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Naringenin mitigates reserpine-induced anxiety-like behavior, neurodegeneration, and oxidative stress in male rats

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Reserpine (Res) induces anxiety-like behaviors, orofacial dyskinesia, and neurodegeneration in animals, the pathophysiology of which has been related to oxidative stress. The purpose of this study was to investigate whether naringenin (NG) could prevent reserpine-induced anxiety-like behaviors, orofacial dyskinesia, and neurodegeneration in male rats. Twenty-eight male rats were distributed into different groups as follows: Control rats; vehicle rats, which received the vehicles (normal saline, orally; acetic acid, intraperitoneally); Res rats (1 mg/kg/day) every other day for 3 days; and Res + NG rats, which received NG (50 mg/kg, orally, pre-treatment for 7 days), followed by Res. Administration of Res significantly increased chewing frequency compared with the control group ($P < 0.01$) and NG reversed the effect of Res on this factor ($P < 0.05$). Res induced an anxiety-like behavior in rats in the plus maze, and pre-treatment with NG improved this behavior. In addition, Res significantly increased the level of oxidative stress markers and degenerated neurons in the striatum; NG was able to ameliorate these damages. The results of this study demonstrated that Res caused behavioral disorders and increased the levels of oxidative stress in male rats; the use of NG was effective in treating these disorders. Therefore, NG should be considered as a preventive agent for reserpine-induced brain damage in male rats.

Keywords Reserpine · Naringenin · Oxidative stress · Anxiety · Rat

Introduction

Tardive dyskinesia (TD) is a complicated hyperkinetic syndrome with rhythmic, abnormal, involuntary, choreiform, and athetoid movements. The most common muscles involved in TD are the face, mouth, and tongue [11]. Vacuous chewing movements (VCMs), which have been widely used as a model for TD, are generally characterized by purposeless mouth openings with or without tongue protrusion [6].

Reserpine (Res) is a common drug used to treat hypertension. It has the ability to decrease biogenic amines and is a potent oxidant [17]. Res is an essential hypertensive drug, but its use is limited due to side effects [1]. Some of the major side

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effects of Res are orofacial dyskinesia, depression, and neurodegeneration [4, 14, 23]. Res is used in rodents to mimic Parkinson's disorders [24] and the Res model mimics the main features of Parkinson's disease (PD) in terms of symptomatology and neurochemistry. For this reason, this model is widely employed for screening anti-Parkinson's drugs.
Naringin (NG) is a flavanone found in citrus fruits. Its neuroprotective, anti-inflammatory, and anti-cancer effects have been proven in several studies [8, 25, 26]. The antidepressant [27] and anti-convulsive [13] effects of NG have also been confirmed in recent studies. An interesting study proved the anti-viral effect of NG and promised to use it against Covid-19 virus [26].
This study was designed to evaluate the effects of NG on reserpine-induced orofacial dyskinesia, anxiety-like behavior, and neurodegeneration in male rats.

Materials and methods

Animals

Twenty-eight male Wistar rats, weighing between 210 and 240 g, were purchased from the animal farm of Kerman University of Medical Sciences, Kerman, Iran. They were kept under light- and temperature-controlled conditions with free access to standard laboratory rodent food and water. All procedures were approved by the Ethics Committee of Kerman University of Medical Sciences (KUMS) (EC/1400-12).

Drugs

Reserpine (methyl reserpate 3, 4, 5-trimethoxybenzoic acid ester) and naringenin were bought from Sigma (St Louis, Missouri, USA).

Experimental design

Animals were distributed into different groups as follows (n=7): Control rats, which received nothing; vehicle rats, which received the vehicles (normal saline, orally; acetic acid, intraperitoneally); Res rats, which received reserpine (1 mg/kg/day) every other day for 3 days; and Res + NG rats, which received NG (50 mg/kg, orally for 7 days) and Res (1 mg/kg/day) every other day for 3 days. Res was dissolved in glacial acetic acid and diluted with distilled water to a final concentration of 0.1% acetic acid. One day after the last drug injection, behavioral tests were performed during the photoperiod of the circadian cycle from 8:00 am to 3:00 pm on the same day. Then the rats sacrificed for biochemical and histological studies.

Elevated plus maze

The elevated plus- maze comprised a black wooden apparatus with arms of equal size. Two of the arms were enclosed by walls (30×15×5 cm) and arranged in a line with 2 opposite open arms (30×5 cm). The maze was 50 cm above the floor. The rats were then placed in the center of the maze facing the open arms. The arena was illuminated by two lamps (100 W). The rats were allowed to explore the maze and their behavior was observed with a camera for 5 min after each test; the apparatus was cleaned with 75% ethanol to remove residual odors. Afterwards, the time spent in the open and closed arms was recorded.

