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Naringenin attenuates behavioral derangements induced by social defeat stress in mice via inhibition of acetylcholinesterase activity, oxidative stress and release of pro-inflammatory cytokines

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ABSTRACT

The effects of naringenin; a dietary flavonoid, with potent anti-oxidant and anti-inflammatory activities on social defeat stress (SDS)-induced neurobehavioral and biochemical changes were evaluated in mice using resident-intruder paradigm. The intruder male mice were distributed into 6 groups (n = 6). Mice in group 1 (control) received vehicle (3% DMSO, i.p), group 2 (SDS-control) were also given vehicle, groups 3–5 received naringenin (10, 25 and 50 mg/kg, i.p.) while group 6 had ginseng (50 mg/kg, i.p) daily for 14 days. However, 30 min after treatment on day 7, mice in groups 2–6 were exposed to SDS for a period of 10 min confrontation with aggressive counterparts for 7 consecutive days. Neurobehavioral phenotypes: spontaneous motor activity (SMA), memory, anxiety and depression were then evaluated on day 14. Malondialdehyde (MDA), glutathione (GSH), catalase and superoxide dismutase (SOD) were then estimated in the brain tissues. Acetylcholinesterase (AChE) activity and the concentrations of tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) were also determined. SDS-induced neurobehavioral deficits were significantly ($p < 0.05$) attenuated by naringenin. The increased brain level of MDA (13.00 ± 0.63 μmol/g tissue) relative to vehicle-control (6.50 ± 0.43 μmol/g tissue) was significantly ($p < 0.05$) reduced to 5.50 ± 0.22 μmol/g tissue by naringenin (50 mg/kg). Mice exposed to SDS had decreased brain GSH level (5.17 ± 0.40 μmol/g tissue) relative to control (11.67 ± 0.84 μmol/g tissue). However, naringenin (50 mg/kg) significantly ($p < 0.05$) elevated GSH content (13.33 ± 0.88 μmol/g tissue) in the brains of SDS-mice. Moreover, 50 mg/Kg of naringenin (38.13 ± 2.38 ρg/mL) attenuated ($p < 0.05$) increased TNF-α level when compared with SDS (49.69 ± 2.81 ρg/mL). SDS-induced increase in brain level of IL-1β (236.5 ± 6.92 ρg/mL) was significantly ($p < 0.05$) reduced by naringenin (219.90 ± 15.25 ρg/mL). Naringenin also elevated antioxidant enzymes and decreased AChE activity in the brains of mice exposed to SDS ($p < 0.05$). These findings suggest that naringenin attenuates SDS-induced neurobehavioral deficits through inhibition of acetylcholinesterase activity, oxidative stress and release of pro-inflammatory cytokines.

1. Introduction

Chronic psychosocial stress has been identified as a major risk factor for the development of psychiatric pathologies such as memory deterioration, depression, anxiety and addictive disorders [1,2]. Psychosocial stress is a common form of stressor in our world today as a result of increased global insecurity, violent acts, joblessness, poverty or economic pressure, which largely increases the risk of psychopathologies [3,4]. The response to stress is different depending on the type of stressor [5,6], and some studies have shown that social stress produces different behavioral and physiological outcomes that are different from nonsocial stress [2,7,8]. Thus, social defeat stress (SDS) is widely used to model the behavioral and neurobiological impact of psychosocial stressors in humans, and for detection of various compounds that can mitigate its neuropsychiatric consequences [2,9].

Social defeat stress has been described as a confrontation and subsequent subordination of an animal by a conspecific counterpart, which serves as a major source of distress to the defeated animal [1,10,11]. Social defeat stress has been found to suppress multiple types of appetitive behaviors; consumption of palatable foods, and courtship or copulatory behavior [10,12,13]. The resident-intruder paradigm has been shown as a suitable model to study the effects of social stress-induced phenotypes and endophenotypes relevant to psychiatric diseases [2,14]. Exposure to a single attack or defeat is known as acute

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social defeat stress whereas repeated exposure for consecutive days or weeks is referred to as chronic social defeat stress [7]. The chronic social defeat stress has been recognized as the most suitable model that closely mimics the psychopathologies of psychosocial stress in humans [9,15,16]. Moreover, chronic social defeat stress has been shown to cause more damage to the brain through induction of oxidative stress and neuroinflammation as a result of deregulation of HPA axis [1,2,14]. Although the deleterious effects of SDS had been known for many years, there is no effective drug yet that could be used to ameliorate its pathological impacts and improve the quality of life of patients recovering from its disabling effects. However, there is increasing awareness that compounds like ginseng with potent antioxidant/neuroprotective and anti-inflammatory activities may be efficacious in protecting the brain cells against the impact of chronic social stress, hence attenuation of neuropsychiatric disorders.

