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Original Article

Quercetin nanoparticles attenuates scopolamine induced spatial memory deficits and pathological damages in rats

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ABSTRACT

Quercetin is a well-known flavonoid, has low bioavailability. Quercetin nanoparticles (NQC) enhance its bioavailability. NQC were not explored for their potential therapeutic activities in Alzheimer's disease (AD). Hence, the present study was performed to evaluate the protective effect of NQC in comparison to free quercetin against scopolamine induced spatial memory impairments.

NQC prepared by anti solvent precipitation method. Quercetin, NQC (30 mg/kg p.o.) and rivastigmine (2 mg/kg i.p.) as a reference drug were administered for 8 consecutive days. At the end of the treatment period memory impairments were induced by a single injection of scopolamine (20 mg/kg; i.p.). Conditioned avoidance and rectangular-maze tests were conducted 30 min thereafter then rats were sacrificed and brain homogenates were used for the estimation of glutathione (GSH), catalase and malondialdehyde (MDA) contents together with acetyl cholinesterase (AchE) activity. In addition, histopathologic studies were also performed.

The size of NQC was observed below 300 nm. NQC significantly reduced the transfer latency and conditioned avoidance response compared to scopolamine treated group ($p < 0.05$). Pretreatment with NQC showed a significant ($p < 0.05$) decrease in MDA, AchE levels and increase in brain catalase and GSH levels to be similar to that observed in the rivastigmine group.

In all the behavioral, biochemical and histological experiments, the rats treated with NQC showed additional distinguished results compared to quercetin group indicating that a preventive strategy against the progression of AD. This approach of quercetin nanoparticles provides the potential therapeutic application in human neurodegenerative disease in future.

1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disorder with the progressive decline in memory [1]. AD is characterized by cerebral oxidative stress accompanied by loss of cholinergic neurons in the basal forebrain and hippocampus [2,3]. Central cholinergic neuronal activity plays an important role in learning and memory [4]. Multiple neurotransmitters and neuronal pathways are involved in the process of memory formation [5]. Functional deficits in the cholinergic system are associated with cognitive impairments observed in AD [6].

Scopolamine a muscarinic cholinergic receptor antagonist has profound amnesic effects in experimental animals. Scopolamine induced amnesia has been widely adopted the experimental animal model to screen for drugs with potential therapeutic values in dementia [7] it interferes with acetylcholine transmission in the central nervous system, leading to cholinergic dysfunction and memory impairments in rats [8,9].

Acetyl cholinesterase inhibitors, such as rivastigmine, galantamine and donepezil, are the most effective and approved pharmacotherapeutic agents for cognitive dysfunction [10]. Rivastigmine is a pseudo irreversible inhibitor of both acetylcholinesterase and butyrylcholinesterase [9]. Despite intensive advancement in research, available drugs are not ideal for clinical use due to their undesirable side effects [11,12] thus, it is necessary to search for alternative or adjuvant anti amnestic therapies. Today, it is best known that medicinal plants attracted attention due to their use in the treatment of cognitive disorders [13].

Quercetin is a well-known flavonoid in the human diet, present in vegetables, herbs, edible fruits and other related products eg. Red wine [14], Ginko Biloba [15] and onions [16]. Quercetin is one of the prominent dietary antioxidants it shows biological

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effects that include protection against certain forms of cancer [17], inflammation [18] and cardiovascular diseases [19]. Despite these medicinal benefits, quercetin has low bioavailability (less than 17% in rats and even 1% in human) [20,21] due to its poor aqueous solubility, as a result, the clinical application of this drug greatly restricted. Therefore, it becomes necessary to develop a system which could increase the solubility of quercetin.

Nanoparticles are particularly suitable for drug delivery for water insoluble compounds such as quercetin. According to Noyes-Whitney equation [22] a decrease in particle size will lead to an increase in effective surface area which results in enhanced bioavailability. In the present study, quercetin nanoparticles (NQC) prepared by anti solvent precipitation method using syringe pump to enhance its bioavailability in therapeutic application. The aim of our present study was to evaluate the protective effect of NQC in comparison to free quercetin against scopolamine induced spatial memory impairments.