Behavioral testing

Rats were placed individually in the cages (20×20×20 cm), which were equipped with mirrors under the floor and behind the back wall of the cage to allow quantification of VCMs when the animal was facing away from the observer. Each individual mouth opening was counted as a single VCM. VCMs were calculated for 3 min after a period of adaptation (3 min).

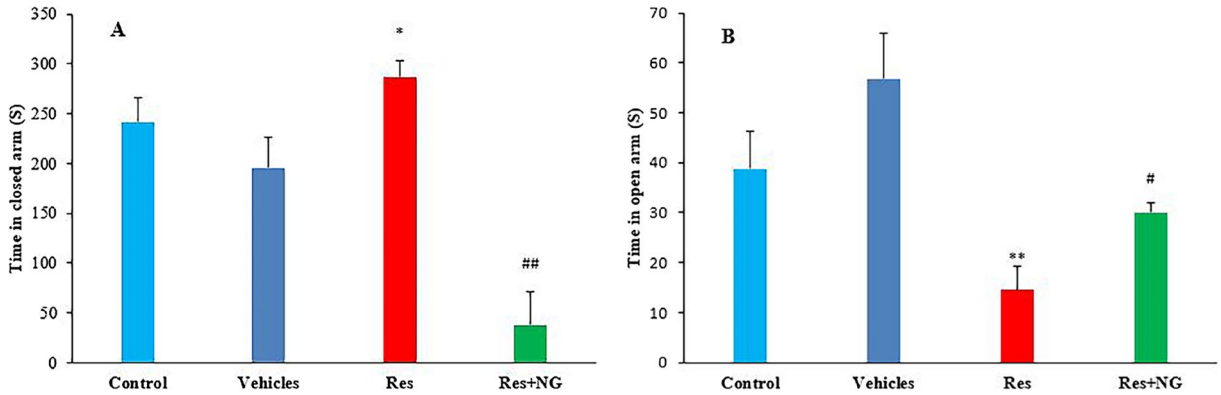

Fig. 1 Anxiety-like effects of Res expressed by the time spent in closed arms (**A**) and open arms (**B**) in the elevated plus maze in male rats. Results are expressed as mean ± SEM (n = 7); * $P < 0.05$ and ** $P < 0.01$ vs. control and vehicle groups. # $P < 0.01$ and ## $P < 0.001$ vs. Res group

Histological procedure

Animals were sampled at the end of the study under deep anesthesia with xylazine (25 mg/kg, IP) and ketamine (90 mg/kg). The brains were placed on ice; one hemisphere of the animal brain (right hemisphere) was selected for histological work and the left hemisphere for biochemical work. Sagittal sections (5 μm thickness) of the brain were collected using a rotary microtome (Leika RM 2145), fixed in 10% buffered- formalin for 72 h, and embedded in paraffin. Samples were stained with hematoxylin and eosin (H&E) to assess morphological changes in the striatum.

Malondialdehyde (MDA) measurement

The level of MDA (as a lipid peroxidation index) was assayed by the thiobarbituric acid (TBA) method. The absorbance of the products was measured at 535 nm.

Glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity

The activities of GPx and SOD were measured by using the Randox kit (UK; Cat NO.SD125). The intracellular GPx level and SOD activity was detected at the wavelength of 340 and 560 nm, respectively.

Statistical analysis

All data were evaluated using the following analyses. Variance (ANOVA) and Tukey's post hoc test. The data are represented as: Mean ± standard error of the mean (S.E.M.) using SPSS software statistical analysis.

Results

Effects of Res on anxiety-like behavior in male rats

Figure 1A shows that Res administration resulted in rats spending significantly more time in the closed arms compared with the control ($P < 0.05$). Figure 1B shows that rats in the Res group spent significantly less time in the open arms compared with the control ($P < 0.01$). NG treatment showed a significant decrease in time spent in the closed arms ($P < 0.001$) and a significant increase in time spent in the open arms in the Res+ NG group compared with the Res group ($P < 0.01$). No significant difference was found in this factor between control and vehicle-treated rats.

