Phytomedicine 23 (2016) 923–930

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.com/locate/phymed

Reversal of diabetes-induced behavioral and neurochemical deficits by cinnamaldehyde

Akshay Jawale[a,1], Ashok Kumar Datusalia[a,1], Mahendra Bishnoi[b,1], Shyam S. Sharma[a,1,*]

[a] Molecular Neuropharmacology Lab, Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S.A.S. Nagar -160062, Punjab, India
[b] National Agri-Food Biotechnology Institute (NABI), S.A.S. Nagar (Mohali)-160071, Punjab, India

ARTICLE INFO

Article history:
Received 8 December 2015
Revised 25 April 2016
Accepted 26 April 2016

Keywords:
Diabetes
Cognititon
Cinnamaldehyde
GABA
Glutamate
IL-6
TNF-α

ABSTRACT

Background: Chronic hyperglycemia during diabetes is associated with altered cognitive function. Cinnamaldehyde showed to have many pharmacological activities indicating anti-diabetic, cognitive enhancer, antiinflammatory etc. In the present study, we have investigated the effects of cinnamaldehyde (CA) on diabetes-induced cognitive deficits.

Methods: Diabetes was induced in Sprague Dawley rats using high fat diet followed by streptozotocin (35 mg/kg, i.p.). High fat diet feeding was continued for 18 week after STZ administration. CA was administered daily during the last 3 weeks (week 16–18) at a doses of 10, 20 and 40 mg/kg (p.o.). Animals were subjected to behavioral tests during 18th week. Neurotransmitter levels (glutamate and GABA), acetylcholine esterase (AChE) activity and inflammatory markers (TNF-α and IL-6) were assessed in the hippocampus and cortex.

Results: Vehicle-treated diabetic rats showed impaired behavior in open field, elevated plus maze and water maze test compared to age-matched control rats. Cinnamaldehyde showed significant reduction in blood glucose levels at dose of 20 and 40 mg/kg. Three weeks treatments of cinnamaldehyde showed significant amelioration of behavioral deficits in diabetic rats. Chronic treatment with cinnamaldehyde showed improvement in brain ChE activity, neurotransmitter levels and reduction in IL-6 and TNF-α levels.

Conclusion: The present study demonstrates that treatment with cinnamaldehyde reverse neuroinflammation and changes in neurotransmitter levels, and consequently improves behavioral deficits in diabetic rats.

Introduction

Diabetes is one of the most common metabolic disorder in human. 382 million people are presently suffering from diabetes which is expected to rise to 592 million by 2035 (Guariguata et al., 2014). Type-2 diabetes (T2D) adversely affects major regulating systems of body to which central nervous system is not an exception. Studies have demonstrated an association between diabetes and a variety of brain alterations including cognitive decline, anxiety, depression, Alzheimer's disease, and Parkinson's disease. 17.5% of total T2D population face moderate to severe behavioral deficits moreover elderly people of age above 65 are more prone to develop diabetes-induced cognitive deficits (DACD) and dementia. (Strachan et al., 2011). In an earlier study, we demonstrated that 15 weeks duration of diabetes significantly impaired the cognitive function in high fat diet/streptozotocin induced rat model of diabetes (Datusalia and Sharma, 2014). Diabetic rats showed significant impairment in spatial memory and avoidance response. Synaptic plasticity in hippocampus has been studied in rat model of diabetes to elucidate the pathology behind cognitive decline. Recent studies on various animal models have given new hope for the treatment of DACD with enalapril, insulin, tocotrienol, C-peptide replacement, vitamine E, sesamol, lycopene, resveratrol, curcumin, PARP inhibitors but none is nearby to definitive

Abbreviations: AChE, Acetylcholine esterase; CA, cinnamaldehyde; DACD, Diabetes-induced cognitive deficits; DTNB, 5,5-dithiobis-2-nitrobenzoic acid; EPM, Elevated plus maze; GABA, γ-amino butyric acid; GLUT4, Glucose transporter 4; HAB, high anxiety-related behaviour; HbA1c, Glycated haemoglobin; HFD, high fat diet; IL-6, Interleukin-6; LTP, long-term potentiation; MWM, Morris water maze; NPD, Normal palate diet; STZ, streptozotocin; T2D, Type-2 diabetes; TNF-α, tumour necrosis factor-α; TRPA1, transient receptor potential cation channel A1.

* Corresponding author.
E-mail addresses: jawalead9970@gmail.com (A. Jawale), ashokdatusalia@gmail.com (A.K. Datusalia), mbishnoi@nabi.res.in (M. Bishnoi), sssharma@niper.ac.in (S.S. Sharma).

[1] **Author contributions**: AJ performed research; AJ and AKD analyzed data; SSS, AKD and MB designed hypothesis and wrote the paper.

http://dx.doi.org/10.1016/j.phymed.2016.04.008
0944-7113/

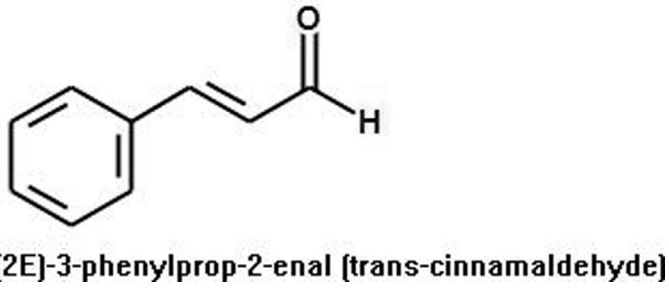

Fig. 1. Structure of cinnamaldehyde.

treatment (Kuhad et al., 2009; Sima et al., 2004; Tiwari et al., 2009). Moreover, most studies focus vascular theory of cognitive decline, taupathy and oxidative stress dependent CNS damage. However, 90% population among the diabetics are suffering from T2DM but still there is a significant gap to address the cognitive deficits in T2DM (American Diabetes Association, 2014).

