HOSTED BY

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://ees.elsevier.com/ejbas/default.asp

Polyphenon-60 ameliorates metabolic risk factors, oxidative stress, and proinflammatory cytokines and modulates apoptotic proteins to protect the heart against streptozotocin-induced apoptosis

Mohamed A. El-Missiry [a,*], *Maher A. Amer* [a], *Azza I. Othman* [a], *Yaseen khashman* [b]

[a] *Zoology Department, Faculty of Science, Mansoura University, Mansoura, Egypt*
[b] *Microbiology, Faculty of Science, Al-Mustansiriya University, Baghdad, Iraq*

ARTICLE INFO

Article history:
Received 3 March 2015
Received in revised form
29 March 2015
Accepted 31 March 2015
Available online 18 April 2015

Keywords:
Green tea
Catechins
Cardiomyopathy
Lipids
Streptozotocin
Heart

ABSTRACT

The role of green tea polyphenon-60 (PP-60) in decreasing metabolic risk factors, oxidative stress, and inflammation and in the amelioration of cardiac apoptosis in experimentally induced diabetes was investigated. After the induction of diabetes in male rats by streptozotocin (STZ; 50 mg/kg, i.p.), PP-60 (100 mg/kg) was orally administered for two weeks. The treatment of the diabetic rats with PP-60 ameliorated hyperglycemia and HbA1c, increased insulin secretion, improved the HOMA-IR level and lipid profile and increased the levels of IL-1b, IL-6, and TNF-α. PP-60 also blunted the increase in the level of MDA and H_2O_2 and the decreases in the levels of GSH, SOD, and CAT in the heart of STZ-treated rats and improved heart function. PP-60 decreased the apoptosis of cardiomyocytes; enhanced Bcl-2 expression; blocked the increases in p53, Bax, caspases 9, 8 and 3; and ameliorated DNA damage in the heart. Our data suggests that PP-60 decreased apoptosis by blocking DNA damage via the action of caspases and the suppression of pro-inflammatory cytokines. The pleiotropic effects of PP-60, including its anti-hyperglycemic effect, hypolipidemic influence, attenuation of oxidative stress reduction of circulating pro-inflammation cytokines and anti-apoptotic action through modulation of its related regulated proteins, contribute to and accompany the beneficial effects of treatment with PP-60 on diabetic heart dysfunction.

1. Introduction

Diabetes mellitus is one of the most common endocrine metabolic disorders and is associated with marked morbidity and mortality due to cardiac complications [1,2]. The pathophysiology underlying diabetes-induced cardiac damage is complex and is induced by several factors, including elevated oxidative stress, high proinflammatory cytokines and dyslipidemia as the main risk factors [3]. It was recently reported

* *Corresponding author.*
E-mail address: maelmissiry@yahoo.com (M.A. El-Missiry).
Peer review under responsibility of Mansoura University.
http://dx.doi.org/10.1016/j.ejbas.2015.03.003

that short-term diabetes induces cardiac antioxidant depletion, increases the oxidative stress levels, and results in cardiac apoptosis as early as three weeks after diabetic induction in rats [4]. A similar finding was reported after the administration of streptozotocin (STZ) for four days [5].

In diabetes, the dysregulation of programmed cell death is a significant factor of cardiomyopathy. In human and animal models of diabetes, cardiac pathology is associated with excessive apoptosis [5,6] that progressively leads to histopathological features and eventually to heart disease [7,8]. Overwhelming evidence indicates that cardiomyocyte apoptosis in diabetes is associated with cytopathological features [9,10], which is likely a direct result of hyperglycemia-triggered caspase 3 activation [3,11]. There are two pathways known to produce cardiac apoptosis, namely the mitochondrial and the death receptor-mediated pathways, and both are influenced by cellular antioxidants [5]. Caspases 8 and 9 are initiator caspases for the extrinsic and intrinsic pathways, respectively, and caspase 3 is activated by both pathways and initiates apoptosis. The early event of myocyte apoptosis is characterized by nuclear damage, which is often undetectable morphologically, but DNA damage can be identified by several techniques, including the comet assay. The initial phase of the apoptotic process is followed by nuclear fragmentation and later by cytoplasmic alterations [12,13]. In diabetes, oxidative stress is the key element in all of mechanisms underlying cardiomyopathy and apoptosis, both of which are associated with hyperglycemia and hyperlipidemia [3].

