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Original article

Effect of diosgenin on cardiac tissue lipids, trace elements, molecular changes, TNF-α and IL-6 expression in CRF rats

Jeganathan Manivannan , Janakiraman Shanthakumar , Krishnamoorthy Rajeshwaran , Pandiyan Arunagiri , Elumalai Balamurugan *

Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar 608 002, Tamilnadu, India

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ABSTRACT

The aim of the present study was to evaluate the effect of diosgenin a proven antioxidant on chronic renal failure (CRF) induced abnormalities linked with oxidative stress in heart. CRF was induced in male Wistar rats by feeding the animals with 0.75% adenine-containing diet and diosgenin was given orally (everyday at the dose of 40 mg/kg). Effect of diosgenin on cardiac tissue lipids, trace elements (iron, zinc, magnesium, copper and manganese) and activity of cardiac mitochondrial enzymes were assayed. Expression of tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) was also assessed. The Fourier transform infrared spectroscopy (FTIR) analysis was employed to indicate the oxidative stress related molecular changes in heart tissue. The outcomes of the study indicated that, diosgenin reduces the tissue lipid abnormalities induced by CRF. Cardiac elemental concentration was not changed in all groups but the plasma zinc was altered and diosgenin have no effect on it. Cardiac mitochondrial enzymes abnormalities and proinflammatory cytokines expression was also significantly reduced by diosgenin. Finally, the molecular and structural changes of proteins were also reduced by diosgenin treatment. The overall study shows that diosgenin with antioxidant function have enough potential to improve cardiac tissue abnormalities.

1. Introduction

Cardiovascular complications are the leading cause of mortality in patients with end-stage renal disease (ESRD). The excess cardiovascular risk and mortality is already demonstrable in early renal disease and in patients with chronic renal failure (CRF), with the highest relative risk of mortality in the youngest patients [1].

Evidence for oxidative stress in CRF was based on the elevation of toxic lipid peroxidation products, which cause destruction and damage to cell membranes and enhance inflammatory burden through the generation of an imbalance between increased production of reactive oxygen species (ROS) and limited or decreased antioxidant capacity [2]. Moreover, CRF induces oxidative stress and fibrosis in heart [3]. Further, CRF is accompanied by characteristic abnormalities of lipid metabolism, which appear as a consequence of nephrotic syndrome or renal insufficiency and are reflected in an altered apolipoprotein profile as well as elevated plasma lipid levels [4]. Moreover, an increased link between elemental concentration deregulation and renal insufficiency was already explored [5].

Diosgenin is a steroidal saponin found in several plants including *Solanum* and *Dioscorea* species. Studies have also suggested a lower incidence for coronary artery diseases and disorders related to estrogen deficiencies in humans who have a high consumption of diet rich in phytoestrogens (i.e., genistein, daidzein, and diosgenin) [6]. Recent studies discovered that diosgenin have antioxidant potential and inhibits the production of intracellular ROS species, aortic remodeling, vascular calcification and lipid peroxidation [7,8].

There was a strong link between oxidative stress, tissue abnormalities and renal failure. Moreover, diosgenin was already proved as good antioxidant. Therefore, the present study was aimed to evaluate the effect of diosgenin on cardiac abnormalities in CRF rats.

2. Materials and methods

2.1. Animals and chemicals

Male albino Wistar rats, 8–10 weeks old (weighing 180–220 g) were procured for this study. This experimental study was approved by the institutional Ethical Committee. Diosgenin was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). All other

* Corresponding author. Tel.: +948 688 0889.
E-mail address: balamurugan_au@yahoo.co.in (E. Balamurugan).

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chemicals used were of analytical grade obtained from Merck and Himedia, India.

2.2. Chronic renal failure rats and diosgenin treatment

CRF in wistar rats was induced by feeding the animals with diet containing 0.75% adenine for 5 weeks. Each of the following groups consisted of six animals. Diosgenin was administered everyday orally by dissolving in corn oil (vehicle). Our previous study found that diosgenin 40 mg/kg body weight (bw) of animals has effective antioxidant activity and protective effect [Mani .8]:

- group I – control (animals fed with rat diet);
- group II – control + diosgenin 40 mg/kg (bw) of animals;
- group III – control animals fed diet with 0.75% adenine – CRF animals;
- group IV – CRF + diosgenin 40 mg/kg bw of animals.

2.3. Tissue lipid profile

Heart tissue lipids were extracted by the method of Folch et al. [9]. The sample was homogenized in cold chloroform-methanol (2:1 v/v) and the contents were extracted after 24 hours. The combined filtrate was washed with physiological saline and the aqueous layer was discarded. The lipid extract was re-dissolved in 3.0 mL of chloroform–methanol (2:1) mixture and aliquots were taken for the estimation of lipids. Total cholesterol, triglycerides (TG), free fatty acids (FFA), and phospholipids (PL) were estimated by the methods of Allain et al. [10], Mcgowan et al. [11], Falholt et al. [12], and Zilversmit and Davis [13], respectively.

