al., 2001).

Flavonoids and triterpine were recently shown to reduce hypertension in experimental animal models (Jalili et al., 2006). It is evident that diosmin supplementation significantly decreased blood pressure in DOCA-salt treated groups may be due to its antioxidant nature which hinders the reactive oxygen species produced by DOCA treatment.

4.2. Body weight, water intake and organ weight

After DOCA-salt treatment, elevated blood pressure in rats was associated with a significant loss of body weight (Hawakawa et al., 1994). After treatment with diosmin, the weight loss improved which might be as a result of its ability to reduce the loss or degradation of structural proteins (Varshavsky, 1997). DOCA-salt rats also showed significantly increased water intake, kidney and heart weight as reported earlier (Chan et al., 2006). On treatment with diosmin reduced the water intake, renal and cardiac hypertrophy that might be due to the blood pressure lowering effect of the diosmin.

4.3. Electrolytes

When renal function is reduced, extracellular volume increases that could increase blood pressure (Ziomber et al., 2008). In our study DOCA-salt hypertensive rats showed increased serum sodium and chloride and decreased potassium levels when compared with control rats. Our results are in line with previous report stating that intracellular sodium overload and potassium depletion may be important in the pathophysiology of hypertension (Leiba et al., 2005). The reduction of sodium and chloride and elevation of potassium in our study may be due to beneficial effect of diosmin in DOCA-salt hypertensive rats.

4.4. Nitric oxide metabolites

The main vaso-relaxing factor produced by endothelial cells is nitric oxide. It is synthesized from l-arginine by the action of endothelial nitric oxide synthase and is important for the regulation of blood pressure (Moncada and Higgs, 1993). Nitric oxide, a free radical is highly unstable and gets converted into an equimolar ratio of its stable metabolites nitrite and nitrate. According to our results plasma total nitrite and nitrate concentration in DOCA-salt rats was lower than control may be due to various mechanisms including decreased nitric oxide synthesis and increased nitric oxide degradation caused by oxidative stress (Schlachl et al., 2007). On the other hand treatment with diosmin elevated plasma nitrite and nitrate levels clearly indicates its ability to protect nitric oxide from free radicals thereby increasing the availability.

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organ weight</td>
<td></td>
<td>Kidney weight (g)</td>
<td>0.845 ± 0.025&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.844 ± 0.023&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.955 ± 0.065&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.614 ± 0.045&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.871 ± 0.021&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heart weight (g)</td>
<td>0.819 ± 0.017&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.817 ± 0.017&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.149 ± 0.048&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.065 ± 0.043&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.851 ± 0.030&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Electrolytes (mEq/L)</td>
<td></td>
<td>Sodium</td>
<td>143.61 ± 7.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>141.23 ± 7.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>179.15 ± 10.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>171.26 ± 9.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>146.51 ± 7.72&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Potassium</td>
<td>6.91 ± 0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.98 ± 0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.09 ± 0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.1 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.45 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chloride</td>
<td>106.21 ± 7.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>104.11 ± 6.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>132.95 ± 9.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>124.32 ± 7.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>108.34 ± 5.81&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Group I – uninephrectomized control; Group II – uninephrectomized control + diosmin (100 mg/kg); Group III – DOCA-salt control (25 mg/kg); Group IV – DOCA-salt + diosmin (25 mg/kg); Group V – DOCA-salt + diosmin (50 mg/kg); Group VI – DOCA-salt + diosmin (100 mg/kg).

Values are means ± S.D. for six rats. Values not sharing a common superscript differ significantly at P<0.05 (Duncan’s multiple range test).
4.5. Lipid peroxides

Lipid peroxidation, arising from the reaction of free radicals with lipids, has been linked with altered membrane structure and enzyme inactivation. Its end products measured as thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes were seen increased in plasma and tissues clearly indicating increased oxidative stress in DOCA-salt rats and it has been previously reported (Nicod et al., 2000). A possible explanation for the enhancement of lipid peroxidation products in DOCA-salt hypertensive rats is due to the increased free radical production and decreased antioxidant system. Treatment with diosmin decreased the levels of lipid peroxidation products in DOCA-salt hypertensive rats. Thus, diosmin inhibits lipid peroxidation may be due to scavenging of free radicals and is attributed to its radical scavenging property (Campanero et al., 2010).

