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PARTICIPATION OF ANTIOXIDANT AND CHOLINERGIC SYSTEM IN PROTECTIVE EFFECT OF NARINGENIN AGAINST TYPE-2 DIABETES-INDUCED MEMORY DYSFUNCTION IN RATS

A. RAHIGUDE,[a] P. BHUTADA,[a]* S. KAULASKAR,[a] M. ASWAR[b] AND K. OTARI[c]

[a] *Sinhgad College of Pharmacy, Post-Graduate Research Department, Off Sinhgad Road, Vadgaon (Bk), Pune 411 041, Maharashtra, India*

[b] *Sinhgad Institute of Pharmacy, Department of Pharmacology, Narhe, Pune 411 041, Maharashtra, India*

[c] *Rajgad Dyanpeeth's College of Pharmacy, Bhor, Pune, Maharashtra, India*

Abstract—Naringenin is a flavone flavonoid possessing antidiabetic, antioxidant and memory improving effects. Therefore, we studied the influence of naringenin against type-2 diabetes-induced memory dysfunction in rats. Type-2 diabetes was induced by high-fat diet and high-fat emulsion for two weeks and a low dose of streptozotocin (35 mg/kg). The memory deficit was assessed by using a novel object recognition paradigm. The changes in oxidative markers and cholinesterase (ChE) levels were evaluated in the hippocampal region. After confirmation of diabetes, naringenin (50 mg/kg) treatment was given to animals as a preventive and in another set of experiments naringenin (25 and 50 mg/kg) or pioglitazone (5 mg/kg) or donepezil (3 mg/kg) treatments were started after long-standing diabetes (4 weeks after confirmation). Both the treatment schedules show significant protection and improvement in cognitive behavior against diabetes-induced memory dysfunction and biochemical changes. Also, treatment with pioglitazone and donepezil improved memory performance in rats. Naringenin was found to decrease oxidative stress by depleting elevated lipid peroxide and nitric oxide and elevating reduced glutathione levels. Cholinergic function was improved by naringenin through the inhibition of elevated ChE activity. In conclusion, the present study suggests that naringenin acts as an antioxidant and ChE inhibitor against type-2 diabetes-induced memory dysfunction.

Key words: type-2 diabetes, memory, cholinesterase, novel object recognition, hippocampus, pioglitazone.

INTRODUCTION

Diabetes mellitus (DM) is the most common endocrine disorder characterized by increased blood glucose levels resulting from defective insulin secretion, resistance to insulin action or both. DM is associated with long-term complications and affects eyes, kidneys, blood vessels, heart, and nerves (Gispen and Biessels, 2000; Northam et al., 2009). DM is strongly associated with degenerative and functional disorders of the CNS (Kim et al., 2003; Northam et al., 2009). These effects of diabetes in the CNS are a series of neurochemical, neurophysiological and structural abnormalities (Biessels et al., 2002; Sima et al., 2004; Bhutada et al., 2012). Diabetics are found to have decreased performance on measurement of memory, cognitive flexibility, rapid information processing and psychomotor efficacy (Pasquier et al., 2006). The association of DM with CNS doubles the risk of Alzheimer's diseases and memory deficits (Arvanitakis et al., 2004; Biessels et al., 2006; Bhutada et al., 2012). This suggests a strong link between diabetes and memory deficits.

The consequences that lead to memory dysfunction in diabetes are impairment in glucose utilization and energy metabolism (Cao et al., 2003), secondary hyperglycemia (Biessels et al., 2002), decreased insulin levels and sensitivity (Steen et al., 2005), increased insulin resistance and inadequate functionality of insulin/insulin receptors (Cosway et al., 2001; Zhao et al., 2004), changes in synaptic plasticity and transmission in the hippocampus (Biessels et al., 1996; Kamal et al., 2006), increased advanced glycation end products (AGE) that increases neurofibrillary tangle formation and therefore amyloid-β deposition (Heitner and Dickson, 1997) and oxidative stress that triggers degenerative events in the hippocampus (Cardoso et al., 2010). Studies also suggest that individuals with a high energy intake are at increased risk of Alzheimer's disease (Luchsinger et al., 2002). Animal studies have shown that high-calorie diets impair the structure and function of the hippocampus, a brain region critical for learning and memory (Greenwood and Winocur, 1990; Eichenbaum et al., 1996; Winocur and Greenwood, 1999; Molteni et al., 2002; Wu et al., 2003; Kanoski et al., 2007; Farr et al., 2004). In animal models, both type-1 and type-2 diabetic animals are reported to induce severe memory deficits (Bhutada et al., 2010, 2011, 2012; Jiang et al., 2012).

Naringenin, a flavone flavonoid is a biologically active molecule possessing a wide range of pharmacological

*Corresponding author. Tel: +91-020-4354720; fax: +91-020-4354721.
E-mail addresses: psbhutada@live.com, pra777us@yahoo.com (P. Bhutada).

Abbreviations: ANOVA, analysis of variance; ChE, cholinesterase; DM, diabetes mellitus; GSH, reduced glutathione; HFD, high-fat diet; HFE, high-fat emulsion; MDA, malondialdehyde; NO, nitric oxide; PPAR, peroxisome proliferator-activator receptor; STZ, streptozotocin.