2.4. Elemental concentration by ICP-AES

Level of trace elements was determined by using inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin-Elmer) by standard digestion method with nitric acid [14]. Level of iron (Fe), magnesium (mg), manganese (Mn), zinc (Zn) and copper (Cu) in plasma and heart tissue was assessed.

2.5. Cardiac mitochondrial enzymes assays

The heart tissues were put into ice cold 50 mM Tris–HCl (pH 7.4) containing 0.25 M sucrose and homogenized. Then, the homogenates were centrifuged at 700 × g for 20 minutes, and then the supernatants obtained were centrifuged at 9000 × g for 15 minutes. Then, the pellets were washed with 10 mM Tris–HCl (pH 7.8) containing 0.25 M sucrose and finally resuspended in the same buffer [15].The activities of isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH), α-ketoglutarate dehydrogenase (α-KGDH) were assayed by the methods of King [16], Slater and Borner [17], Mehler et al. [18], Reed and Mukherjee [19] respectively.

2.6. Gene expression analysis

After the study period, the heart tissues were subjected to total RNA extraction using RNA isolation kit (Fluka, Sigma Aldrich). The integrity, quality and quantity of the RNA were determined by nano-drop spectrometer. RNA was used for quantitative real time polymerase chain reaction (qRT-PCR) using SYBR Green qRT-PCR kit (sigma Aldrich, USA). Primer sequences used were as follows, TNF-α FP: GTACTAACTCCCAGAAAAGCAAGC and RP: CAGTAGACAGAAGAGCGTGGTG; IL-6 FP: ACCACTTCACAAGTCGGAGGC and RP: GGTCTTGGTCCTTAGCCACTCC; glyceraldehydes-3-phosphate dehydrogenase (GAPDH) FP: ACCACAGTCCATGCCAT CAC and RP: TCCACCACCCTGTTGCTGTA. The amplification specificity of all the primers was confirmed through resolved the PCR products by agarose gel electrophoresis. The relative fold change method was employed for calculating the differential expression between samples [20]. Fold change values were averaged from six reactions.

2.7. Fourier transform infrared spectroscopy analysis of heart tissue

FTIR analysis was carried out as described previously [8]. A small and equal amount of heart tissue samples were homogenized and potassium bromide (KBr) pellets was prepared for use in FTIR spectrometer. FTIR spectra of the region 4,000–400 cm^{-1} were recorded at the temperature of 25 ± 1 °C on a Nicolet-Avatar-360 FTIR spectrometer.

2.8. Statistical analysis

Values are given as mean ± SD for six rats. Data were analyzed by one-way analysis of variance followed by Duncan's multiple range test using SPSS version 11.5 (SPSS, Chicago, IL). The limit of statistical significance was set at $P < 0.05$.

3. Results

3.1. Effect of diosgenin on tissue lipids

CRF rats showed significant ($P < 0.05$) increase in the levels of cholesterol, TG and FFA with a significant ($P < 0.05$) decrease in PL. On treatment with diosgenin, the levels of all the above parameters brought to normal level (Table 1).

3.2. Effect of diosgenin on cardiac mitochondrial enzymes

The activities of mitochondrial enzymes were significantly ($P < 0.05$) down regulated in CRF heart whereas diosgenin treatment significantly reverses the above changes (Fig. 1).

3.3. Effect of diosgenin on plasma and tissue elements

CRF significantly ($P < 0.05$) decreased the concentrations of Zn in plasma and there was no change in other elements, diosgenin treatment did not affect the above. Further, there was no significant change in cardiac tissue elemental profile (Table 2).

3.4. Effect of diosgenin on cardiac TNF- α and IL-6 expressions

The quantitative PCR analysis have shown that, expression of TNF- α and IL-6 was significantly ($P < 0.05$) elevated in cardiac tissue

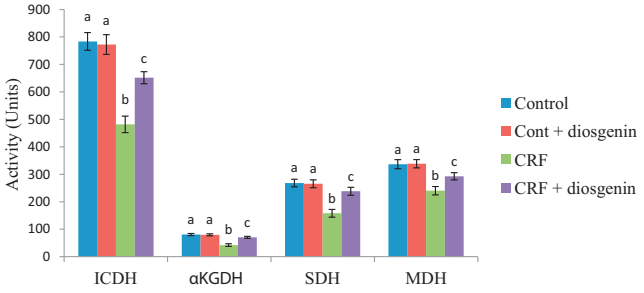

Fig. 1. Effect of diosgenin on mitochondrial Krebs cycle enzymes activity. Activity is expressed as nM of NADH oxidized/h/mg protein for ICDH; nM of ferrocyanide formed/h/mg protein for α-KGDH; nM of succinate oxidized/min/mg protein for SDH; nM of NADH oxidized/min/mg protein for MDH. Each value is mean ± SD for six rats in each group. Values not sharing a common letter differ significantly with each other ($P < 0.05$, Duncan's multiple range test).