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Therapeutic evaluation of solid lipid nanoparticle of quercetin in pentylenetetrazole induced cognitive impairment of zebrafish

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ABSTRACT

Background: Quercetin is a major flavonoid in various plants. It possesses the multiple pharmacological actions like vascular integrity and regulatory action of the blood-brain barrier.
Aims: The present study is focused on evaluating the role of solid lipid nanoparticle of quercetin in pentylenetetrazole (PTZ) induced cognitive impairment of *Danio rerio* species.
Main methods: The memory impairment of zebrafish was induced by exposing of PTZ in 7.5 mM solution. The pretreatment of solid lipid nanoparticle of quercetin (SLN-Q; 5 and 10 mg/kg) was administered by single intraperitoneal (*i.p.*) injection. The reference control i.e., donepezil (10 mg/kg) was administered by single intraperitoneal (*i.p.*) injection. The learning and memory levels were evaluated with different tests like light and dark chamber test; partition preference test; and three (horizontal) compartment tests. In addition, the PTZ induced biochemical changes such as acetylcholinesterase activity, lipid peroxidation, and reduced glutathione levels were assessed in the brain of zebrafish.
Key findings: The solid lipid nanoparticle of quercetin found to possess the attenuating effect in PTZ induced neurocognitive impairments along with amelioration of biochemical changes. This effect is similar to that of donepezil pretreated group.
Significance: Therefore, this solid lipid nanoparticle of quercetin can be used as future nanomedicine for various neurodegenerative disorders like Alzheimer and Parkinson disorders due to its potential anti-oxidative, anti-lipid peroxidative and acetylcholinesterase inhibitory actions.

1. Introduction

Learning is the process of acquisition from any new information about the event occurring in the surroundings; whereas, the retrieving of this information is referred as memory [1,2]. The various animal models like rat mice, fruit fly (*Drosophila melanogaster*) and *Caenorhabditis elegans* are employed for the screening of nootropic drugs [3]. Currently, zebrafish (*Danio rerio*) is also employed in the assessment of neurodegenerative disorders [4]. This model closely mimics the human gene as well as neurophysiology and neuropharmacological actions [5,6]. This model is widely accepted for multiple neurological disorders including memory dysfunction [7,8]. Neurological system is one of the high energy utilizing tissue. The lack of nutrients and abundant generation of free radicals are well documented to produce the neuronal damage as well as neurodegeneration [9,10]. Pentylenetetrazole (PTZ) is one of the potent CNS stimulant and epileptogenic agent. However, this agent is able to damage the neuron via over-activation of free radicals and apoptotic process. In the rodent, it is used for the induction of convulsant [11]. In addition, it also modulates the neurotransmitters levels in the brain especially acetylcholine levels [12,13]. Therefore, it causes the transient impairment of motor impairment as well as memory dysfunction.

Quercetin is a flavonoid and it has polyphenolic chemical substructure. In a biological system, it acts as antioxidants and it is known to protect the tissue from free radicals [14]. Currently, it is identified as potent anti-cancer, anti-inflammation, cardioprotective and neuroprotective actions [15,16]. Further, the quercetin has lower bioavailability and partial permeability of blood-brain barrier (BBB) [17,18]. Therefore, the clinical approach of quercetin utilization is delayed. However, newer formulation of quercetin with potent bioavailability and blood-brain barrier crossing ability may improve the therapeutic potency and efficacy to ameliorate the various disorders [19]. Recently, nanoparticle formulation plays a promising role in the crossing of BBB and neuro-specific action [20]. Solid lipid nanoparticles are known to produce target specific action and also able to cross the BBB [21,22]. Therefore, the present study designed to evaluate the solid lipid nanoparticle of

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quercetin (SLN-Q) in PTZ induced cognitive impairment in a zebrafish model.

2. Materials and methods

2.1. Animals

Wild-type adult (< 8-month-old male) Zebrafish was used in this study. In adult age; female zebrafish has variable reproductive hormone and it was documented to produces the negative results in the assessment neuropsychological behavior [23,24]. Hence, the male zebrafish animal species were selected in this study; to prevent the negative results in the assessment of neurocognitive behavior. The animal kept in 10 l (potable water) housing tank. Further, the tank was maintained with aerator for aeration (for making oxygen-enriched environment) process; the temperature i.e., 25 ± 2 °C was maintained with aquarium temperature; and 11 to 12 light and dark cycle of photoperiod for maintaining the normal circadian rhythmicity. The home cage tank was closed with rubber board to avoid diving outside of the tank and damage to the tissue. All the animals were acclimatized for 2 weeks before the performance of cognitive study test. All behavioral observation was performed between the 09.00 AM to 01.00 PM to avoid the hormone associated neurobiological interaction and neurobehavioral abnormalities.

2.2. Drugs and chemicals

Trichloroacetic acid, 5,5′-Dithiobis(2-nitrobenzoic acid), reduced glutathione, thiobarbituric acid, 1,1,3,3-Tetramethoxypropane, acetylthiocholine iodide, quercetin ($C_{15}H_{10}O_7$) and pentylenetetrazole ($C_6H_{10}N_4$) were purchased from Sigma chemical, India. Other chemicals (analytical grade) were procured from SD fine chemicals Ltd., Mumbai. Triple distilled water was used in the experiment. Quercetin ($C_{15}H_{10}O_7$) and pentylenetetrazole ($C_6H_{10}N_4$) were procured from Sigma chemical; and donepezil ($C_{24}H_{29}NO_3$) was procured from Intas pharmaceutical Ltd., India.

