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Original research article

Possible involvement of nitric oxide mechanism in the neuroprotective effect of rutin against immobilization stress induced anxiety like behaviour, oxidative damage in mice

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ABSTRACT

Dietary supplements are widely used to manage stress and related consequences. However, the exact pathological mechanism and cellular cascades involved in the action of these supplements are not properly understood so far. Therefore, the present study has been designed to explore the neuroprotective mechanism of rutin against immobilization stress-induced anxiety-like behavioural and oxidative damage in mice. Laboratory Animal Centre A-strain (laca) mice were used in the present study. Rutin (20, 40, and 80 mg/kg), L-arginine (100 mg/kg), L-nitroarginine methyl ester (L-NAME) (5 mg/kg) and vitamin-E (50 mg/kg) were administered for 5 days before 6 h immobilization stress on 6th day. Various behavioural parameters (mirror chamber test, locomotor activity) followed by biochemical parameters (lipid peroxidation, nitrite concentration, reduced glutathione and catalase) in brain and then serum corticosterone level were assessed. 6 h immobilization stress produced anxiety-like behavioural in mirror chamber test, raised corticosterone level and oxidative stress (as evidenced by rise in lipid peroxidation, nitrite concentration, depletion of reduced glutathione and catalase activity) significantly as compared to naive group. 5 days pre-treatment with rutin (40 and 80 mg/kg) causes a significant attenuation of locomotor activity, corticosterone level, oxidative stress as compared to control. Further, L-arginine (100 mg/kg) pre-treatment significantly reversed the protective effect of rutin (40 mg/kg) in 6 h immobilized animals. However, L-NAME (5 mg/kg) pre-treatment with rutin (40 mg/kg) potentiated their protective effect which was significant as compared to their effect *per se*. The present study suggests the involvement of nitric oxide mechanism in the neuroprotective effect of rutin against immobilization stress-induced anxiety-like behaviour and oxidative damage in mice.

Introduction

Stress has been well known to cause several neuropsychiatric diseases with clinical pathological alterations in discrete areas of the brain. Psychological and physical stress causes depression, cognitive dysfunction and anxiety [15,38,47]. Stress has also been recognized as one of the precipitating factors for causing anxiety and related problems in rodents [22]. Anxiety has been implicated in several psychiatry disorders such as depression, panic attack, phobias, generalized disorder, obsessive compulsive disorder and post traumatic disorders [2,9,25,46,47,58]. Restraint stress activates sympatho–adrenomedullary system (SAS) and hypothalamic–pituitary–adrenal (HPA) axis causing the release of catecholamines and stress hormones namely glucocorticoids and corticosterone from the adrenal gland [5,30,50]. Corticosteroids act through specific mineralocorticoid and glucocorticoid receptors (GRs) localized in hippocampus and amygdale that are involved in the regulation of fear and anxiety-like behaviour [32,42]. Serum corticosterone is a well known marker for stress [19]. Immobilization stress has been well known to increase corticosterone level [10].

Immobilization stress produced anxiety-like behaviour in the elevated plus maze (EPM), open field test (OFT) [23], mirror chamber (MC) test and reduced locomotor activity. Immobilizations stress a well-established and widely used experimental model to study anxiety-like behaviour [7,53]. Imbalance between antioxidant defence and production of excessive free radicals occurs during

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oxidative stress which provides impetus to brain damage and neurodegenerative disorders. The brain consumes large amount of oxygen and therefore comparatively produces a large amount of free radicals as by-products. Brain tissue is particularly susceptible to oxidative damage attributed to its high oxygen content, low level of antioxidant defence, and high level of polyunsaturated fatty acids. Anxiety has been linked with oxidative stress [45]. Besides, Kumar and his team also proposed that targeting oxidative damage is one of the most promising strategies in the treatment of stress-induced anxiety-like behaviour [33].

Nitric oxide (NO) is a versatile molecule with diverse physiological functions. It may act both as pro-oxidant and antioxidant depending upon the simultaneous production of superoxide radicals [40]. NO plays a complex physiological role in CNS and in the regulation of neuroendocrine functions. Besides, restraint stress has been reported to increase the expression of nitric oxide in experimental animals [27,48]. NO is an important signalling molecule contributing to stress response involved with regulation of anxiety and related to HPA axis regulation [4]. In addition, it has been shown that nitric oxide regulates activity of HPA axis that has an impact on the synthesis of stress hormones such as glucocorticoids [8,51]. However, reports on the role of nitric oxide on stress mediating effect have been contradictory [43].