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effects. It is abundantly found in citrus fruits such as grapefruits, oranges and tomatoes (Wilcox et al., 1999). Naringenin possesses various biological activities such as antidiabetic (Borradaile et al., 2002, 2003; Ortiz-Andrade et al., 2008; Mulvihill et al., 2009; Rayidi, 2011), antiatherogenic (Wilcox et al., 1999; Goldwasser et al., 2010), antidepressant (Zbarsky et al., 2005; Olsen et al., 2008), immunomodulatory (Wilcox et al., 1999), antitumor (Park et al., 2010a), anti-inflammatory (Ribeiro et al., 2008), DNA protective (Oršolić et al., 2011), hypolipidaemic (Mulvihill et al., 2009; Rayidi, 2011), anti-oxidant (Heo et al., 2004a,b) and peroxisome proliferator-activated receptors (PPARs) activator (Goldwasser et al., 2010). In addition, naringenin also exhibits inhibitory effect on acetylcholinesterase and therefore it may have potential in the treatment of dementia (Heo et al., 2004b). These properties of naringenin suggest that it may have beneficial effects against diabetic memory dysfunction. But, the literature revealed that study related to the influence of naringenin in diabetic memory deficits is not performed and therefore the present study was designed.

EXPERIMENTAL PROCEDURES

Experimental animals

Young male Sprague–Dawley rats (150–200 g) were procured from the National Institute of Biosciences, Pune, India. The animals were maintained under standard laboratory conditions at temperature 23 ± 2 °C, relative humidity 55 ± 10% and 12:12 h light (08:00–20:00 h)/dark cycle maintained throughout the experiment. Animals had free access of water and standard laboratory feed *ad libitum* prior to the dietary manipulation. The animal studies were approved by the Institutional Animal Ethics Committee (protocol No. SCOP/2011-12/15), constituted for the purpose of control and supervision of experimental animals by the Ministry of Environment and Forests, Government of India, New Delhi, India. Animals were naive to drug treatments and experimentation at the beginning of all studies. All tests were conducted between 08:00 and 14:00 h.

Drugs and solutions

Naringenin (SRL, India), streptozotocin (STZ) (Enzo Life Sciences, UK), pioglitazone (Wockhardt Pharmaceuticals Ltd, India) and donepezil hydrochloride (Alkem Laboratories Ltd, India) was used. Naringenin and pioglitazone were suspended in 1% carboxy methylcellulose in distilled water. STZ was dissolved in citrate buffer (pH 4.4) and donepezil hydrochloride was dissolved in distilled water. Drug solutions/suspensions were prepared fresh. All the other agents used were of analytical grade. Doses of naringenin (25 and 50 mg/kg, p.o.) were selected on the basis of previous literature (Ortiz-Andrade et al., 2008; Rayidi et al., 2011).

Experimental induction of diabetes

Type-2 diabetes was induced in rats by earlier reported methods with slight modifications (Srinivasan et al., 2005; Xu et al., 2012). In brief, rats were fed with a combination of high-fat emulsion (HFE) and manipulated normal pelleted diet to high-fat diet (HFD). In both, HFE and HFD, total obtained calories from fat was 60%. After two weeks of feeding with HFD and HFE, low dose of STZ (35 mg/kg) was administered. Blood samples were taken from the tail vein 48 h after STZ injection to measure glucose levels. Animals with fasting blood glucose levels above 200 mg/dl were considered diabetic and used for further study. The details of the composition of HFD and HFE are given in Tables 1 and 2.

Table 1. Constituent for high-fat diet

Constituents	% of calories
Carbohydrates	23.85
Proteins	12.35
Fats	60
Fibers and other	4.8

Table 2. Constituent for high-fat emulsion

Constituents	% of calories (ml)
Ghee	40
Groundnut oil	20
Water	25
Tween 80	10
Propylene glycol	5

Treatments schedule of naringenin

Treatment 1. After confirmation of diabetes, separate groups of rats ($n = 6$) were administered with naringenin (50 mg/kg, p.o.) or vehicle (1 ml/kg, p.o.) twice daily (08:00 and 20:00 h) for the next 58 days (day 1–58), and at the end of the treatment schedule rats were subjected to object recognition test and memory was evaluated.

Treatment 2. Four weeks after diabetes confirmation, separate groups of rats ($n = 6$) were administered with naringenin (25, 50 mg/kg, p.o.) or pioglitazone (50 mg/kg, p.o.) or donepezil (3 mg/kg, p.o.) or vehicle (1 ml/kg, p.o.) twice daily (08:00 and 20:00 h) for the next 30 days, and at the end of treatment schedule, rats were subjected to object recognition test. Similar treatments were given to control (non-diabetic) rats. Learning and memory were evaluated on days 59 and 60 after the confirmation of diabetes.

Assessment of cognitive function

Novel object recognition test. At the end of the treatment schedules, rats were tested in the novel object recognition test. This test is based on the natural propensity of animals to spend more time exploring a new rather than a formerly encountered object. Memory was evaluated at two retention intervals (30 min and 24 h) as described earlier (de la Tremblaye and Plamondon, 2011; Bhutada et al., 2012). Rats were transported from the animal vivarium to the testing laboratory and allowed to acclimatize to the testing environment for at least 60 min before behavioral testing began. Testing was monitored by an overhead camera. The test was performed in the open-field arena 72 cm × 72 cm × 36 cm as previously described (Pascalis et al., 2009). Each rat was exposed to three experimental conditions in the open field. In the initial trial (T1), one object-stimulus (O1) was placed in one corner of the open field and the rat positioned in the opposite corner of the arena, and time spent exploring the object (touching the object with