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

Lalit Machawal, Anil Kumar *

Pharmacology Division, University Institute of Pharmaceutical Sciences, UGC Centre of Advanced Study, Panjab University, Chandigarh, India

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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.

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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 sympato–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
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.

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

![Fig. 1. Chemical structure of rutin.](image-url)
malondialdehyde (MDA), a measure of lipid peroxidation was measured by reaction with thiobarbituric acid at 532 nm using Perkin Elmer lambda 20 Spectrophotometer (Norwalk, CT, USA). The values were calculated using molar extinction coefficient of chromophore ($1.56 \times 10^5$ M$^{-1}$ cm$^{-1}$) and expressed as percentage of naïve.

**Estimation of nitrite**

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO), was determined with a colorimetric assay with Greiss reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by Green [18]. Equal volumes of supernatant and Greiss reagent were mixed, and then the mixture was incubated for 10 min at room temperature in the dark. The absorbance was determined at 540 nm with Perkin Elmer lambda 20 spectrophotometer. The concentration of nitrite in the supernatant was determined from a sodium nitrite standard curve and expressed as percentage of naïve.

**Catalase estimation**

Catalase activity was assayed by the method of Luck [36], wherein the breakdown of hydrogen peroxides ($H_2O_2$) is measured at 240 nm. Briefly, assay mixture consisted of 3 ml of $H_2O_2$ phosphate buffer and 0.05 ml of supernatant of tissue homogenate (10%), and change in absorbance was recorded at 240 nm. The results were expressed as micromole $H_2O_2$ decomposed per milligram of protein/min.

**Glutathione (GSH) assay**

Reduced glutathione in the brain was estimated according to the method of Ellman (1959). Homogenates were precipitated with 1.0 ml of 4% sulfosalicylic acid by keeping the mixture at 4 °C for 1 h and the samples were immediately centrifuged at 1200 × g for 15 min at 4 °C. The assay mixture contained 0.1 ml of supernatant, 2.7 ml of phosphate buffer of pH 8 and 0.2 ml of 0.01 M dithiobisnitrobenzoic acid (DTNB). The yellow colour developed was read immediately at 412 nm on spectrophotometer (Perkin Elmer lambda) [11]. The results were expressed as nano moles of reduced glutathione per milligram of protein.

**Protein estimation**

Protein estimation was done by Gornall method [16] using bovine serum albumin as standard.

**Preparation of serum and corticosterone estimation**

At the end of behavioural assessments, blood samples were collected through retro-orbital route and allowed to clot at room temperature. The tubes were then centrifuged at 2000 rpm for 10 min; straw coloured serum was separated and frozen at −20 °C. Serum corticosterone was estimated by the method of Katyar [28]. Fluorescence was measured on F-2500 fluorescence spectrophotometer (Hitachi, Japan) at 472 nm excitation and 523 nm wavelength emission.

**Statistical analysis**

The results were expressed as the mean ± standard error of means (SEM). The results were analyzed using one-way ANOVA followed by post hoc analysis using Tukey’s Multiple Comparison Test. The $p$ value $< 0.05$ was considered to be statistically significant.

**Results**

**Effects of rutin and its influence by L-arginine and L-NAME pre-treatments on locomotor activity**

In the present study, 6 h acute immobilization stress causes a significant decrease in locomotor activity as compared to naive group. The dose of rutin (40, 80 mg/kg) and vitamin-E (50 mg/kg) for five days pre-treatment causes a significant amelioration in locomotor activity as compared to that in control group (Fig. 3). Rutin (20 mg/kg) pre-treatment for five days did not produce any significant effect on locomotor activity as compared to control ($p < 0.05$) (Fig. 3). L-Arginine (100 mg/kg) pre-treatment causes a significant reversal in protective effect of rutin (40 mg/kg) in 6 h immobilized animals. L-NAME (5 mg/kg) pre-treatment with rutin (40 mg/kg) for five days potentiated their protective effect which was significant as compared to their effect per se. Rutin (80 mg/kg), L-NAME (5 mg/kg), and L-arginine (100 mg/kg) per se treatment group did not produce any significant effect on locomotor activity as compared to naive group (data not shown).

