Biomedicine & Preventive Nutrition 4 (2014) 519–525

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Original article

Neuroprotective effect of *Prunus avium* on streptozotocin induced neurotoxicity in mice

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ARTICLE INFO

Article history:
Received 27 June 2014
Accepted 4 August 2014

Keywords:
Neurotoxicity
Cognition
Oxidative stress
Acetylcholinesterase
Streptozotocin
Corticosterone

ABSTRACT

Objective: To evaluate the anti-amnesic and neuroprotective activity of ethanolic extract of *Prunus avium* (EEPA) on streptozotocin (STZ) induced neurotoxicity in mice.
Methods: The mice were pre-treated with EEPA at selective doses (200, 400 mg/kg, p.o.) for a period of 3 weeks followed by intracerebroventricular injection (ICV) of STZ (0.5 mg/kg). Neurobehavioral-alterations were evaluated using Y-maze and elevated plus maze. Biochemical markers, such as acetylcholinesterase (AChE), corticosterone, thiobarbituric reactive substances (TBARS), tissue nitrite, antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase were estimated.
Results: Obtain results revealed those 28 days of treatment with EEPA was effective in averting neurotoxicity. EEPA supplementation significantly reduced AChE, corticosterone, TBARS, tissue nitrite levels and ameliorated the deficits in learning and memory impairment with increased levels of antioxidants.
Conclusions: These results envisage that ethanolic extract of *Prunus avium* exhibit cognitive improvement which is most likely related, at least in part, to its antioxidant and neuroprotective activity. Further studies are suggested to evaluate the isolated bioactive *Prunus avium* fruits to identify the molecular mechanism involved in modulation of cholinergic transmission.
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1. Introduction

Alzheimer's disease (AD) type of dementia is associated with neurodegeneration due to accumulation of neurofibrillary tangles, senile plaque deposits and neuroinflammation leading to progressive decline in cognition. The neurotransmitter acetylcholine imperatively plays a role in the hippocampus of brain for learning and memory, and in contrast, the loss of cholinergic activity correlates with impairment of cognition. The amyloid hypothesis emphasizes that increased beta amyloid (Aβ) production or failure of Aβ clearance which induces gradual Aβ accumulation, result in the formation of plaques. Aβ aggregates induce changes in calcium influx [1], increases oxidative stress [2], and activates inflammatory mediators, such as microglia and astrocytes [3,4]. Activated microglia cells in AD release a variety of pro-inflammatory mediators, such as cytokines, reactive oxygen species (ROS), and nitric oxide which can contribute to neuronal dysfunction and cell death [5].

Oxidative stress is one of the major causes of cell damage in central nervous system, and is seen in many neurodegenerative disorders, including AD. The free radical produced during oxidative stress causes oxidation of DNA and RNA indicated by elevated levels of 8-hydroxyl-2-deoxyguanosine and 8-hydroxy guanosine [6,7], increased levels of protein carbonyl residues [8,9] and the lipid peroxidation (LPO) is marked by higher levels of thiobarbituric acid reacting substances (TBARS), malondialdehyde (MDA), 4-hydroxy *trans* neoneal (HNE) and isoprostrane [10,11]. Impaired insulin signaling leads to increased oxidative stress (Agarwal et al., 2010). Cholinergic neurons are among the earliest to succumb to the neurotoxic actions. Oxidative stress also increases the expression of AChE, decreases the activity of choline acetyltransferase, the enzyme that synthesizes acetylcholine activity [12]. Moreover, oxidative stress stimulates hypothalamic–pituitary–adrenal (HPA) axis, which release the glucocorticoids from adrenal cortex. Dysregulation of the HPA axis results in elevated circulating cortisol levels [13–15], which leads to shrinkage in hippocampal volume and memory impairment.

Prunus avium is a deciduous tree belonging to the family Rosaceae, commonly called wild cherry. The fruit of *P. avium* is a drupe, bright red to dark purple, edible, variably sweet to astringent and bitter to eat. The fruits are rich sources for flavonoids. The

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http://dx.doi.org/10.1016/j.bionut.2014.08.004
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medicine prepared from the stalks of the fruits possesses diuretic, astringent, anti-tussive property. Fruits have been reported for its antioxidant activity, antiproliferative and anti-cancer properties, anti-inflammatory effects, protection against cardiovascular diseases and retarding of aging process [16–18]. Herein, the study was designed to evaluate the anti-amnesic and neuroprotective activity of EEPA on STZ induced neurotoxicity in mice. Pharmacological investigations were carried out by assessing the behavioural parameters, such as Y-maze in video tracking system, elevated plus maze and open field exploration. The biochemical changes in brain were evaluated by estimating the corticosterone level, AChE level and tissue nitrite and antioxidant enzymes after the treatment with EEPA.

2. Materials and methods

2.1. Drugs and reagents

Streptozotocin, 5,5′-dithiobis (2-nitrobenzoic acid), eserine, acetylthiocholine iodide, reduced glutathione, NADPH, pyrogallol, dexamethasone and DPPH were procured from Sigma Aldrich, USA. All other chemicals were purchased from S. D Fine chemicals Pvt LTD, India.