Cinnamomum verum J.Presl, *Cinnamomum zeylanicum* Blume (Lauraceae) and other species of the genus Cinnamomum are used worldwide as household spices and food and condiment additives. In addition to its culinary uses, cinnamon bark has been used in medieval times to treat various conditions such as coughing, arthritis, sore throats and gynecological aliments (Ranasinghe et al., 2013). In current clinical and preclinical research, this spice has been shown to have potential as cognitive enhancer, anti-diabetic, anti-hypertensive, immunomodulatory, anti-arthritic, anti-inflammatory, anti-microbial and anti-parasitic activities and in reducing risk of colonic cancer (Frydman-Marom et al., 2011; Leach and Kumar, 2012; Ranasinghe et al., 2013). Cinnamon bark contained essential oil with *trans*-cinnamaldehyde (Figure 1) as the major component 60–90% of the total essential oil (Wong et al., 2014). Cinnamaldehyde has gained recognition for the management of diabetes, lipid disorders and diabetes-induced hypertension (Li et al., 2012). The treatment of diabetic subjects with cinnamon was investigated in several clinical trials and its insulin-like effects were present in type-2 diabetic patients. In addition, cinnamaldehyde showed improvement of brain insulin sensitivity in mouse models of obesity (Sartorius et al., 2014). Interestingly, it was also described as beneficial in Alzheimer's disease by reducing β-amyloid oligomerization and cognitive decline, and further prevented glutamate-induced neuronal death in cultured cerebellar granule cells (Frydman-Marom et al., 2011; Shimada et al., 2000). These reports provide evidences that, cinnamaldehyde (a principal component of cinnamon oil) is of high ethnopharmacological importance and exerts a variety of biological–pharmacological effects including diabetes and brain disorders. However, the effect of cinnamaldehyde on diabetes-induced change in neurobehavior and neurochemistry has not yet explored.

In the present study, we have explored the effect of cinnamaldehyde in diabetes-induced behavioral deficits using open field, elevated plus maze, passive avoidance paradigms and morris water maze. We have also investigated the effects of cinnamaldehydye against the diabetes-induced alteration in the inflammatory cytokine (IL-6), acetylcholinesterase activity and neurotransmitter levels (glutamate and GABA) in the hippocampus and cerebral cortex of diabetic rats.

Materials and methods

Animals

Studies were carried out in male Sprague-Dawley rats (weighing around 130–160 g; 5–6 weeks of age). Animals were procured from central animal facility, National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. Nagar and provided with standard diet and water *ad libitum*. The experimental study protocol was approved by the Institutional Animals Ethics Committee (IAEC), NIPER, and all the guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Govt. of India were followed. Animals were housed in room maintained at approximately 24 ± 1°C temperature and humidity of 55 ± 5% with 12 h light/dark cycle. Animals were acclimatized for at least 7 days before the initiation of the experiment and were observed for any sign of disease. Animal's body weight was determined on a weekly basis.

Drugs and chemicals

Cinnamaldehyde (C80687; $\geq$99%; Lot no. STBC3013), streptozotocin, acetylthiocholine, phenylmethanesulfonyl chloride, L-tyrosine, *o*-phthalaldehyde were precured from Sigma Aldrich, USA. All other chemicals used in the study were of analytical grade. The composition of high fat diet is given in Supplementary Table 1.

Induction of diabetes and experimental design

Type-2 diabetes was induced by combination of high fat diet (HFD) feeding and low dose of streptozotocin (STZ) treatment as described elsewhere (Srinivasan et al., 2005). Rats were fed with HFD for two weeks and then injected with single low dose of STZ (35 mg/kg, *i.p.*) to induce type-2 diabetes. Plasma glucose level was analysed at the end of 2 weeks of STZ administered and only those rats with plasma glucose level of >250 mg/dl were considered diabetic and selected for the study.

At the end of 15 weeks of STZ administration animals were randomized and divided into following groups; (1) Control (NPD) ($n=8$), (2) diabetic Control (HFD + STZ + Veh) ($n=8$), (3) Normal + Cinnamaldehyde (40 mg/kg) ($n=6$), (4) Diabetic + Cinnamaldehyde (10 mg/kg) ($n=7$), (5) Diabetic + Cinnamaldehyde (20 mg/kg) ($n=7$), (6) Diabetic + Cinnamaldehyde (40 mg/kg) ($n=7$). Cinnamaldehyde treatment was given for three weeks as daily single oral dose, from 16th week to 18th week. Olive oil was used as a vehicle. Animals were assessed for behavioural parameters during 18th week of STZ administration.

Behavioral studies

Open field test

Open field test was used for evaluating animal's spontaneous behaviour in response to a novel environment. An open field arena was constructed of plywood. Dimensions of arena was 70 × 70 × 37 cm and divided into two zone as peripheral and central zone. Experiments were carried out in diffused light at day time. All the experiments were recorded and analysed using ANYMAZE software. Movement of rat in peripheral and central zone was recorded (Eilam, 2003).

Elevated plus maze

The elevated plus maze (EPM) apparatus consisted of a four-arm cross-formed wooden maze with two opposite open arms (15 × 46 cm) and two opposite closed arms (15 × 46 × 23 cm), which all extended at an angle of 90° from a central area (15 × 16 cm). The EPM was placed on a support frame located 70 cm above the floor and was placed in the center of an experimental room with a distance of at least 1 m to the adjacent walls. Experiments were carried out in diffused light and analysed using ANYMAZE software. At the time of testing, the rat was placed in the centre of the plus maze facing a closed arm and was allowed to explore the maze for 5 min, after that it was removed from the maze. Time