**Fig. 2.** Diagrammatic view of study.

**Fig. 3.** Effect of rutin on locomotor activity against immobilization stress in mice. Values are expressed mean ± SEM (ANOVA followed by Tukey test) ($n = 6$). *$p < 0.05$ as compared to naive, *$p < 0.05$ as compared to IS; *$p < 0.05$ as compared to R (40), *$p < 0.05$ as compared to L-NAME (5), R - rutin, IS – immobilization stress, Vit E (50) – vitamin E, and R (40) – rutin 40 mg/kg.
Effects of rutin and its influence by l-arginine and l-NAME pre-treatments on anxiety-like behaviour

In the present study, 6 h acute immobilization stress causes a significant behaviour insult as anxiety-like behaviour which was evidenced by delayed time latency to enter mirror chamber, decreased number of entries and time spent in mirror chamber as compared to naive group. The dose of rutin (40 and 80 mg/kg) and vitamin-E (50 mg/kg) causes a significant attenuation in anxiety-like behaviour [i.e. shortening of time latency to enter mirror chamber (Fig. 4), increased number of entries (Fig. 5) and duration in mirror chamber (Fig. 6)] as compared to control. Rutin (20 mg/kg) did not produce any significant effect on anxiety-like behaviour as compared to control (p < 0.05) (Figs. 4–6). L-Arginine (100 mg/kg) pre-treatment significantly causes a reversal in protective effect of rutin (40 mg/kg) as evidenced by an increase in latency time, and reduces the number of entries and time spent in mirror chamber as compared to their effect per se in 6 h immobilized mice. l-NAME (5 mg/kg) pre-treatment with rutin (40 mg/kg) for five days potentiated that their protective effect was significant as compared to their effect per se (Figs. 4–6). Rutin (80 mg/kg), l-NAME (5 mg/kg), and l-arginine (100 mg/kg) per se treatment groups did not produce any significant effect on anxiety-like behaviour as compared to naive animals (data not shown).

Effects of rutin and its influence by l-arginine and l-NAME pre-treatments on plasma corticosterone level

Six hour acute immobilization stress causes a significant increase in serum corticosterone level as compared to naive group. The dose of rutin (40 and 80 mg/kg) and vitamin-E (50 mg/kg) for five days pre-treatment causes a significant attenuation in serum corticosterone level as compared to control group (Fig. 7). However, rutin (20 mg/kg) did not produce any significant effect on serum corticosterone level as compared to control. L-Arginine (100 mg/kg) pre-treatment causes a significant reversal in

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**Fig. 4.** Effect of rutin on latency to enter in mirror chamber against immobilization stress in mice. Values are expressed mean ± SEM (ANOVA followed by Tukey test) \((n = 6)\). \(^{a}p < 0.05\) as compared to naive, \(^{b}p < 0.05\) as compared to IS, \(^{c}p < 0.05\) as compared to R (40), \(^{d}p < 0.05\) as compared to l-NAME (5), R − rutin, IS − immobilization stress, Vit E (50) − vitamin E, and R (40) − rutin 40 mg/kg.

**Fig. 5.** Effect of rutin on number of entries into mirror chamber against immobilization stress in mice. Values are expressed mean ± SEM (ANOVA followed by Tukey test) \((n = 6)\). \(^{a}p < 0.05\) as compared to naive, \(^{b}p < 0.05\) as compared to IS, \(^{c}p < 0.05\) as compared to R (40), \(^{d}p < 0.05\) as compared to l-NAME (5), R − rutin, IS − immobilization stress, Vit E (50) − vitamin E, and R (40) − rutin 40 mg/kg.

**Fig. 6.** Effect of rutin on time spent in mirror chamber against immobilization stress in mice. Values are expressed mean ± SEM (ANOVA followed by Tukey test) \((n = 6)\). \(^{a}p < 0.05\) as compared to naive, \(^{b}p < 0.05\) as compared to IS, \(^{c}p < 0.05\) as compared to R (40), \(^{d}p < 0.05\) as compared to l-NAME (5), R − rutin, IS − immobilization stress, Vit E (50) − vitamin E, and R (40) − rutin 40 mg/kg.

