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Effect of hesperidin on neurobehavioral, neuroinflammation, oxidative stress and lipid alteration in intracerebroventricular streptozotocin induced cognitive impairment in mice

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ABSTRACT

Recent attention is given to the influence of dietary supplementation on health and mental well-being. Oxidative stress is associated with many diseases including neurodegenerative disorders. Dietary flavonoids exert chemopreventive and neuroprotective effects and comprise the most common group of plant polyphenols that provide much of the flavour and colour of the vegetables and fruits. Hesperidin is a flavanone glycoside found abundantly in citrus fruits, has been reported to have antioxidant, hypolipidaemic, analgesic and anti-hypertensive activity. Pretreatment of hesperidin (100 and 200 mg/kg body weight orally once daily for 15 days) to Swiss male albino mice has prevented the cognitive impairment. The cognitive impairment was developed by giving single intracerebroventricular-streptozotocin (ICV-STZ) injection (2.57 mg/kg body weight each side) bilaterally. Hesperidin pretreatment improved memory consolidation process as tested by Morris water maze possibly through modulation of acetylcholine esterase activity (AChE). Moreover, hesperidin attenuated the depleted content of reduced glutathione (GSH) and elevated level of thiobarbituric acid reactive substances (TBARS) and also augmented lipid alteration significantly following ICV-STZ injection. We also demonstrated that the flavonoid hesperidin modulates neuronal cell death by inhibiting the overexpression of inflammatory markers like nuclear factor κB, inducible nitric oxide synthase, cyclooxygenase-2 and glial fibrillary acidic protein positive astrocytes. The results from the present study open the possibility of using flavonoids for potential new therapeutic strategies for sporadic dementia of Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia in elderly people and in western industrialised nations. AD is characterized by two classical pathological hallmarks in the brain: extracellular neuritic plaques composed of a dense amyloid core of β-amyloid peptide (Aβ) and (ii) intracellular neurofibrillary tangles (NFTs) composed of an abnormally phosphorylated form of the tau protein. In addition, AD is also associated with neuronal loss and increased neuro-inflammation [1]. Neuronal loss is usually prominent in the hippocampus, especially the CA1 region, and is further detected throughout the cerebral cortex, increasing with disease progression [2]. In addition, postmortem studies have also demonstrated significant inflammatory changes in the brain tissue from AD patients [3]. It is well established that AD pathology is closely related to altered lipid metabolism [4]. In neurons, pathogenic Aβ production, low levels of gangliosides and lipid abnormalities may contribute to the pathological conditions in AD [5]. Gangliosides concentrations were found significantly lower in patients with dementia of the Alzheimer's type than the concentrations in normal patients [6].

Intracerebroventricular (ICV) administration of sub-diabetogenic dose of betacytotoxic drug streptozotocin (STZ) produces long-term and progressive learning and memory deficits in rats. It also impairs cerebral glucose/energy metabolism and ATP generation that leads to oxidative stress and inflammation, which resemble to those found in the brain of sporadic Alzheimer's disease (SAD) patients [7,8]. Furthermore, ATP depletion leads to degradation of membranous

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phospholipids [9]. ICV-STZ leads to the hyperphosphorylation of tau protein in the hippocampus, and some reports suggested β-amyloid accumulation in the meningeal capillaries was observed, that further supports the resemblance of this experimental model to human sAD [10,11]. Nowadays, it is a well established model to study the therapeutic intervention for sporadic dementia of Alzheimer's type (SDAT).

The bioflavonoid, hesperidin is a specific glycoside which is frequently found in oranges and lemons. Hesperidin possesses significant antioxidant [12], analgesic [13], hypolipidemic [14], anti-hypertensive and diuretic activity [15]. Further, hesperidin significantly contributes to the intracellular antioxidant defence system and has been reported to act as a powerful agent against superoxide, singlet oxygen and hydroxyl radicals [16]. Besides that, this compound has an important neuroprotective property related to diverse neuronal insults such as ischemia, oxidative-induced damage and dopamine-induced neurotoxicity [17–19]. The *in vitro* data suggested that hesperidin acts as a neuroprotective agent against amyloid β induced toxicity [17], but its neuroprotective efficacy in animal model of SDAT is still undetermined. In the light of the above properties of this compound, we have attempted to investigate the potential anti-inflammatory, antioxidative and neuroprotective effects of hesperidin on ICV-STZ induced mouse model of SDAT. This study could lead to open a new therapeutic intervention, since there is still no curative therapy for AD and other neurodegenerative diseases.

