Biomedicine & Pharmacotherapy 107 (2018) 203–211

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Original article

The effects of safranal, a constitute of saffron, and metformin on spatial learning and memory impairments in type-1 diabetic rats: behavioral and hippocampal histopathological and biochemical evaluations

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ARTICLE INFO

Keywords:
Safranal
Diabetes
Learning and memory impairments
Metformin
Rats

ABSTRACT

Safranal is one of saffron constituents and has antioxidant and neuroprotective properties. Metformin is used as an anti-diabetic drug. This study was planned to investigate the separate and combined treatment effects of safranal and metformin on diabetes-induced learning and memory impairments by behavioral and hippocampal histopathological and biochemical evaluations. Diabetes was induced by intraperitoneal injection of streptozotocin (STZ), treatments with safranal (0.025, 0.1 and 0.4 mg/kg), metformin (50 and 200 mg/kg), and a combination of low doses of this chemicals were initiated after confirmation of diabetes and continued for 37 days. Blood glucose concentration was measured before and on days 15, 25 and 35 after injection of streptozotocin. Learning and memory tested using Morris Water Maze (MWM) on days 40–45 and on day 45 hippocampal specimens were collected for determination of malodialdehyde (MDA), tumor necrosis factor-alpha (TNF-α) and Caspase-3 levels and superoxide dismutase (SOD) activity. The hippocampus was also designed for light microscopy evaluation. Hyperglycemia, spatial learning and memory impairments, hippocampal neuron loss, increase of hippocampal MDA, TNF-α and caspase-3 levels and decrease of SOD activity were observed in diabetic rats. Safranal (0.1 and 0.4 mg/kg), metformin (200 mg/kg) and safranal (0.025 mg/kg) with metformin (50 mg/kg) improved the above-mentioned behavioral, histopathological and biochemical changes. Safranal and metformin and their combination improved learning and memory impairments in STZ-induced diabetic rats. Antioxidant, anti-inflammatory and antiapoptotic mechanisms might be involved. It is recommended that safranal be considered for diabetes management.

1. Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both [1]. Diabetes can cause many complications such as gastrointestinal disorders, cardiomyopathy, retinopathy, nephropathy and neuropathy [2]. Diabetes threatens human brain health by brain function decline leading to neurodegenerative diseases [3]. Neurogenesis and dendritic remodeling of the hippocampus are impaired and neuronal apoptosis is increased in diabetes mellitus which cause learning and memory decline [4].

Crocus sativus L. (saffron), as an important medicinal plant, is widely used for medicinal purposes, and the main components of this plant are crocin, crocetin and safranal [5]. Saffron and its main constituents produce relaxant effects on smooth muscle by activation of β2-adrenoceptors and inhibition of histamine H1 and muscarinic cholinergic receptors [6]. Safranal (2,6,6-trimethyl-1, 3-cyclohexadien-1-carboxaaldehyde) as the most abundant chemical in saffron essential oil accounts for 60–70% of volatile fraction [7]. Pharmacological studies have suggested antinociceptive, anti-inflammatory, anti-oxidant, anti-apoptotic, ant-cancer, anti-epileptic, immunomodulatory and tissue protective properties of safranal [5,8–11]. Safranal produced a neuroprotective effect on diabetic peripheral neuropathy by sciatic nerve histopathological changes attenuation and its MDA content restoration

Abbreviations: MDA, malondialdehyde; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-alpha; DM, diabetes mellitus; MWM, Morris water maze; STZ, streptozotocin; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus

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https://doi.org/10.1016/j.biopha.2018.07.165
Received 2 June 2018; Received in revised form 28 July 2018; Accepted 31 July 2018

[12]. In nephropathy complication of T2DM, treatment with safranal reduced renal tissue damage and dysfunction and cytokine level as well as oxidative stress index [13]. Chronic treatment with safranal recovered metabolic changes such as hyperglycemia and hyperlipidemia in T1DM [14].

Metformin, a simple and inexpensive biguanide molecule is frequently used an oral antihyperglycemic drug for the treatment of type 2 diabetes mellitus [15]. Recent interest has been generated in using metformin in type 1 diabetes mellitus [16]. In this context, administration of metformin normalized metabolic, oxidative and histopathological changes in STZ-induced type 1 diabetic rats [17]. In addition, metformin reduced neuroinflammation and improved spatial memory in mice [18].

Despite the therapeutic benefits for the treatment of DM, most of the drugs can produce some undesirable side effects [19]. Medical plants and their bioactive substances are more affordable and have fewer side effects compared with synthetic drugs, and are more effective in treatment of diabetes mellitus and its complications [20,21]. Regarding the fact that saffron and its main constituents, crocin and safranal exerts more beneficial effects on central nervous system disorders such as depression, convulsion and Alzheimer's disease [22], this study was planned to investigate the effect of separate and combined treatments of safranal and metformin on learning and memory impairments caused by diabetes using the Morris water maze (MWM) tasks. Histopathology and biochemistry of the hippocampus were also designed to this study. The MWM is the most frequently used laboratory tool to investigate spatial and long term memory in rodents by observing and recording escape latency, distance moved, and velocity during the time spend in the MWM water tank [23].