**Fig. 7.** Effect of rutin on serum corticosterone after immobilization stress. Values are expressed mean ± SEM (ANOVA followed by Tukey test) \((n = 6)\). \(^{a}p < 0.05\) as compared to naive, \(^{b}p < 0.05\) as compared to IS, \(^{c}p < 0.05\) as compared to R (40), \(^{d}p < 0.05\) as compared to l-NAME (5), R − rutin, IS − immobilization stress, Vit E (50) − vitamin E, and R (40) − rutin 40 mg/kg.
Table 1
Effect of rutin on oxidative stress parameter against immobilization stress in mice.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>LPO (mmol of MDA/mg protein) (% of naive)</th>
<th>Nitrate (μmol of nitrite/mg protein) (% of naive)</th>
<th>Catalase (μmol of H2O2/min/mg protein) (% of naive)</th>
<th>GSH (μmol of GSH/mg protein) (% of naive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>0.102 ± 0.002 (100)</td>
<td>262 ± 17.2 (100)</td>
<td>0.07 ± 0.006 (100)</td>
<td>1.07 ± 0.14 (100)</td>
</tr>
<tr>
<td>IS</td>
<td>0.60 ± 0.06a (588.23)</td>
<td>843 ± 45.6b (321.75)</td>
<td>0.012 ± 0.001a (17.14)</td>
<td>0.27 ± 0.06a (25.23)</td>
</tr>
<tr>
<td>Vit E (50)+IS</td>
<td>0.119 ± 0.09b (116.66)</td>
<td>284 ± 16.4d (108.39)</td>
<td>0.059 ± 0.005b (84.28)</td>
<td>0.92 ± 0.03b (85.98)</td>
</tr>
<tr>
<td>R (20)+IS</td>
<td>0.490 ± 0.02 (480.39)</td>
<td>812 ± 36.3c (309.92)</td>
<td>0.017 ± 0.001c (24.28)</td>
<td>0.36 ± 0.08c (33.64)</td>
</tr>
<tr>
<td>R (40)+IS</td>
<td>0.143 ± 0.01c (140.19)</td>
<td>312 ± 26.9d (119.08)</td>
<td>0.051 ± 0.004d (72.08)</td>
<td>0.82 ± 0.09d (76.63)</td>
</tr>
<tr>
<td>L-Arg (100)+IS</td>
<td>0.582 ± 0.045 (570.58)</td>
<td>831 ± 65d (317.17)</td>
<td>0.0128 ± 0.002d (18.28)</td>
<td>0.28 ± 0.04d (26.16)</td>
</tr>
<tr>
<td>L-NAME (5)+IS</td>
<td>0.594 ± 0.049 (582.35)</td>
<td>839 ± 72d (320.22)</td>
<td>0.0131 ± 0.002d (18.71)</td>
<td>0.30 ± 0.07d (28.03)</td>
</tr>
<tr>
<td>L-Arg (100)+R (40)+IS</td>
<td>0.423 ± 0.039d (414)</td>
<td>829 ± 56d (316)</td>
<td>0.019 ± 0.007d (27.14)</td>
<td>0.40 ± 0.03d (37.38)</td>
</tr>
<tr>
<td>L-NAME (5)+R (40)+IS</td>
<td>0.132 ± 0.02e,d (129.41)</td>
<td>306 ± 24d (116.79)</td>
<td>0.06 ± 0.004e,d (80)</td>
<td>0.87 ± 0.056e,d (81.30)</td>
</tr>
</tbody>
</table>

Values are expressed mean ± SEM (ANOVA followed by Tukey test) (n=6).

R, rutin; IS, immobilization stress; Vit E (50), vitamin E; R (40), rutin 40 mg/kg.

a, b, c, d, e, f p < 0.05 as compared to naive.

Effects of rutin and its influence by l-arginine and l-NAME pretreatments on oxidative stress parameters

Six hour acute immobilization stress causes a significant increase in lipid peroxidation, nitrate concentration, depletion of reduced glutathione level and catalase activity as compared to naive. The dose of rutin (40, 80 mg/kg) and vitamin E (50 mg/kg) for 5 days pre-treatment causes a significant attenuation in elevated lipid peroxidation, nitrate concentration and restored reduced glutathione and catalase activity as compared to the control group (p < 0.05) (Tab. 1). However, rutin (20 mg/kg) pre-treatment for five days did not produce significant effect on oxidative stress parameters as compared to control (p < 0.05) (Table 1). L-Arginine (100 mg/kg) pre-treatment causes a significant reversal in protective effect of rutin (40 mg/kg) in 6 h immobilized animals. l-NAME (5 mg/kg) pre-treatment for five days potentiated the protective effect of rutin (40 mg/kg) which was significant as compared to their effect per se (Table 1). Rutin (80 mg/kg), l-NAME (5 mg/kg), and l-arginine (100 mg/kg) per se treatment groups did not produce any significant effect on oxidative stress parameters as compared to naive animals (data not shown).

Discussion

Immobilization stress has been known to produce both psychological stress and physical stress, which results in a wide range of behavioural and physiological alterations including reduced locomotor activity, anxiety-like behaviour, secretion of stress hormones (corticosterone) and oxidative stress in brain [36,49]. Dietary supplements, antioxidants, yoga/meditation therapy are widely used to manage stress and its consequences. The utility values of antioxidants as well as dietary supplements are now gaining importance these days against day-to-day life stress. Rutin is one of the flavonoids known for its antioxidant and anti-inflammatory properties. Therapeutic potentials of rutin have been well demonstrated experimentally in several neurological problems such as Alzheimer disease [26], learning and memory impairment [30], and cognitive impairment [50,55]. Exact neuroprotective mechanism of rutin in neurological problem is still not clear. Besides, its effect on inducible nitric oxide mechanism has also been demonstrated in different experimental models of Alzheimer disease, and in hepatoprotective conditions [45]. Therefore, the present study investigated the neuroprotective effect of rutin and its possible nitric oxide mechanism against immobilization stress-induced anxiety-like behaviour and oxidative damage in mice.