Beyond the beverage use of tea, naturally derived catechins have been recognized to have numerous health benefits. However, the molecular mechanisms through which these molecules mediate their effects remain largely unknown [14,15]. Green tea is obtained from the fresh leaves of the *Camellia sinensis* plant and is processed to prevent oxidation of the polyphenolic constituents [16]. Experimental and clinical studies have confirmed the efficacy of phytochemicals in the modulation of oxidative stress-induced cardiovascular diseases associated with diabetes [17,18]. Catechins are flavanols that constitute the majority of soluble solids of green tea. EGCG [(−)-epigallocatechin-3-gallate] is the main catechin [19] and ECG [(−)-epicatechin-3-gallate] is the second most concentrated catechin component of green tea. Other major catechins found in green tea include (−)-epicatechin (EC) and (−)-epigallocatechin (EGC) [20]. Catechins afford an antioxidant effect by inducing antioxidant enzymes, inhibiting prooxidant enzymes and scavenging free radicals [16]. The potential mechanisms underlying the antioxidant effects of tea as effective scavengers of physiologically relevant reactive oxygen and nitrogen species were reviewed previously. The aromatic phenol rings present in these molecules cause them to behave as antioxidants by reducing free radicals and chelating metals in the body [21].

Catechins of green tea, particularly EGCG, have antioxidant activity [22], antidiabetic activity and antiobesity [23] as well as anti-inflammatory activities [24,25], and can modulate apoptotic processes in the liver [26]. EGCG binds to low-density lipoproteins (LDL) and protects them from glycation and oxidation under high-glucose medium, which mimics diabetes. Oxidized LDL and soluble vascular cell adhesion molecule-1 are markedly decreased after 30 days of green tea ingestion [27]. Oral treatment with green tea polyphenol offers cardioprotection against ischemia-reperfusion injury in an isolated rat heart model [28]. Plyphenol-60 (PP-60) has a therapeutic effect on acne by suppressing inflammation, specifically by inhibiting IL-8 secretion [29].

The beneficial effects of green tea are related to catechins, particularly EGCG [30]. However, no previous study has investigated the efficacy of polyphenon-60, which contains pure catechins, mainly EGCG, in diabetes-induced cardiomyopathy in rats. In addition, the mechanisms involved in its beneficial effects are not fully understood. Therefore, the present study was designed to evaluate the cardioameliorative effects of polyphenon-60 in STZ-induced early cardiac injury in rats and attempts to understand the mechanism underlying the therapeutic effects of this compound with reference to biochemical and molecular markers.

2. Materials and methods

2.1. Chemicals

Green tea polyphenol (polyphenon-60) and streptozotocin (STZ) were purchased from Sigma (Sigma, St. Louis, MO, USA).

2.2. Experimental design and animal groups

Male Wistar rats weighing approximately 250–300 g were obtained from the Laboratory Animal Maintenance Unit, Faculty of Science, Mansoura University and acclimatized to the laboratory conditions for two weeks. They were maintained at 25 ± 2 °C and a 12-h light/12-h dark cycle. The rats were given standard commercial rat chow and water *ad libitum*. All of the experiments were conducted in accordance with protocols approved by the local ethical committee for *in vivo* animal experiments. The animals were divided into four groups of eight rats each. The first group served as a control. The second group received an oral dosage of polyphenon-60 (100 mg/kg body wt) every other day for a period of two weeks. The third group received a single i.p. injection of STZ (40 mg/kg body wt). The diabetic state of the rats was confirmed by determination of the blood glucose level after two days of STZ injection. The fourth group received STZ followed by green tea polyphenon-60 at the same doses as the second and third groups.

After two weeks of treatments, overnight fasted rats were anaesthetized with sodium pentobarbital (50 mg/kg i.p.), and their blood was collected by cardiac puncture. The sera were separated by centrifugation (500× g) for 5 min for biochemical determinations. The heart samples were homogenized in 10 volumes of 50 mM sodium phosphate buffer (pH 7.4) at 4 °C for 30 s. The homogenate was centrifuged (1000× g) for 5 min in a refrigerated centrifuge. The resulting supernatant was used for the biochemical determinations.

2.3. Biochemical analysis

The blood glucose, insulin and HbA1c levels were estimated using kits supplied by Spinreact (St. Esteve d'en Bas Girona, Spain), Abcam (Cambridge, MA, USA) and BioSystems