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Naringenin and its nanocarriers as potential phytotherapy for autism spectrum disorders

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ABSTRACT

The aim of the study was to investigate pharmacotherapeutic potential of Naringenin and its brain targeted nanoformulations in Autism Spectrum Disorders (ASD).

ASD like phenotype was induced by infusion of 1 M Propanoic acid into anterior portion of lateral ventricle in rats. Naringenin (25, 50 and 100 mg/kg), uncoated and coated (glutathione & tween 80) naringenin loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles (25 mg/kg) and minocycline (50 mg/kg) were administered orally for 29 days. Neurobehavioural, biochemical, blood-brain barrier permeability, TNF-α, MMP-9, HSP-70 and P-glycoprotein tests were performed.

Naringenin and its nanoparticles significantly restored behavioural and biochemical deficits in ASD phenotype. Glutathione and tween 80 coated nanoparticles enhanced brain delivery of NGN by inhibition of P-glycoprotein. Naringenin (100 mg/kg) and its nanocarriers (25 mg/kg) demonstrated pharmacological efficacy comparable to minocycline (50 mg/kg).

Naringenin and its coated nanocarriers have strong clinical potential as an adjunct neurotherapeutic moiety in attenuating neuropsychopathology associated with ASD.

1. Introduction

AUTISM SPECTRUM DISORDERS (ASD) is a neurodevelopmental disorder mainly affecting the social interaction ability and communication skills of an individual along with development of pervasive, restricted and stereotypic behaviour (Helverschou, Bakken, & Martinsen, 2011; American Psychiatric Association, 2013). Oxidative stress and mitochondrial dysfunction is either a result of prenatal or postnatal exposure to harmful environmental pollutants, toxins as well as viral or bacterial infections leading to epigenetic modifications or as a consequence of microglial activation and release of pro-inflammatory cytokines as a consequence of ROS generation and immune system activation (Rossignol & Frye, 2014).

Gut-brain axis is one the most common and important pathway that is very closely associated with ASD. Gut bacteria such as Clostridia, Desulfovibrio, Sutterella and Ruminococcus species produce short-chain fatty acids (SCFAs) such as propanoic acid (PPA) as a result of breakdown of dietary carbohydrates and amino acids (Finegold et al., 2002; Wang et al., 2013). Ingestion of carbohydrate rich-foodstuffs or those having PPA as preservative results in worsening of the behavioural as well as gastrointestinal complications associated with ASD children (Horvath, Papadimitriou, Rabsztyn, Drachenberg, & Tildon, 1999). PPA alters serotonin, dopamine and glutamate levels, stimulates inflammatory cytokines release, reduces glutathione, superoxide dismutase, enhances lipid peroxidase and mitochondrial dysfunction. It disrupts gap junction coupling (MacFabe et al., 2007; Shultz et al., 2009; Macfabe, 2012; Aldbass, Bhat, & El-Ansary, 2013). Intracerebroventricular administration of PPA in adolescent rats induced ASD phenotype (MacFabe et al., 2007; Bhandari and Kuhad, 2015, 2017).

(±)-Naringenin (5,7-Dihydroxy-2-(4-hydroxy phenyl) chroman-4-one) is a flavanone which is abundantly found in grapefruit as well as in oranges and tomato skin (Felgines et al., 2000). Naringenin possesses strong neuroprotective potential by virtue of its direct/indirect action on various pathological pathways (Kumar and Tiku, 2016; Wu et al., 2016). Despite its promising therapeutic potential, naringenin's clinical utility is limited due to its poor bioavailability and brain penetration (Yen, Wu, Lin, Cham, & Lin, 2009). In order to improve the bioavailability and enhance brain uptake, we developed naringenin loaded PLGA nanoparticles and further coated these with reduced glutathione (GSH) and tween 80 (our unpublished data).

With this background, our aim was to investigate therapeutic potential of naringenin as well as its coated and uncoated PLGA nanoparticles in the experimental paradigm of ASD.

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2. Material and methods

2.1. Animals and drugs

Three-Four months old male Sprague-Dawley rats (250–280 g) bred in the Central Animal House Facility of Panjab University, Chandigarh (India) were used. The experimental protocol was approved by Institutional Animal Ethics Committee of Panjab University, Chandigarh and was conducted according to Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines for the use and care of experimental animals.

2.2. Preparation of NGN-PLGA uncoated as well as coated nanoparticles

The NGN-PLGA nanoparticles were prepared by nanoprecipitation method reported by Fessi, Puisieux, Devissaguet, Ammoury, and Benita (1989) with minor modifications. The nanoparticles were lyophilized and suspended in 0.5% sodium carboxymethylcellulose for in-vivo studies (our unpublished data).

2.3. Induction of ASD phenotype and treatment schedule

ASD was induced in rats by administering an intracerebroventricular injection of 1 M Propanoic acid according to the procedure of Macfabe (2012) and Macfabe et al. (2007) with some modifications (Bhandari & Kuhad, 2015, 2017). The experimental protocol is explained in Table 1.