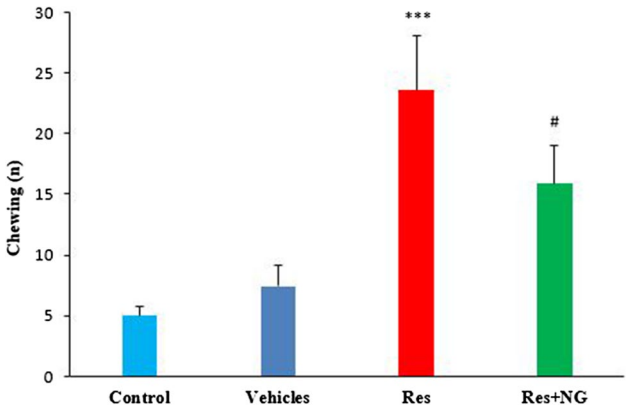

Fig. 2 Effects of Res and NG on the number of chewing in male rats. Results are expressed as mean ± SEM (n = 7); *** P < 0.01 vs. control and vehicle gropus. # P < 0.05 vs. Res group

Effects of Res on orofacial movements in male rats

As shown in Fig. 2, Res treatment led to an increase in VCM compared with the control group (P < 0.01) and NG in the Res+ NG group significantly reversed the effect of Res (P < 0.05). In other words, the total number of VCMs was significantly higher in Res-treated animals than in the other groups, and treatment with NG significantly decreased the number of VCMs in treated rats.

Effects of Res on neurodegeneration in male rats

Histological evaluation depicted that Res could induce neurodegeneration in the striatum of male rats (Fig. 3). NG ameliorated the effect of Res. Neurons in the control and vehicle groups had intact morphology, consisting of light stained cytoplasm and prominent nucleus. In the Res groups, neurons had dark cytoplasm and wrinkled nucleus.

Effects of Res on MDA and antioxidant enzymes of the brain of male rats

The effect of Res on MDA level is shown in Table 1. The biochemical assay revealed a significant increase in MDA in rats treated with Res. NG could reverse this effect.
On the other hand, Res treatment markedly diminished the GPx and SOD levels in treated rats and NG reversed these changes (Table 1).

Discussion

The main finding of the current study was that NG could ameliorate the orofacial disorders and increased levels of oxidative stress induced by Res in male rats, so it had the potential to counteract reserpine-induced injuries.
VSM is an orofacial movement used as an extrapyramidal symptoms model in rodents [5]. Res is a monoamine-reducing agent that exerts its damaging effects via blockade of the vesicular monoamine transporter in the central nervous system (CNS) [3], leading to an increase in monoamine turnover, mostly in the basal ganglia, which is related to extrapyramidal symptoms. The increase in the metabolism of monoamine through the monoamine oxidase activity can produce hydrogen peroxide that may interact with transition metals that are present in the basal ganglia and create free radicals [15]. Various antioxidants have been revealed to prevent the appearance of VCM in the Res model [18, 20]. NG reversed the increase in VCM number caused by exposure to Res. It was depicted that in PD models, the treatment with NG resulted in increasing the nuclear factor E2-related factor 2 (Nrf2) protein level and promoted the antioxidant response element. In an oxidative stress condition, Nrf2 (a transcription factor) is translocated from the cytoplasm to the nucleus [21]. In an experimental study, NG protects neurons against neurodegeneration and oxidative injury in PD [16].