2. Materials and methods

2.1. Animals

We used healthy adult male Wistar rats, weighing 180–210 g throughout the study. Rats were maintained in a light-dark cycle (light on at 07:00 h) at a controlled ambient temperature (22 ± 0.5 °C) with ad libitum food and water. All experiments were performed between 10:00 h and 15:00 h. Veterinary Ethics Committee of the Faculty of Veterinary Medicine of Urmia University approved research and animal care procedures (AECVU-161-2018).

2.2. Chemicals

The following chemicals were used in the present study: safranal (Kosher, purity of ≥ 88%), and metformin hydrochloride. Safranal and metformin were purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. Analytical chemicals such as sodium dodecyl sulphate, acetic acid and thiobarbituric acid were purchased from Merck Chemical Co., Darmstadt, Germany.

2.3. Treatment groups

Rats were divided into the following groups:

Control groups (n = 11): Three days after an intraperitoneal injection of citrate buffer, six rats received intraperitoneal injection of normal saline plus two drops of Tween 10% (as a vehicle of safranal) and the other five rats received intra-gastric administration of normal saline (as a vehicle of metformin) for 37 days, respectively.

Diabetes groups (n = 11): Three days after an intraperitoneal of STZ, six rats received intraperitoneal injection of normal saline plus two drops of Tween 10% and the other five rats received intra-gastric administration of normal saline for 37 days.

Safranal (0.025 mg/kg) groups (n = 11): Six diabetic rats induced by STZ and five non-diabetic rats received safranal at a dose of 0.025 mg/kg for 37 days.

Safranal (0.1 mg/kg) groups (n = 11): Six diabetic rats induced by STZ and five non-diabetic rats received safranal at a dose of 0.1 mg/kg for 37 days.

Safranal (0.4 mg/kg) groups (n = 11): Six diabetic rats induced by STZ and five non-diabetic rats received safranal at a dose of 0.4 mg/kg for 37 days.

Metformin (50 mg/kg) groups (n = 11): Six diabetic rats induced by STZ and five non-diabetic rats received metformin at a dose of 50 mg/kg for 37 days.

Metformin (200 mg/kg) groups (n = 11): Six diabetic rats induced by STZ and five non-diabetic rats received metformin at a dose of 200 mg/kg for 37 days.

Safranal (0.025 mg/kg) plus metformin (50 mg/kg) groups (n = 11): Six diabetic rats induced by STZ and five non-diabetic rats received safranal (0.025 mg/kg) plus metformin (50 mg/kg) for 37 days.

The purpose of adding five rats to each group was to explore the effects of safranal and metformin and their combination treatments in non-diabetic rats on respective parameters. The results of these groups were compared with control group. In our previous studies in which the effects of histidine and n-acetylcysteine have been investigated on doxorubicin-induced cardiomyopathy and peripheral neuropathy, additional rats were used to clarify the effects of histidine and n-acetylcysteine on doxorubicin non-treated rats [24,25].

Safranal was dissolved in normal saline with adding two drops of Tween 10% and intraperitoneally injected at a volume of 2 ml/kg. A suspension of metformin in normal saline was prepared and gave by gavage at a constant volume 0.3 ml/rat. During learning training and memory session (days 40–45), the animals did not receive any treatments (Fig. 1). The doses of safranal and metformin used here were designed according to previous studies in which safranal (0.05-0.8 mg/kg) and metformin (25–100 mg/kg and 300 mg/kg) were used [26–28]. Time table and treatments schedule used in the present study are shown in Fig. 1.

2.4. Induction and confirmation of diabetes

Diabetes mellitus was induced in 12 h-fasted rats by a single intraperitoneal injection of freshly prepared STZ (55 mg/kg). STZ was dissolved in sodium citrate buffer (0.1 M, pH 4.5). STZ (2-deoxy-3-(3-methyl-3-nitrosourea)-1-D-glucopyranose) is a naturally occurring compound produced by the soil bacterium *Streptomyces achromonogenes* actinomycetes [29]. STZ has been used alone or in combination with other chemicals or with dietary manipulations for induction of T1DM

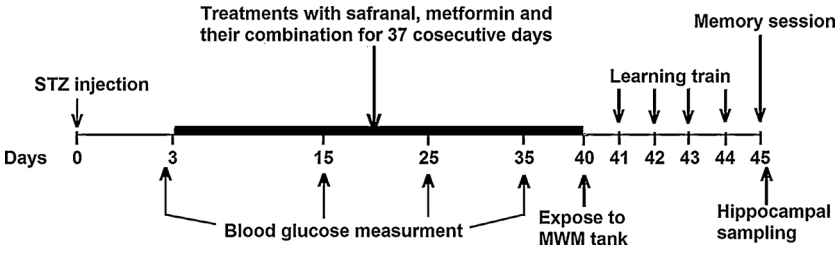

Fig. 1. Time table and treatments schedule used in the present study. STZ was administered on day 0. Diabetes was confirmed on days 3 and then treatment schedule was begun and continued for 37 days. Animals had no treatment during MWM tank exposing, learning training and memory session. Blood glucose levels were measured on days 15, 25 and 35 in 12 h-fasted rats.