4.6. Enzymatic antioxidants

Free radical scavenging enzymes such as superoxide dismutase, catalase, and glutathione peroxidase are the first line of cellular defense against oxidative injury. Superoxide dismutase reduces superoxide anion to form H2O2 and oxygen. Catalase removes H2O2 by breaking it down directly to oxygen (Frank and Massaro, 1980). The observed declined activities of superoxide dismutase, catalase in DOCA-salt rats may be due to the involvement of free radicals which is consistent with a previous study (Nicod et al., 2000). A decrease in activity of these enzymes leads to accumulation of superoxide anion and H2O2 which in turn can form the toxic hydroxyl radical. Our results show that diosmin prevented the decrease in the activities of enzymic antioxidants in DOCA-salt hypertensive rats.

The selenium-containing enzyme glutathione peroxidase detoxifies H2O2 by utilizing reduced glutathione and H2O2 as substrates to yield H2O and oxidized glutathione. The decreased activity of these enzymes in DOCA-salt treated rats is due to the decreased concentration of their substrate, glutathione. The observed decrease in glutathione content might be due to increased utilization in protecting proteins from peroxidative damage by scavenging peroxides and other lipid derived oxidants. Oral treatment with diosmin increases the concentration of glutathione and the activity of glutathione peroxidase in DOCA-salt hypertensive rats. This effect clearly exposed the antioxidant nature of diosmin (Campanero et al., 2010).

4.7. Non-enzymatic antioxidants

The second line of defense consists of non-enzymatic antioxidants namely, vitamin C, vitamin E, and reduced glutathione which scavenge the residual free radicals escaping from decomposition by the antioxidant enzymes (Seifi et al., 2010). The major antioxidant of the aqueous phase is vitamin C, which acts as the first line of defense during oxidative stress. Vitamin E appears to be the most effective lipid soluble antioxidant in the biological system. Glutathione plays a marked role in detoxification reaction because it is a direct radical scavenger (Kitts et al., 1998). Our results depict that the levels of non-enzymatic antioxidants that were decreased in DOCA-salt rats might be due to their increased utilization for the neutralization of free radicals and lipid peroxidation (Newaz and Nawal, 1999). Treatment with diosmin enhanced the levels of these antioxidants and suggests that this compound might be potentially useful in counteracting free-radical mediated oxidative stress caused by lipid peroxidation.

4.8. Hepatic marker enzymes

Liver plays a fundamental role in metabolism, toxicity and elimination of endogenous and exogenous components (Kitts et al., 1998). Assessment of liver function can be made by estimating the activities of serum hepatic marker enzymes. In our study the levels

Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiobarbituric acid reactive substances (mmol/100 g wet tissue)</td>
<td>Plasma (mmol/dL)</td>
<td>0.19 ± 0.03a</td>
<td>0.18 ± 0.02a</td>
<td>0.47 ± 0.04a</td>
<td>0.39 ± 0.01b</td>
<td>0.21 ± 0.05b</td>
<td>0.34 ± 0.02c</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>0.88 ± 0.11a</td>
<td>0.83 ± 0.13b</td>
<td>2.4 ± 0.13c</td>
<td>1.98 ± 0.28d</td>
<td>0.98 ± 0.15e</td>
<td>1.73 ± 0.24f</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>1.45 ± 0.12a</td>
<td>1.43 ± 0.15a</td>
<td>4.02 ± 0.24b</td>
<td>3.15 ± 0.21c</td>
<td>1.65 ± 0.31d</td>
<td>1.65 ± 0.20e</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>0.58 ± 0.11a</td>
<td>0.53 ± 0.08b</td>
<td>3.05 ± 0.20b</td>
<td>2.28 ± 0.11c</td>
<td>0.75 ± 0.13d</td>
<td>1.85 ± 0.18e</td>
</tr>
<tr>
<td></td>
<td>Aorta</td>
<td>0.6 ± 0.09a</td>
<td>0.52 ± 0.07b</td>
<td>2.45 ± 0.18b</td>
<td>1.8 ± 0.23c</td>
<td>0.83 ± 0.12d</td>
<td>1.55 ± 0.12e</td>
</tr>
<tr>
<td>Lipid hydroperoxides (mmol/100 g wet tissue)</td>
<td>Plasma (mmol/dL)</td>
<td>9.58 ± 0.35a</td>
<td>9.04 ± 0.53b</td>
<td>22.3 ± 0.49b</td>
<td>18.03 ± 1.21c</td>
<td>12.26 ± 0.36a</td>
<td>16.25 ± 0.86d</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>81.54 ± 5.72a</td>
<td>80.35 ± 5.41a</td>
<td>104.16 ± 4.74a</td>
<td>91.45 ± 2.68a</td>
<td>85.71 ± 3.19a</td>
<td>89.28 ± 3.19a</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>68.45 ± 7.63a</td>
<td>67.26 ± 5.25a</td>
<td>162.5 ± 22.44a</td>
<td>131.54 ± 8.27a</td>
<td>72.61 ± 5.83a</td>
<td>103.57 ± 4.51a</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>72.02 ± 4.17a</td>
<td>71.42 ± 5.05a</td>
<td>138.69 ± 8.27a</td>
<td>107.14 ± 7.49a</td>
<td>76.19 ± 4.87a</td>
<td>97.61 ± 13.10a</td>
</tr>
<tr>
<td></td>
<td>Aorta</td>
<td>74.40 ± 5.72a</td>
<td>72.61 ± 4.32a</td>
<td>122.61 ± 14.04a</td>
<td>90.45 ± 6.55a</td>
<td>77.97 ± 6.93a</td>
<td>94.64 ± 8.67a</td>
</tr>
<tr>
<td>Conjugated dienes (mmol/100 g wet tissue)</td>
<td>Plasma (mmol/dL)</td>
<td>0.75 ± 0.13a</td>
<td>0.72 ± 0.08a</td>
<td>2.21 ± 0.56a</td>
<td>1.81 ± 0.29a</td>
<td>0.92 ± 0.03c</td>
<td>1.53 ± 0.30d</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>68.72 ± 10.60a</td>
<td>67.13 ± 13.53a</td>
<td>130.87 ± 13.47a</td>
<td>104.07 ± 7.40a</td>
<td>73.45 ± 5.92a</td>
<td>93.5 ± 10.17d</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>20.32 ± 2.56a</td>
<td>19.15 ± 3.10b</td>
<td>40.37 ± 6.64b</td>
<td>32.11 ± 9.02b</td>
<td>24.21 ± 6.83c</td>
<td>29.9 ± 4.04d</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>44.67 ± 7.45a</td>
<td>42.2 ± 4.61b</td>
<td>81.47 ± 9.26b</td>
<td>67.57 ± 6.18b</td>
<td>48.60 ± 9.21c</td>
<td>60.2 ± 10.17d</td>
</tr>
<tr>
<td></td>
<td>Aorta</td>
<td>42.1 ± 5.79a</td>
<td>40.65 ± 3.22b</td>
<td>78.36 ± 5.53c</td>
<td>65.22 ± 4.89d</td>
<td>45.91 ± 10.88c</td>
<td>58.8 ± 8.51d</td>
</tr>
</tbody>
</table>

Group I – uninephrectomized control; Group II – uninephrectomized control + diosmin (100 mg/kg); Group III – DOCA-salt control (25 mg/kg); Group IV – DOCA-salt + diosmin (25 mg/kg); Group V – DOCA-salt + diosmin (50 mg/kg); Group VI – DOCA-salt + diosmin (100 mg/kg).

