Biomedicine & Pharmacotherapy 87 (2017) 280–287

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Tinospora cordifolia extract attenuates cadmium-induced biochemical and histological alterations in the heart of male Wistar rats

Lohanathan Bharathi Priya[a,1], Rathinasamy Baskaran[a,b,1,2], Pitchai Elangovan[a], Velumani Dhivya[a], Chih-Yang Huang[c,d,e], Viswanadha Vijaya Padma[a,b,c,e,*]

[a] *Translational Research Laboratory, Department of Biotechnology, School of Biotechnology and Genetic Engineering, Bharathiar University, Coimbatore 641046, Tamil Nadu, India*
[b] *DRDO-BU Centre for Life Sciences, Bharathiar University, Coimbatore 641046, Tamil Nadu, India*
[c] *Graduate Institute of Basic Medical Science, China Medical University, Taichung, Taiwan*
[d] *Graduate Institute of Chinese Medical Science, China Medical University, Taichung, Taiwan*
[e] *Department of Health and Nutrition Biotechnology, Asia University, Taichung, Taiwan*

ARTICLE INFO

Article history:
Received 3 November 2016
Received in revised form 19 December 2016
Accepted 22 December 2016

Keywords:
Cadmium
Tinospora cordifolia
Oxidative stress
Heart
Antioxidant enzymes

ABSTRACT

Persistence of cadmium (Cd) in the environment causes serious ecological problems. *Tinospora cordifolia* is a medicinal herb used in Ayurveda for treating various metabolic disorders and toxic conditions. The present study investigates the protective effect of *T. cordifolia* stem methanolic extract (TCME) on a heavy metal, Cd-induced cardiotoxicity in male Wistar rats. Male albino Wistar rats were divided into four groups (n = 6). The animals after treatment for 28 days with Cd and TCME were analysed for biochemical and histological changes in the serum and heart tissues. Cd induced lipid peroxidation and protein carbonylation was significantly reduced by TCME. TCME also reduced the histological alterations induced by Cd treatment in the heart tissues with diminished loss of myocardial fibers. Administration of TCME effectively prevented the altered levels of serum marker enzymes (creatine kinase and lactate dehydrogenase), antioxidants, such as superoxide dismutase, catalase, glutathione, glutathione peroxidase and glutathione-S-transferase, and glycoproteins contents such as hexose, hexoseamine, fucose, and sialic acid by Cd intoxication. TCME also offered protection against the change in levels of Na^+K^+ATPase, Mg^{2+}ATPase and Ca^{2+}ATPase activities against Cd toxicity. The study suggests TCME as a potent cardioprotective agent against Cd induced toxicity.

1. Introduction

The heavy metal Cd is a major environmental toxicant and human carcinogen [1]. Occupational and environmental exposure to Cd happens *via* smelters, pigment plants, battery factories and consumption of crops and vegetables contaminated by Cd through application of fertilizer and sewage sludge to farm land. Smoking, contaminated drinking water and dietary habits are other risk factors of Cd exposure [2]. The increased biological half-life of Cd (*i.e.*, 20–30 years) leads to its bioaccumulation in the upper levels of food chain [3]. Cd induced toxicity is mediated by generation of free radicals and subsequent ROS accumulation, which plays a vital role in tissue damage [4]. Oxidative stress, cell cycle progression, DNA damage and apoptosis are the major events triggered due to cadmium poisoning [5]. Cadmium exposure in animals resulted in the pathogenesis of hypertension and cardiotoxicity [6,7]. Increasing recent evidences suggest Cd as an independent factor responsible for various cardiovascular disorders like myocardial infarction, peripheral arterial disease, hypertension and atherosclerosis in humans [8,9].

Remedies with natural products proved effective with less or no side effects for treating various disorders. The Indian herb, *Tinospora cordifolia* (TC) belongs to the family Menispermaceae popularly known as 'Guduchi' in Ayurveda [10]. TC has been widely used in veterinary folk medicine/Ayurveda as a tonic, vitalizer, as a remedy for various metabolic disorders [11]. It possesses several pharmacological properties like immunomodulatory, hepatoprotective, gastroprotective, cholesterol-lowering, anti-inflammatory,

* Corresponding author at: Department of Biotechnology, School of Biotechnology and Genetic Engineering, Bharathiar University, Coimbatore 641 046, Tamil Nadu, India.
E-mail address: vvijayapadma@rediffmail.com (V.V. Padma).
[1] Both the authors contributed equally to this work.
[2] Present address: National Institute of Cancer Research, National Health Research Institutes, Zhunan, Miaoli County 35053, Taiwan.

http://dx.doi.org/10.1016/j.biopha.2016.12.098
0753-3322/

anti-allergic, anti-arthritic, diuretic, anti-stress anti-neoplastic, and anti-diabetic activities [12]. Antioxidant and cardioprotective activity of TC against streptozotocin induced diabetic rats was reported [13]. Inhibition of Fenton reaction through the free radical scavenging and metal chelation property of TC against radiation mediated damage were reported [14]. Accumulating evidences prove the beneficial effects of natural/herbal formulations for its protection against heavy metal mediated toxicity. Our previous findings revealed the protective effect of TCME on Cd induced toxicity in kidney [15]. Although, there are several reports on the Cd-induced cardiotoxicity, the cardioprotective studies are very limited. Since, heart is more vulnerable to oxidative stress compared to other organs and with knowledge of *T. cordifolia* as a medicinal plant, the present study was designed to evaluate the protective role of TCME on Cd-induced oxidative stress and impairment on tissue architecture by analyzing histology, membrane bound ATPases and glycoprotein content in the heart of male Wistar rats.