2.4. Behavioural tests

Briefly, neurobehavioural tests for sociability like reciprocal social interaction (Silverman, Yang, Lord, & Crawley, 2010), three-chamber test for social preference and social novelty preference (Karvat and Kimchi, 2012), repetitive self-grooming (Moretti, Bouwknecht, Teague, Paylor, & Zoghbi, 2005): partition test (Silverman et al., 2010) and marble burring test (Thomas et al., 2009) were performed. For communication deficit olfactory habituation/dishabituation (Silverman et al., 2010; Yang and Crawley, 2010), with minor modifications was done. Sensorimotor dysfunction and locomotor activity were assessed by rotarod test (Dunham & Miya, 1957) and actophotometer (Sachdeva, Kuhad, & Chopra, 2011). Associated Behaviours like anxiety and depression were evaluated by elevated plus-maze (Sharma and Kulkarni, 1992), open-field activity (Raghavendra, Chopra, & Kulkarni, 1999) and forced swim test (Slattery & Cryan, 2012). Memory & perseverative behaviour were assessed by novel object recognition test (Antunes & Biala, 2012) and Morris Water maze test (Morris, Garrud, Rawlins, & O'Keefe, 1982; Tuzcu & Baydas, 2006).

2.5. Biochemical assessments

Lipid peroxidation (Wills, 1966, glutathione (Jollow et al., 1974), superoxide dismutase (Kono, 1978), Catalase (Claiborne, 1985), nitrite (Green et al., 1982), NADH dehydrogenase (complex I) (King and Howard, 1967), succinate dehydrogenase (complex II) (King, 1967), complex-III (MTT activity) (Liu, Peterson, Kimura, & Schubert, 1997), cytochrome oxidase (complex IV) (Sottocasa et al., 1967) and blood brain barrier (BBB) permeability were assessed (Manaenko, Chen, Kammer, Zhang, & Tang, 2011; Kumar and Sharma, 2016). Tumor necrosis factor-alpha (TNF-α) Matrix Metalloproteinases-9 (MMP-9), heat shock protein (HSP-70) and permeability glycoprotein (Pgp) were by ELISA.

Material and methods have been described in detail in the supplementary section.

2.6. Statistical analysis

Results have been expressed as mean ± S.E.M. For all behavioural tests, intergroup variation was measured by one-way analysis of variance (ANOVA) (except in few cases where two-way analysis of variance (ANOVA) was used), followed by Tukey's post-hoc test. Statistical

Table 1
Protocol design for the study.

Groups (n = 5)	Behavioural tests (21st Day)	Tests for estimation of Biochemical, Mitochondrial and molecular parameters
• SHAM Control • 1 M PPA • NGN-25 mg/kg • NGN-50 mg/kg • NGN-100 mg/kg • Minocycline (50 mg/kg) • NGN-PLGA-NP (25 mg/kg) • GLU-NGN-PLGA-NP (25 mg/kg) • Tween 80-NGN-PLGA-NP (25 mg/kg) • Blank-NP Treatment was started from 2nd day post surgery and was continued till 29th day. Daily three doses of naringenin & its nanocarriers were administered (8 hourly). Each group consisted of five animals. 50 animals were used for behavioural, biochemical, mitochondrial complex estimation and ELISA studies. In addition, 50 animals were subjected to same treatment and was evaluated for Blood Brain Barrier Permeability Test	Neurobehavioural tests • Reciprocal Social Interaction • Three-Chamber test • Partition test • Repetitive Self-grooming • Marble burying • Olfactory habituation/dishabituation Sensorimotor dysfunction and Locomotor activity • Rotarod • Actophotometer Associated Behaviours like anxiety and depression • The Elevated plus-maze • Open-field Test • Forced swim Test (Day 14th) Memory & Perseverative Behaviour • Novel Object Recognition test • Morris Water Maze (MWM) (Day 21st-Day 28th) All the behavioural tests were done on 21st day as statistically significant changes in behaviour were observed on this day as shown in our previous studies, except FST which did not show any difference in the results of 14th day and 21st day. All behavioural tests were performed over two consecutive days. MWM was performed between 21st and 28th day	Animals were sacrificed after dosing on 29th day under deep anesthesia. Blood was collected from tail-vein and plasma was separated. Whole brain was removed. Samples of both plasma and brain were stored at −80 °C until further processed.Biochemical Assessment (Brain homogenate) • LPO • Glutathione • SOD • Catalase • Nitrite Mitochondrial Complex Estimation (Brain homogenate) • Complex I • Complex II • Complex III • Complex IV Blood Brain Barrier Permeability Test ELISA STUDIES Plasma • TNF-α • MMP-9 • HSP-70 Brain homogenate • P-gp