In the present study, 6 h immobilization stress significantly impaired locomotor activity (motor function) which is improved by rutin pre-treatment, suggesting its therapeutic effect. Stress significantly influences brain functions and causes long-term changes in multiple neural systems. Further, 6 h immobilization stress significantly caused anxiety-like behaviour as evidenced by delayed in time latency to enter mirror chamber, decrease number of entries and duration in the mirror chamber. Restraint stress has been reported to enhance anxious behaviour in rodents [22]. In the present study, five days pre-treatment with rutin significantly attenuated anxiety-like behaviour suggesting its antianxiety like properties [56]. The protective effect was compared to that of Vit E. Antistress properties of rutin have been well documented in different experimental system [26,41]. However, its mechanism of action is not clearly defined so far. Immobilization stress produces 300–500% increase in plasma corticosterone level [1]. In the present study, 6 h immobilization stress significantly raised serum corticosterone level which is attenuated by rutin pre-treatment further supporting its antianxiety like effect. Further, it is well reported that corticosterone level increases after immobilization stress [29]. Immobilization stress activates HPA axis which
primarily releases corticotropin-releasing factor (CRF) in the region of the locus coeruleus (LC) that results in anxiety-like behaviour [21]. It seems that rutin might have some therapeutic potential against stress-induced anxiety-like behaviour. However, its mechanism against anxiety-like behaviour is not clear so far.

Oxidative stress is one of the strong mediators of brain damage in acute stress because brain is highly vulnerable to oxidative stress due to its high O2 consumption, its modest antioxidant defences and its lipid-rich consumption [9]. Immobilization stress has been well reported to cause oxidative damage, neuroinflammation and raised cytokines levels in discrete areas of the brain. Supporting to our previous observations, 6 h immobilization stress-induced oxidative stress as evidenced by raise in lipid peroxidation, nitrite concentration, reduced glutathione and catalase activity suggesting oxidative damage. Numerous studies suggest that the excessive production of free radicals and poor antioxidant defences is involved in the pathogenesis of several neurodegenerative diseases [25,54]. Further, in the present study, rutin and Vit E pre-treatment for five days significantly attenuated oxidative stress suggesting its antioxidant like potential which has also been supported by several studies [32]. Further, it has been reported that rutin prevents lipid peroxidation due to its metal chelating action [32]. Rutin is a phenolic compound, known for its free radicals scavenging property and accountable for its antioxidant properties [13,14,30]. It seems that antioxidant properties of rutin might have contributed to the attenuation of its anxiety-like effect against immobilization stress.

Role of nitric oxide has been well reported in stress and related conditions [37]. However, its exact role in stress-induced pathology such as anxiety-like behaviour is still far from our understanding. HPA axis is critically involved in the stress related pathologies and nitric oxide regulates its activity of HPA axis which has an impact on synthesis of stress hormones such as CRF and ACTH and glucocorticoids [20]. Nitric oxide plays a complex physiological role in CNS and in the regulation of neuroendocrine function. Systemic inhibition of nitric oxide synthesis can prevent stress-induced behaviour changes in rodents. Nitric oxide has also been proposed to influence neuronal function and related behaviours possible by brain derived neurotrophic factor [35]. In the present study, nitric oxide modulators (l-NAME and l-arginine) significantly altered immobilization stress induced anxiety-like behaviour and oxidative damage suggesting the involvement of nitric oxide in stress pathways. Pre-treatment of l-arginine significantly reversed the neuroprotective of rutin as evidenced by impaired locomotor activity, anxiety-like behavioural and oxidative damage. Further, l-NAME pre-treatment significantly potentiated the protective effect of rutin suggesting the involvement of nitric oxide mechanism. Nitric oxide mechanism of rutin has also been demonstrated in different experimental systems such as depression and cognitive dysfunction [55].

In conclusion, the present study provides an evidence that nitric oxide modulatory mechanism could be involved in the neuroprotective effect of rutin against 6 h immobilization stress-induced anxiety-like behaviour. This study further provides a hope that rutin could be used as an effective therapeutic option in the treatment of stress and related problems.

Conflict of interest

Authors do not have any conflict of interest.

Funding

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[22] Higashi T, Ninomiya Y, Iwaki N, Yamauchi T, Akahori A, Yamauchi A. Antioxidant activity of Rutin, a compound, known for its free radicals scavenging property and accountable for its antioxidant properties [13,14,30]. It seems that antioxidant properties of rutin might have contributed to the attenuation of its anxiety-like effect against immobilization stress.

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