Naringenin (4_5, 7-trihydroxyflavanone) is a natural dietary flavanone found in fruits and vegetables like oranges, mandarins, grapefruit and lemons [17]. It is also found in cereals and regular consumption of high amounts of fruits and vegetables rich in naringenin have been reported to be of great benefits for the prevention of chronic diseases such as obesity, cardiovascular disorders, diabetes, depression and neurodegenerative disorders [17]. Naringenin has been shown to readily cross the blood brain barrier to exert its various central nervous system (CNS) activities [17–21]. Several studies have shown that naringenin possessed potent anti-inflammatory and antioxidant activities [17,18]. It was shown to reduce inflammatory mediators such as tumor necrosis factor-alpha, cyclooxygenase-2, and inducible nitric oxide synthase in 3-nitropropionic acid–treated rats [19]. Naringin and naringenin are well known potent scavengers of free radicals and have been found to prevent lipid peroxidation [18]. Naringenin has been shown to lower adiposity and total triglycerides contents in adipose tissue in rats through promotion of gene expression and adiponectin protein secretion from 3T3-L1 adipocytes [20]. It has also demonstrated hepatoprotective effect via inhibition of oxidative stress and inflammatory cytokines in the liver [21]. However, pharmacokinetic studies conducted in rats revealed that naringenin is rapidly metabolized in the liver and converted to glucuronide intermediates [17]. Hence, the high first-pass metabolism has limited the oral bioavailability of naringenin *in vivo* [17].

Several CNS activities of naringenin have been reported in literature, which indicate its therapeutic potentials in neurological disorders [22–24]. Previous studies have shown that naringenin improved brain insulin signaling and cognitive functions, and also ameliorated Alzheimer's disease (AD)-type neurodegeneration due to intracerebroventricular injection of streptozotocin [22,25]. Attenuation of type-2 diabetes-induced memory dysfunction was partly ascribed to its antioxidant property and inhibition of cholinesterase activity in the hippocampus [22]. Part of these beneficial effects of naringenin were also attributed to its ability to reduce Aβ level and inflammatory processes in the hippocampus. Moreover, the inhibition of proinflammatory cytokines and neuroprotective effect have been shown to be involved in the potential benefit of naringenin in chronic inflammatory-related disorders like AD [23,24]. Naringenin demonstrated neuroprotective effect against stroke in experimental model via suppression of NF-kappa B signaling pathway [23]. It was found to improve functional disturbances in ischemic brain via inhibition of NF-κB-mediated neuroinflammation [23]. In addition, naringenin exhibited neuroprotective effect against 6-hydroxydopamine and iron overload-induced neurotoxicity through inhibition of oxidative stress [26]. Moreover, naringenin has been reported to exhibit antidepressant activity through inhibition of monoamie oxidase, a well known target enzyme for treatment of major depression [27]. The beneficial effects of naringenin in chronic neurological diseases associated with oxidative stress and neuroinflammation have been ascribed to inhibition of NF-kappa B signaling pathway and decreased caspase-3 activation with concomitant fall in concentrations of proinflammatory markers [17,23]. However, this present study was carried out to investigate its effects on social defeat stress-induced neurobehavioral and biochemical changes in mice.

2. Materials and methods

2.1. Laboratory animals

Male Swiss mice (22–27 g) used in this study were purchased from the Central Animal House, University of Ibadan and housed in plastic cages at room temperature. The animals had free access to rodent pellet diet and water *ad libitum.* They were acclimatized for two weeks to the laboratory conditions before commencement of the study. The animals were handled in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

2.2. Drugs and chemicals

Naringenin-NARIN, ginseng-GINS and dimethyl sulphoxide (DMSO) were obtained from Sigma Aldrich, Germany, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB; Sigma Aldrich, USA), triochloroacetic acid (Sigma Aldrich, USA), thiobarbituric acid (Sigma Aldrich, USA),sodium carbonate (BDH Poole, England), potassium carbonate (BDH Poole, England), and sodium chloride (BDH Poole, England) were used in this study.

2.3. Drug preparation and treatments

NARIN was dissolved in DMSO and this solution was further diluted with distilled water. The final concentration of DMSO in the solution used for the study did not exceed 3%. GINS was dissolved in distilled water from which further dilutions of required concentrations were made immediately before use. All drugs were administered intraperitoneally (i.p.). The doses of NARIN (10, 25 and 50 mg/kg) used in the study were selected based on the results obtained from preliminary investigations. The dose of GINS (50 mg/kg), which served as positive control was selected based on previous study [28].

2.4. Induction of chronic social defeat stress using the resident-intruder paradigm

The social defeat stress (SDS) was carried out using the resident-intruder model according to a previously described procedure [14]. Briefly, male resident mice were made aggressive by housing them individually with each female counterpart for 3 weeks. However, the male mice that served as intruders were housed in groups and randomly divided into 6 treatment groups (n = 6). Mice in group 1, serving as non-stress control, were given DMSO (10 mL/kg, i.p.), group 2, which served as SDS control also received DMSO (10 mL/kg, i.p.), groups 3–5 were treated with NARIN (10, 25 and 50 mg/kg, i.p.) whereas group 6 received GINS (50 mg/kg, i.p) daily for 14 consecutive days. However, SDS was carried out 30 min after treatments on days 7 to 14 by subjecting each intruder mouse in groups 2–6 to a 10 min confrontation in the home cage of an aggressive resident mouse. The intruders were subjected to social defeat stress from different aggressive residents each day, so as to enhance aggression of the residents and reduce familiarity between the resident and intruder on daily basis [29]. Social defeat was observed each day in the intruders as shown by upright defensive postures, submissive postures, flight and vocalizations. Mice were returned to their home cage after each social defeat session.

2.5. Effect of NARIN on SDS-induced behavioral derangements

The effect of NARIN on SDS-induced behavioral derangements such as altered spontaneous motor activity (SMA), memory deficit, anxiety- and depressive-like behaviors were evaluated in this sequence after the