2.3. Preparation of solid lipid nanoparticle of quercetin (SLN-Q)

The solid lipid nanoparticle of quercetin (SLN-Q) was prepared by solvent evaporation method as described by [25,26]. Briefly, about 4 mg of poloxamer-188 was dissolved in 10 ml of water. The poloxamer was added as a surfactant. And, 3 ml of co-solvent i.e., propylene glycol was added with surfactant solution. This solution was used for the lipid formulation with 50 mg of stearic acid. Then, 10 mg of quercetin was dissolved in the 10 ml of ethanol and mixed with above lipid mixture. This formulation was kept for 45 min in magnetic stirrer. Thereafter, the formulation was sonicated for further 15 min and centrifuged at 11,200 g-force at room temperature (37 °C) for 15 min. The solid pellet was kept for freeze drying. The prepared SLN-Q was stored at 4 °C for further physiochemical testing (i.e., particle size, zeta potential, entrapment efficiency and drug loading capacity) and therapeutic evaluation.

2.4. Particle size and zeta potential of SLN-Q

The particle size and zeta potential of SLN-Q were assessed by dynamic light scattering method by using Microtrac Nanotrac Wave Nanotechnology Particle Size Analyzer (Betatek Inc., Toronto, Canada). The mean diameter of ultrafine particles of SLN-Q was analyzed by Nanotrac FLEX software with Nanotrac Wave-Particle Size Analyzer. The laser light frequency was shifted according to the Doppler Effect corresponding to the relative velocity of the particle. Laser diode light was scattered in all directions including 180 degrees backward. The power spectrum interference signal was calculated with high-speed FFT (Fast Fourier Transform) with digital signal processors. Particle size was evaluated corresponding to the volume of distribution. Further, the zeta potential of SLN-Q was analyzed by using same Microtrac Nanotrac Wave-Particle Size Analyzer (Betatek Inc., Toronto, Canada) with optical electric probes device. The pair of optical electric probes was arranged opposite manner to the sample cell. This probe was creating the electric field and induce the particle movements towards the opposite electrode charge. The motion of particle was analyzed by dynamic light scattering method. Even, the particle size distribution was determined by the velocity of particle distribution in dispersing medium. For zeta potential assessment, the particle electrophoretic mobility was calculated by this additional velocity component. Zeta potential was calculated by measurement of particle mobility and zeta potential relationships. This relationship was calculated by Smoluchowski equation: $\zeta = \mu\eta / \varepsilon$. Hence, ζ is zeta potential; μ is mobility of particles; η is the viscosity of particle; and ε is dielectric constant [ε = Vacuum permittivity: 8.854×10^{-12} F/M (farads per meter)] at 25 °C in water.

2.5. Entrapment efficiency and drug loading capacity of SLN-Q

The SLN-Q was separated from the free drug by using the Sephadex G-50 minicolumn centrifugation technique [27,28]. Briefly, 0.3 ml of SLN-Q suspension was placed in Sephadex G-50 minicolumn. This column was presaturated with empty nanoparticles with the same composition of suspension. The mini column was centrifuged at 112 g-force for 1 min, and the column washed seven times with 0.1 ml of distilled water. Elute contains the drug-loaded nanoparticles. The elute volume was adjusted to 25 ml with ethanol solution. These solutions were vortexed for 3 min for the breakdown of SLN-Q and complete dissolving of quercetin in the medium. Then, the mixture was centrifuged at 16,128 G- a force for 10 min to precipitate the crystallized salt and lipid particles. The amount of entrapped quercetin was assessed in the supernatant liquid by using an ultraviolet-visible spectrophotometer (DU 640B, UV-Spectrophotometer, Beckman Coulter Inc., CA, USA) at a wavelength of 373 nm. The entrapment efficiency and drug-loading of quercetin in the nanoparticles were calculated according to the following equations [29].

$$EE = \frac{W(\text{Entrapped drug})}{W(\text{Initial drug})} \times 100\%$$

$$DL = \frac{W(\text{Entrapped drug})}{W(\text{Nanoparticles})} \times 100\%$$

where W (Initial drug) represented the initial amount of quercetin added. W (Entrapped drug) represented the amount of quercetin entrapped in the nanoparticles. W (Nanoparticles) represented the total weight of nanoparticles with all components.

2.6. In-vitro drug release effect of quercetin from SLN-Q

The release of quercetin from SLN-Q was assessed by an in-vitro method using diffusion cell apparatus (EMFD-08, Orchid scientific & Innovative India Pvt. Ltd. Nasik, Maharashtra, India) with a dialysis membrane (molecular weight cutoff 10,000 Da). Briefly, the membrane was kept in double distilled water for 24 h. Then, the membrane was fixed in diffusion cell apparatus. The 2 ml suspension of SLN-Q was loaded in donor compartment and receptor compartment was filled with the dissolution medium i.e., phosphate buffer at pH 7.4. The temperature was maintained at 35 ± 0.5 °C with the continuous stirring process at 100 rpm. The 2 ml of aliquots were withdrawn at different time points 0, 1, 2, 3, 6 and 12 h. Thereafter, aliquots were filtered and replenished with 2 ml of the fresh buffer medium. The aliquots solution was mixed with 250 ml of ethanol and shaking for 15 min in ultrasonic bath; then, filtered through Whatman (No. 1) filter paper. The obtained solution is diluted with 1:1 ratio of 50% v/v ethanol and water mixture. The quercetin concentration was estimated by spectrophotometrically (DU 640B Spectrophotometer, Beckman