Values are means ± S.D. for six rats. Values not sharing a common superscript differ significantly at P < 0.05 (Duncan’s multiple range test).
Effects of diosmin on hepatic marker enzymes and renal markers in serum of uninephrectomized control and DOCA-salt hypertensive rats.

Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate aminotransferase (IU*/mg protein)</td>
<td>Erythrocyte (IU*/mg Hb)</td>
<td>7.51±0.55*</td>
<td>7.64±0.69*</td>
<td>3.09±0.36b</td>
<td>3.94±0.52b</td>
<td>6.95±0.84b</td>
<td>4.33±1.20b</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>8.12±1.19</td>
<td>8.58±0.94</td>
<td>4.21±0.34a</td>
<td>4.47±0.41a</td>
<td>7.04±0.82a</td>
<td>5.65±0.44a</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>14.24±1.87*</td>
<td>14.70±1.86*</td>
<td>7.23±0.51b</td>
<td>8.87±0.73b</td>
<td>13.05±2.10b</td>
<td>10.30±2.20b</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>5.29±0.37*</td>
<td>5.42±0.49*</td>
<td>2.98±0.25c</td>
<td>3.15±0.23c</td>
<td>4.82±0.47c</td>
<td>3.71±0.27c</td>
</tr>
<tr>
<td></td>
<td>Aorta</td>
<td>5.06±0.55*</td>
<td>5.22±0.49*</td>
<td>2.79±0.35b</td>
<td>2.95±0.32b</td>
<td>4.66±0.56b</td>
<td>3.50±0.37b</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td></td>
<td>7.19±0.46*</td>
<td>7.16±0.70*</td>
<td>3.77±0.31b</td>
<td>3.76±0.34b</td>
<td>6.83±0.68b</td>
<td>5.13±0.62b</td>
</tr>
</tbody>
</table>

Diosmin controlled the activity of these enzymes. It shows that diosmin, to an extent, prevents the functional capacity of the liver owing to its antioxidant capacity (Campanero et al., 2010).

Table 4

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C (μg/mg protein)</td>
<td>Plasma (mg/dL)</td>
<td>2.12±0.13*</td>
<td>2.38±0.19*</td>
<td>0.92±0.08b</td>
<td>1.17±0.10b</td>
<td>2.02±0.15b</td>
<td>1.35±0.05b</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>0.83±0.07b</td>
<td>0.85±0.07b</td>
<td>0.48±0.04b</td>
<td>0.53±0.06b</td>
<td>0.79±0.08b</td>
<td>0.61±0.04b</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>0.72±0.06b</td>
<td>0.74±0.04b</td>
<td>0.35±0.03b</td>
<td>0.43±0.03b</td>
<td>0.69±0.05b</td>
<td>0.52±0.03b</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>0.54±0.05b</td>
<td>0.57±0.08b</td>
<td>0.27±0.01b</td>
<td>0.33±0.02b</td>
<td>0.50±0.04b</td>
<td>0.38±0.02b</td>
</tr>
<tr>
<td></td>
<td>Aorta</td>
<td>0.52±0.04b</td>
<td>0.54±0.05b</td>
<td>0.28±0.01b</td>
<td>0.32±0.02b</td>
<td>0.51±0.03b</td>
<td>0.40±0.02b</td>
</tr>
<tr>
<td>Vitamin E (μg/mg protein)</td>
<td>Plasma (mg/dL)</td>
<td>1.96±0.17b</td>
<td>2.04±0.17*</td>
<td>0.93±0.09b</td>
<td>1.19±0.11b</td>
<td>1.85±0.11b</td>
<td>1.37±0.16b</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>6.19±0.44b</td>
<td>6.28±0.42b</td>
<td>3.28±0.21b</td>
<td>3.83±0.23b</td>
<td>5.88±0.36b</td>
<td>4.45±0.29b</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>4.13±0.27*</td>
<td>4.28±0.33*</td>
<td>1.61±0.11b</td>
<td>2.06±0.19b</td>
<td>3.88±0.23b</td>
<td>2.69±0.20b</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>4.12±0.36*</td>
<td>4.21±0.36*</td>
<td>1.84±0.15b</td>
<td>1.96±0.17b</td>
<td>3.86±0.29b</td>
<td>2.44±0.14b</td>
</tr>
<tr>
<td></td>
<td>Aorta</td>
<td>4.00±0.30*</td>
<td>4.11±0.32b</td>
<td>1.59±0.14b</td>
<td>1.90±0.13b</td>
<td>3.80±0.27b</td>
<td>2.46±0.12b</td>
</tr>
<tr>
<td>Glutathione (μg/mg protein)</td>
<td>Plasma (mg/dL)</td>
<td>33.60±1.47b</td>
<td>35.02±2.56*</td>
<td>19.02±1.05b</td>
<td>22.22±1.84b</td>
<td>31.47±1.47b</td>
<td>26.13±0.89b</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>13.04±0.97a</td>
<td>13.98±1.15b</td>
<td>6.22±0.59b</td>
<td>7.45±0.59b</td>
<td>11.45±1.26b</td>
<td>8.99±0.65b</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>10.49±1.03b</td>
<td>11.28±1.03b</td>
<td>4.54±0.36b</td>
<td>5.69±0.37b</td>
<td>9.28±0.70b</td>
<td>6.59±0.51b</td>
</tr>
</tbody>
</table>