2. Materials and methods

2.1. Chemicals

Cadmium chloride ($CdCl_2$) was obtained from Sigma Aldrich Pvt. Ltd., Bangalore, India. All other chemicals used were of analytical grade and were purchased from HiMedia laboratories, Mumbai, India.

2.2. Plant material

Crude extract from the stem of *T. cordifolia* was extracted based on our previous report [15]. Stem of *T. cordifolia* was collected from the Western Ghats near Palaghat, Kerala, India, during the season of November to January. Plant material was authenticated by a taxonomist and submitted in herbarium collections at Bharathiar University, Coimbatore, India for reference. Dried and powdered stem of *T. Cordifolia* was subjected for soxhlet extraction defatted with petroleum ether to remove lipids and fats, and then the residue was extracted again with methanol. The extract solution was evaporated under reduced pressure at 40 °C, concentrated using a rotary vapor evaporator, and used for further assays and experiments. Methanolic extract of TC stem was prepared by soxhlet extraction after defatting with petroleum ether.

2.3. Animals and experimental design

Adult male albino Wistar rats weighing 200 ± 20 g were purchased from small animal breeding station, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala and used for the experiments. The animals were acclimatized, grouped into six rats per cage and maintained at a temperature of 25 ± 2 °C, with a natural light/dark cycle and were allowed for free access to commercially available pelleted rat chow (Sai Durga private limited, Bangalore) and water *ad libitum*. The animal treatment protocols were done according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and regulations, and the study was approved by the Institutional Animals Ethics Committee at Bharathiar University, Coimbatore, India. The animals were divided randomly into 4 groups with a minimum of 6 rats in each group. The Cd used was 5 mg/kg of body weight dissolved in normal saline and TCME was 100 mg/kg of body weight dissolved in 0.3% carboxy methyl cellulose (CMC). Group I served as control received normal saline. Group II received Cd in normal saline. Group III were treated with TCME. Group IV received a co-treatment of TCME and Cd. The rats were exposed to the respective treatment as mentioned in the groups for 28 days by oral intubation method.

After the completion of treatment period, the animals were deprived of food overnight, anesthetized by exposing to diethyl ether and euthanized by cervical decapitation. Blood was collected from jugular vein; serum was separated and used for cardiac marker assays. The heart tissue was dissected out, washed in ice-cold saline, patted dry and weighed. Of this, 100 mg of tissue was homogenized in chilled 0.1 M Tris-HCl buffer in a Potter-Elvejhem Teflon homogenizer for biochemical investigation and a small portion of the tissue was stored in 10% formalin for histopathological examination. The remaining tissue was stored in −80 °C until examined.

2.4. Estimation of total phenolic and flavonoid content of TCME

The total phenolic content was determined by the method of Malik and Singh [16] with reference to the standard gallic acid (100–1000 μg/ml) and the total soluble flavonoid content by aluminium chloride colorimetric method of Woisky and Salatino [17] with quercetin as standard.

2.5. DPPH free radical scavenging and FRAP assay

The free radical scavenging activity of TCME was determined using the method of Moon and Terao [18]. The ferric reducing antioxidant power of TCME was determined by the modified method of Benzie and Strain [19].

2.6. Analysis of serum cardiac markers

Blood collected was allowed to clot at room temperature and serum was separated by centrifuging at 1200 g for 15 min and used for analyzing creatine kinase and LDH according to the method of Okinaka et al. [20] and King [21].

2.7. Estimation of lipid peroxidation, protein carbonyl content and GSH levels

Lipid peroxidation in heart tissue homogenates were determined according to the method of Ohkawa et al. [22] and Levine et al. [23] respectively. GSH content in heart tissue homogenates was measured using the method of Moron et al. [24]

2.8. Assay of cellular antioxidant enzymes

Antioxidant status of the heart tissues were measured for superoxide dismutase (SOD) [25], catalase (CAT) [26], glutathione peroxidase (GPX) [27] and glutathione-s-transferase (GST) [28].

2.9. Histopathological examination

The heart tissues were qualitatively analysed for histological alterations after fixing in 10% formalin. The tissues were then processed for dehydration and clearing of fixative and embedded in paraffin wax. Sections of heart (3–5 μm thickness) were cut and stained with hematoxylin and eosin (H and E) dyes for morphological observation under the microscope.

2.10. Assay of membrane bound ATPases

Activities of the membrane-bound ATPases namely Na^+K^+ ATPase [29], Ca^{2+}ATPase [30], and Mg^{2+}ATPase [31] were measured in the tissue homogenates.