2.2. Plant material and extraction

The *P. avium* fruits were collected from commercial distributor, Hyderabad and authenticated by pharmacognosist Dr. Babashankar Rao, School of Pharmacy, Hyderabad and a voucher specimen (No: LCP/COG/3256) was deposited in department of Pharmacognosy. The obtained fruits were deseeded and shade dried. An amount of 1 kg of dried sample was homogenized with sufficient amount of double distilled absolute ethanol (99.9%) at room temperature. After homogenization, it was filtered and concentrated using rotary vacuum evaporator (Heidolph: 5690005000). The resultant thick consistent drug material was placed in an airtight container; the yield was noted as 5% and stored in refrigerator to avoid microbial contamination.

2.3. Experimental design

2.3.1. In vitro evaluation

2.3.1.1. DPPH radical scavenging activity assay. The free radical scavenging activity of crude extracts on 2, 2-di-phenyl-2-picrylhydrazyl (DPPH•) radical was measured by reduction of DPPH• to DPPHH (Di-phenyl picryl hydrazine) [19,20]. IC_{50} value was determined as the inhibitory concentration of extract that could scavenge 50% of the DPPH radicals. Ascorbic acid was used as positive reference.

2.3.1.2. Reducing power assay. An aliquot of the extract of *P. avium* (125 μL) was mixed with125 μL of sodium phosphate buffer (0.2 M, pH 6.6) and 125 μL of 1% $K_3Fe(CN)_6$ followed by incubation at 50 °C for 20 min. After adding 125 μL of 10% trichloroacetic acid, the mixture was centrifuged at 3750 *g* for 10 min. The supernatant solution (100 μL) was mixed with 100 μL of double distilled water and 20 μL of 1% ferric chloride to react for 10 min. Subsequently, the absorbance was measured at 700 nm. The EC_{50} value is the concentration of sample at which the absorbance is 0.5. L-Ascorbic acid was used as thr positive reference [21].

2.3.1.3. Assay of nitric oxide scavenging activity. Nitric oxide scavenging activity can be estimated by the use of Griess Ilosvoy reaction [22]. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. Inhibition of nitrite formation by the EEPA extract and the standard antioxidant ascorbic acid were calculated relative to the control. Inhibition data (percentage inhibition) were linearized against the concentrations of each extract and standard antioxidant. IC_{50}, which is an inhibitory concentration of extract required to reduce 50% of the nitric oxide formation, was determined.

2.3.2. In vivo pharmacological study

2.3.2.1. Experimental animals. Male Swiss albino mice, weighing 20–25 g, at the age 4–5 weeks were used in the study and given standard laboratory pellet chow diet; Provimi limited (India), provided water *ad libitum* and were kept under standard conditions at 23–25 °C, 35 to 60% humidity, and 12 h light/dark cycle. The mice were acclimatized to the laboratory conditions a week prior to experiment. The experimental protocol (No: I/IAEC/LCP/001/2012/SM/24) was duly approved by institutional animal ethics committee (IAEC).

2.3.2.2. Acute toxicological studies. The procedure was followed by using OECD 423 annexure (D) acute toxic class method [23]. The acute toxic class method is a stepwise procedure with three animals of a single sex per step. The starting dose level of EEPA was 2000 mg/kg body weight p.o. using water as vehicle. Drug was administered to overnight fasted female mice. Food was withheld for a further 3–4 hours after administration of EEPA and observed for signs of toxicity.

2.3.2.3. Induction of neurotoxicity and grouping. Neurotoxicity was induced by ICV injection of STZ (0.5 mg/kg) by identifying the bregma point on skull; in brief, bregma point was identified according to [24,25]. Approximately 1–3 mm rostral to the line is drawn through anterior base of ears, then at 45° angle, the needle was inserted 2 mm lateral to midline and STZ was injected. Animals were divided into four groups of each six. Group I was treated with ICV injection of phosphate buffered saline (PBS) alone; group II was injected with two doses of STZ (0.5 mg/kg) ICV injection bilaterally in PBS. The second dose was administered after 48 hours of the first dose. Group III and group IV were pre-treated with EEPA 200 mg/kg and 400 mg/kg p.o., respectively for 21 days. On 21st day, group III and group IV were injected with STZ ICV and the second dose was administered after 48 hours of the first dose and the treatment with EEPA was continued for 28 days. All the behavioural parameters are evaluated two days before termination of the study. On 28th day, the animals were sacrificed and the brains was isolated for biochemical estimations.

2.4. Behavioural evaluation

2.4.1. Video tracking in Y-Maze

Video tracking (VJ, Instruments) in Y-maze is assisted with software was used to measure the spatial working memory in mice for 8 minutes. Mice tend to explore maze systematically, entering each arm in turn. The series of arm entries, including possible returns into the same arm is recorded by using the video tracking system and the percentage alteration is obtained by the assistance of the software. Alteration is defined as the successive entries into the three arms, on overlapping triplet sets. [26].

2.4.2. Elevated plus maze test

This task has been widely validated to measure anxiety-like behaviour in rodents. The entire maze is elevated to height of 50 cm above the floor level. Mice were housed in pair for 10 days prior to testing in the apparatus. During this time, the mice are handled by the investigator on alternate days to reduce the stress. One hour after oral administration of the extract, each mouse was placed in the center of the maze facing towards one of the open arm. During

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