Rutin is a natural flavonoid (Fig. 1). Flavonoids are widely found in vegetables, fruits, juices, tea and are consumed widely as a dietary supplement. Flavonoids possess divalent metal chelation, antioxidant, anti-inflammatory like properties, and readily permeates blood–brain barrier (BBB) [24].

Rutin is well known antioxidant and its neuroprotective properties have been well demonstrated against multiple disease states, including cancer, cardiovascular disease, ischaemia–reperfusion brain injury [39] and neurodegenerative disorders [12,31,33,44,52]. These therapeutic benefits of rutin have been proposed due to its antioxidant and free radical–scavenging properties [34]. Besides, rutin's structure contains sugar as a side chain which has been suggested as important for its neuroprotective activities [41]. Rutin has been demonstrated to decrease inducible nitric oxide and cytokines activity in experimental model of Alzheimer diseases [52]. However, its exact mechanism is still not clear.

Therefore, the aim of the present study was to investigate the neuroprotective effect of rutin and its possible nitric oxide mechanism against acute immobilization stress induced anxiety-like behaviour and oxidative damage in mice.

Materials and methods

Animals

Male albino mice (laca strain) weighing between 22 and 30 g bred in Central Animal House (CAH) facility of the Panjab University, Chandigarh, India were used. The animals were housed under standard laboratory conditions, maintained on natural light and dark cycle and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. Each group consists of minimum 6 animals. All the experiments were carried out between 09:00 h and 15:00 h. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) [IAEC/282, 30th August 2012] and conducted according to the CPCSEA guideline.

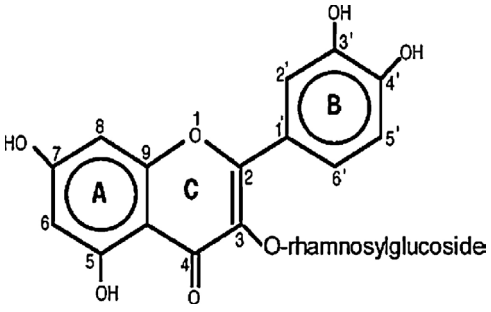

Fig. 1. Chemical structure of rutin.

Drugs and treatment

Rutin (20 mg/kg, 40 mg/kg and 80 mg/kg, *po*), L-arginine (100 mg/kg, *ip*), L-NAME (5 mg/kg, *ip*) and vitamin-E (50 mg/kg, *po*) were administered daily for 5 days before 6 h immobilization challenge on 6th day. L-Arginine and L-NAME were administered 30 min before rutin treatment. Rutin (Sigma Chemicals, USA) was prepared in normal saline. The entire drug treatment protocol has been described in Fig. 2.

Immobilization stress

Animals were immobilized individually for 6 h in modified restrainers' wooden box with dimensions of 7.5 cm length, 3 cm width, and 4 cm height, properly ventilated with small opening at end through which tail of the mice was kept fixed with zinc oxide hospital tape which further restricted the movement of animal. Release was affected by unravelling the tape after moistening with acetone in order to minimize pain or discomfort. In unstressed group, the mice were kept in animal cage with soft bedding in the same experimental condition.

Behavioural assessments

Mirror chamber test

The mirror chamber consists of a wooden chamber having a mirror chamber enclosed within it. During the 5 min test session, following parameters were noted (a) latency to enter the mirror chamber, (b) total time spent in mirror chamber, and (c) number of entries in mirror chamber. Animals were placed individually at the distal corner of the mirror chamber at the beginning of the test. An anxiogenic response was defined as decreased number of entries and time spent in the mirror chamber [33].

Locomotor activity

Locomotor activity was assessed by using actophotometer (IMCORP, Ambala, India). Animals were placed individually in the activity chamber for 3 min as a habituation period before recording actual motor activity for next 5 min. Total activity was expressed as counts per 5 min. The apparatus was placed in a darkened, light and sound attenuated and ventilated testing room during the experimental session [17].

Biochemical tests

Animals were sacrificed by cervical dislocation immediately after last behavioural assessment as well as blood collection from retro-orbital puncture. The brains were removed, rinsed in isotonic saline and weighed. A 10% (w/v) tissue homogenates were prepared with 0.1 M phosphate buffer (pH = 7.4). The post nuclear fraction was obtained by centrifugation of the homogenates at 12,000 × *g* for 20 min at 4 °C.

Measurement of lipid peroxidation

The quantitative measurement of lipid peroxidation was performed according to the method of Wills [57]. The amount of