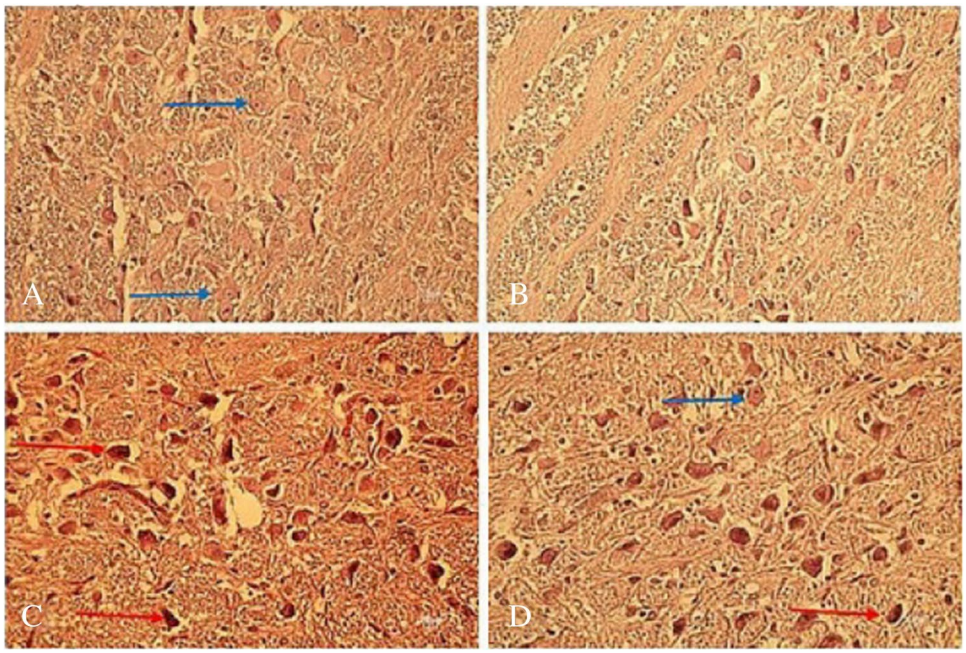

Fig. 3 Photomicrograph of the general histology of the striatum in male rats showing intact pyramidal neuron (blue arrow) and degenerated neuron (red arrow). **Notes:** (**A**) (Control); (**B**) (Vehicles); (**C**) (Res): (**D**) (Res+NG); [Staining H&E, X400]

On the other hand, rats administered with Res and subjected to elevated plus maze spent more time in closed arm and less time in open arm than other groups. These findings suggested the presence of anxiety-like symptoms in the Res-treated animals. This finding was in accordance with other studies [2, 10]. Interestingly, NG improved anxiety-like symptoms in male rats. There is ample evidence that abnormalities of the monoaminergic systems may underlie depression and anxiety disorders [12, 22]. In addition, several lines of evidence indicate the involvement of oxidative stress in the pathophysiology of anxiety [9]. A recent study has shown that the anti-oxidative and anti-inflammatory effects of NG can mediate depressive-like and anxiety-related behaviors in mice [19].

Biochemical analysis showed that MDA was significantly elevated in Res-treated animals. This finding was in agreement with the former study by Naidu [18]. This result demonstrated that Res administration led to reactive oxygen species (ROS) formation and cytotoxicity. In addition, Res injection resulted in decreasing the GPx and SOD (antioxidant defense enzymes) levels in male rats. NG administration significantly reduced the lipid peroxidation marker (MDA) and restored the decreased SOD and GPx levels in the treated rats. It has been shown that NG improves locomotor and increases glutathione with decrease MDA content in the brain tissue of the PD rat model [7].

Our histological evaluation confirmed that Res induced neurodegeneration in the striatum of male rats and NG administration could protect neurons against Res-induced insults. Studies have demonstrated that NG could prevent neuronal degeneration via its anti-apoptotic and anit-inflammatory properties [28]. The anti-inflammatory mechanism of NG is not yet fully understood, but recent studies show that NG suppresses inflammatory cytokine production through both

Table 1 The effects of NG on MDA and antioxidant enzymes of the brain in Res-treated rats

Groups	MDA (nmol/mg tissue)	GPx (U/mg protein)	SOD (U/mg protein)
Control	0.019±0.001	7.27±0.73	0.08±0.007
Vehicles	0.020±0.000	7.04±0.02	0.08±0.005
Res	0.024±0.001**	5.58±0.44**	0.05±0.002*
Res+NG	0.021±0.001$^{\#}$	6.79±0.31$^{\#}$	0.069±0.001$^{\#\#\#}$

Res markedly increased the level of MDA in the brain of male rats. Res diminished the GPx and SOD levels in the treated animals. Treatment with NG considerably decreased MDA in the treated animals. The treatment could improve the GPx and SOD levels in rats. * $P < 0.05$ vs. control and vehicle groups; ** $P < 0.01$ vs. control and vehicle groups; # $P < 0.05$ vs. Res. ###P < 0.0001 vs. Res.

Res reserpine, *NG* naringenin, *MDA* malondialdehyde, *GPx* glutathione peroxidase, *SOD* superoxide dismutase

transcriptional and post-transcriptional mechanisms. Amazingly, NG not only prevents mRNA expression of cytokines, but also stimulates the degradation of lysosome-dependent cytokine proteins [29].
Our obtained data pointed out that NG can be considered as a potential neuroprotective agent against Res-induced injuries via its antioxidant and anti-inflammatory activities. However, further studies are needed to determine the exact mechanism of NG in improving motor and affective disorders induced by Res.

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Authors contribution All the authors met the standard writing criteria based on the recommendations of International Committee of Medical Journal Publishers.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest in the current study.

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