2. Materials and methods

2.1. Materials

Rivastigmine used as a reference drug obtained from Vasudha Pharma Chemicals Ltd, Hyderabad, India. Scopolamine hydrobromide used as a dementia inducing agent obtained from Boehringer Ingelheim, Quercetin, 5,5-dithio-bis2-nitro benzoic acid, (Ellman's reagent), acetylthiocholine iodide, were purchased from Sigma–Aldrich (USA), the absolute ethanol (99.5–99.8%) was obtained from Merck, Mumbai, India. All other reagents used were also of analytical grade.

2.2. Preparation of NQC [23]

Quercetin was dissolved in the solvent (ethanol) at a concentration of 5 mg/ml. The syringe was filled with the prepared solution and secured onto a syringe pump. The drug solution was quickly injected at a fixed flow rate (8 ml/min) into the anti-solvent (deionized water) of definite volume under magnetic stirring (1000 rpm). Ethanol to water volume ratios used was 1:25. The quercetin nanoparticles were filtered and vacuum dried.

2.3. Particle morphology

The particle size and morphology of samples was observed using a Scanning Electron Microscope (SEM) Zeiss EVO 18-EDX special edition machine compatible with EDX machine. The powder samples were spread on a SEM stub and sputtered with gold before the SEM observations. The particle size and texture of nanoparticles can be analyzed by using image magnification software compatible with SEM and helps in determining the presence and formation of NQC. Five SEM pictures were used to find the average range of particle diameter.

2.4. Animals

The study was performed on male Albino Wistar rats (150–250 g). All animals were procured from Mahaveera enterprises, Hyderabad. The animals were maintained under a controlled 12 h light/dark cycle. Handling and experimentation were conducted in accordance with the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi and the experimental protocol was approved by Institutional Animal Ethical Committee (IAEC), Kakatiya University Warangal.

2.5. Treatments

In both behavioral tasks such as conditioned avoidance test and rectangular maze, rats were randomly allocated into five groups (6 rats each) as follows: group I received saline and served as control while group II received scopolamine (20 mg/kg, i.p.). Groups III–V received rivastigmine (2 mg/kg, i.p.), Quercetin (30 mg/kg, p.o.), NQC (30 mg/kg, p.o.). The animals were trained for 7 days. During which they do not receive any drug. The completely trained animals were chosen for the study. These animals were dosed once in a day with the respective drugs for 8 days along with daily training trial. Scopolamine was administered as a single dose 30 min after the last administration in groups II–V.

2.6. Behavioral experiments

2.6.1. Conditioned avoidance test

The test was carried out using a shuttle box as described by Hinrichs et al. [24]. In this experiment, the rat is placed in a two-compartment shuttle box and presented with a conditioned stimulus such as a light, followed after a short delay by an aversive unconditioned stimulus foot-shock. After injection of scopolamine, each rat was placed in the shuttle box and allowed to adapt for 3 min. Following adaptation, the conditioned stimulus was presented for 20 s prior to the unconditioned stimulus. If the rat crossed to the next compartment during 20 s of conditioned stimulus the electric shock was avoided otherwise, failure of avoidance was recorded.

2.6.2. Rectangular maze test

Rectangular maze is used for studying learning, memory in animals. The maze consists of completely enclosed rectangular box divided into chamber A, in which the rat is placed and has a sliding door that is opened to allow the rat to enter the maze; chamber C. The maze, animal has to explore and reward chamber B, at the other end of maze in which the reward is kept. Well-trained animals were taken for the experiment. Transfer latency (Time taken in seconds by the animal to reach reward chamber from chamber A) was recorded. For each animal, four readings were taken and the average is taken as learning score (transfer latency) for that animal. Lower scores of the assessment indicate efficient learning while higher scores indicate poor learning in animals [25].

2.7. Brain homogenate preparation

Immediately after performing the behavioral tests, rats were sacrificed by decapitation. The brains were removed; a (10% w/v) homogenate was prepared in ice-cold 50 mM phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 15 min and aliquots of supernatant were separated and used for biochemical estimation.

2.8. Histopathologic examination of brain tissues

The histopathologic assessment was performed on the brains of different groups. Brains were removed from the skull and post-fixed overnight in paraformaldehyde. Coronal sections of 5 μm thickness were cut using microtome and the sections were stained with hematoxylin and eosin and examined microscopically.

2.9. Determination of biochemical markers in brain homogenate

The method for the assessment of MDA content in the brain homogenates was based on that of Ruiz-Larrea et al. [26] the supernatant was read spectrophotometrically at 532 nm and MDA brain content was expressed as nmol/mg tissue. GSH level

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