2. Materials and methods

2.1. Chemicals and reagents

Reduced glutathione (GSH), thiobarbituric acid (TBA), trichloroacetic acid (TCA), streptozotocin (STZ), acetylthiocholine iodide, 5-5′-dithio-bis-2-nitrobenzoicacid (DTNB), resorcinol, N-acetyl neuraminic acid and hesperidin were purchased from Sigma-Aldrich, Chemicals Pvt. Ltd., India. Cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) were purchased from Santa Cruz Biotechnology, Santa Cruz, CA, USA. Glial fibrillary acidic protein (GFAP) and nuclear factor- κB (NF-κB) were from Abcam, Cambridge, MA, USA. Goat anti rabbit secondary antibody and normal goat serum was purchased from Jackson Immuno Research, West Grove, PA, USA. Avidin biotin complex (ABC kit) and 3,4-diaminobenzidine (DAB) were from Vector Laboratories Ltd. UK. Other chemicals were of analytical reagent grade.

2.2. Animals

The experiments were carried out in one-year-old Swiss male albino mice, weighing 35–40 g, obtained from the Central Animal House Facility of Hamdard University. They were kept in colony cages and maintained under standard housing conditions (room temperature of 25 ± 2 °C and relative humidity 45–55%) with 12 h light/dark reverse cycles. The standard rodent pellet diet and water were available *ad libitum*. Experiments were conducted in accordance with the Animal Ethics Committee of Hamdard University chaired by a Government of India nominee.

2.3. Experimental procedure

2.3.1. Experiment I

This experiment was carried out to evaluate the pre-treatment effect of hesperidin (H) (100 and 200 mg/kg body weight (b. wt.) intraperitoneally in normal saline once daily for 15 days) on the contents of TBARS, GSH and activity of AChE.

The mice were divided into five groups of 10 animals each.

Group I Vehicle injected control (C).
Group II ICV-STZ infused and vehicle treated lesion (L).
Group III ICV-STZ infused H 100 mg/kg b. wt. pre-treated (H100 + L).
Group IV ICV-STZ infused H 200 mg/kg b. wt. pre-treated (H200 + L).
Group V Vehicle injected H pre-treated (H200 + C).

2.3.2. Experiment II

This experiment II was carried out to evaluate the pre-treatment effect of H on lipid profile and immunohistochemical study of iNOS, GFAP, COX-2 and NF-κB in ICV-STZ-infused mice. The mice were divided as in experiment I and each group has 12 mice.

2.4. Intracerebroventricular (ICV) infusion of streptozotocin

Swiss albino mice weighing 35–40 g were anaesthetized with 685.71 mg/kg b. wt. of chloral hydrate i.p. and placed on a dual manipulator stereotaxic frame and skull was exposed. The stereotaxic coordinates for the lateral ventricle were measured accurately as anterio-posterior −0.8 mm, lateral ±1.0 mm and dorso-ventral −3.0 mm relative to bregma and ventral from dura with the tooth bar set at 0 mm. Burr hole was made in the skull by automatic micro drilling machine attached on one arm of the stereotaxic apparatus. Through the hole, a 28-gauge Hamilton® syringe of 10 μl attached to another arm of stereotaxic apparatus micro-injector unit and piston of the syringe was lowered manually into each lateral ventricle. The lesion groups received a bilateral ICV injection of STZ (2.57 mg/kg, b. wt. in saline, 2 μl/injection sites). The control groups underwent the same surgical procedures, but same volume of saline was injected instead of STZ.

2.5. Behavioural testing

The behavioural tests were started 2 weeks after ICV-STZ infusion. The experiment was performed between 9.00 A.M. and 4.00 P.M. at standard laboratory conditions and data were analysed by the blind observer of the experimental conditions (Fig. 1).

2.5.1. Morris water maze test

Spatial learning and memory of animals were tested in Morris water maze [20]. It consisted of a circular water tank (132 cm diameter and 60 cm height) that was filled with 30 cm of water (25 ± 2 °C). A non-toxic white paint was used to render the water opaque and mice were coloured with black hair dye. The pool was divided virtually into four equal quadrants, labelled north–south–east–west. An escape platform (10 cm in diameter) was hidden 1 cm below the surface of water on a fixed location in one of the four quadrants of the pool. The platform remained in the same quadrant throughout experiment. Before the training started, mice were allowed to swim freely into the pool for 60 s without platform. They were given four trials (once from each starting position) per session for 5 days, each trial having a ceiling time of 60 s and a trial interval of approximately 30 s. After climbing on to the platform, the animal remained there for 30 s before the commencement of the next trial. If mice failed to reach the platform within the maximum allowed time of 60 s, it was gently placed on the platform and allowed to remain there for the same interval of time. An overhead video camera was connected to a video monitor and computer software was used to calculate the escape latency.

On the 6th day, a probe test was conducted by removing the platform. Mice were allowed to swim freely into the pool for 60 s. The time spent in the target quadrant, which had previously contained hidden platform was recorded. The time spent in the target quadrant indicated that the degree of memory consolidation has taken place after learning.