Assay of hepatic marker enzymes increased in DOCA-salt rats as compared to control rats. The reason behind this elevation may be due to the necrotic and oxidative action of liver tissues which cause leakage of these enzymes from hepatocytes as a result of membrane damage (Bhattacharjee et al., 2007). Treatment with diosmin controlled the activity of these enzymes. It shows that diosmin, to an extent, prevents the functional capacity of the liver owing to its antioxidant capacity (Campanero et al., 2010).
4.9 Renal markers

The hypertensive rats induce elevation of the serum levels of urea, uric acid and creatinine which are considered as significant markers of renal function (Kang et al., 2002). Urea is the major nitrogen containing metabolic product of protein metabolism; uric acid is the major product of purine bases, adenine and guanine; creatinine is endogenously produced and released into body fluids and its clearance
is measured as an indicator of glomerular filtration rate (Burris and Ashwood, 1996). Renal marker levels were significantly increased in the serum of DOCA-salt hypertensive rats and these marker levels were reduced significantly upon treatment of diosmin, which could be due to reduction in the disturbance of protein and nucleic acid metabolism as evidenced by blood pressure lowering effect of the diosmin.

4.10. Histopathology

Histopathology of diosmin treated DOCA-salt induced heart and kidney showed reduced cardiac and renal damage. Thus, histopathological findings confirmed the biochemical observations of this study.

5. Conclusion

In conclusion, the present biochemical findings showed that diosmin possesses an antihypertensive effect which is evidenced by lowered blood pressure, lipid peroxides, improved nitric oxide availability and antioxidant status. Scavenging of superoxide anions by diosmin is attributed to its antioxidant effect and leads to the increase in nitric oxide availability which infers relaxation of the vessel wall and controls blood pressure. We believe that the low dose (25 mg/kg body weight) might not be sufficient to scavage the radicals, yet the high dose (100 mg/kg body weight) might interact with some other molecules instead of radicals. Thus, we conclude that the maximum efficacy is with the medium dose (50 mg/kg body weight).

Acknowledgment

The financial support to Mr. T. Silambaranas as project fellow from the University Grants Commission, New Delhi, India is gratefully acknowledged.

References

Roe, J.H., Kuether, C.A., 1943. The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J. Biol. Chem. 11, 145–164.