Anti-stress effects of the hydroalcoholic extract of *Rosa gallica officinalis* in mice

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ABSTRACT

Rosa gallica, a plant of the *Rosa* genus, has been used widely since the 13th century and is cultivated in many areas as a medicinal plant for the preparation of herbal medicines. However, details of the neuropsychological effects of *R. gallica* remain unclear; therefore we aimed to investigate the neuropsychological effects of a water-soluble extract of *R. gallica* in male C57BL/6N mice under normal conditions and under chronic stress. We administered a water-soluble extract of *R. gallica* to mice and performed a series of behavioral experiments to compare the treated animals with the untreated controls. No significant differences in activity level, anxiety-like behavior, depression-like behavior, body weight, and body temperature were observed between *R. gallica*-treated mice and control mice. However, in mice subjected to chronic stress, the observed decrease in activity was smaller in the *R. gallica*-treated mice than in the control mice. The oral administration of *R. gallica* did not affect the normal behavior of mice. However, when the mice were subjected to stress, *R. gallica* exerted an anti-stress effect. Therefore, *R. gallica* has potential as a medicinal plant for the purpose of stress prevention.

1. Introduction

The acquisition costs, supply shortages, and side effects accompanying the use of synthetic drugs have increased the interest in the use of plants. The World Health Organization (WHO) estimates that 80% of emerging economies depend on medicinal plants for primary care needs. In addition, the use of essential oils, which are extracts from plants, as alternative medicines has increased. However, the scientific basis for many medicinal plants and essential oils is not clear (Zhang et al., 2013). Although it has been suggested that essential oils are effective in treating mental illness, many scientists have doubted the effectiveness of essential oils as there is no scientific basis for any claim (Lis-Balchin and Hart, 1997; Buckle, 2014) . Therefore, the need to clarify the scientific basis for medicinal plants, especially plants proposed to have a beneficial effect on serious diseases, is rapidly growing (de Sousa et al., 2015). In addition, medicinal plants are an important source of new chemicals with potential therapeutic effects.

Plants have been widely used for medicinal purposes for thousands of years and the reported scientific evidence on their biological effects has increased in recent years (Masek et al., 2017). Flowers contain various beneficial compounds, such as phenolic acid, flavonols, and anthocyanins, which confer beneficial features, including antioxidant properties (Mohebitabar et al., 2017).

Rosa species, commonly known as roses (from the *Rosaceae* family) are known worldwide and are used widely as medicinal plants (Bitis et al., 2017). Roses originated from the Middle East, but are now cultivated worldwide (Krussman, 1981). *Rosa gallica* was bred by the indigenous peoples of central Europe, the south of Europe, and the Caucasus region. This rose species, which was first recorded in the 13th century, was the foundation of large-scale industry. A large number of cultivars have been generated from this species by crossing (Koczka et al., 2018). This rose variety is proposed to be effective for many diseases. For this reason, this species is cultivated for a wide range of functions, including the extraction of the essential oil and the preparation of herbal

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medicines. However, the neuropsychological effects of *R. gallica* have not been clearly demonstrated compared to those of *Rosa damascena*, which have been examined in detail (Boskabady et al., 2011). To clarify the effects on *R. gallica* on behavior may lead to the development of new therapeutic agents and should be a useful illustration of the scientific basis of aromatherapy. Rose hips, a pseudo-fruit from the plants of the *Rosa* genus, is a widespread wild plant that has been used traditionally as a medicinal compound for the treatment of various diseases (Mármol et al., 2017). Rose oil, extracted from rose hips, are known to have antidepressant, analgesic, and anxiolytic effects, and can also be used for psychological alleviation and improvement of sexual dysfunction (Mohebitabar et al., 2017). Transdermal administration of rose oil from *Rosa damascena* Mill causes significant reductions in respiration rate, blood oxygen saturation, and systolic blood pressure in humans, indicating a decrease in autonomic arousal (Hongratanaworakit, 2009). Oral administration of *Rosa damascena* Mill with rose oil to rats has been shown to be protective against stress and depression (Nazıroğlu et al., 2013).

Depression is a disease associated with exposure to stressful life events and is an obstacle to maintaining human health. Among psychiatric disorders, major depressive disorder (MDD) is the most common mental disorder worldwide and of importance (Josefsson et al., 2014). After consideration of recurrent relapse, high morbidity, comorbidity, and mortality, it is estimated that MDD will be the largest health burden in the world by 2020. Despite the emergence of many new antidepressants, many cases of depression are undiagnosed and untreated. Furthermore, as antidepressant drugs may have side effects such as dysuria, digestive dysfunction, and sexual dysfunction, the development of safe and effective antidepressant drugs and the development of anti-stress medicines as a prophylactic drug are desired. As a medicinal plant, *Acorus tatarinowii* Schott (Araceae) has been shown to be an antidepressant in mice (Han et al., 2013). The antidepressant effect of *Perilla frutescens*, used for centuries to treat various conditions including depression (Ji et al., 2014), has also been reported in mice (*Perilla* leaf). It has been reported that oil from *R. damascena* can suppress the activity of the hypothalamus and pituitary system of rats and mice (Dolati et al., 2013). Water-soluble extracts of *R. gallica* may also have antidepressant and anti-stress effects and this should be investigated.

As stated, many of the neuropsychological effects of *R. gallica* have not yet been clarified. The purpose of this study is to clarify the neuropsychological effects of water-soluble extracts from *R. gallica* in mice and to evaluate the anti-stress effect of *R. gallica* in mice subjected to chronic stress.

2. Materials and methods

2.1. Animals

All animal experiments were performed in accordance with the U.S. National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised in 1996) and approved by the Committee for Animal Experiments at Kawasaki Medical School Advanced Research Center (17–26) and all regulations were strictly followed. All efforts were made to minimize the number of animals used and their suffering. Fifteen-week-old male C57BL/6N mice were purchased from Charles River Laboratories (Kanagawa, Japan) and housed in cages (five animals per cage) at 23°C–26 °C, with ad libitum access to food and water, and subjected to a 12 h light/dark cycle. All behavioral tests were conducted in behavioral testing rooms between 08.00 and 18.00 h during the light phase of the circadian cycle. The mice were randomly divided into the experimental and control groups (n = 10 for each group).

2.2. Plant materials

Air-dried petals of *Rosa gallica officinalis* were purchased from Ohtsuya Co., Ltd (Tokyo, Japan). A voucher specimen (No. 1712) was deposited at the herbarium of Nakamura Gakuen University Junior College.

2.3. Preparation of extracts

The dried petals (200 g) were submitted to maceration in 70% ethanol during 28 days at room temperature. Thereafter, the extract was filtered and then concentrated under reduced pressure (at approximately 40 °C). The maceration was repeated twice. After removing the solvent by lyophilization, this procedure gave 27.8 g of a red purple solid and dry hydroalcoholic crude extract (13.9 % w/w yield). The extract was stored in a closed bottle at 4 °C for further use. The extract was dissolved in 1% Tween-80 and used for the investigation of the anti-stress effects of the hydroalcoholic extract of *R. gallica officinalis* in mice.

2.4. Experimental design

First, the aqueous and ethanol extracts of the aerial parts of *R. gallica officinalis* were administered in a volume of 5 mL/kg body weight. The oral administration of reagents was performed every day for 16 days. The details of the experimental design are shown in Fig. 1.

2.5. Behavioral tests

All behavioral tests were performed during the light phase of the circadian cycle (09:00–16:00). Each behavioral test was separated from the next by at least 1 day. The mice were tested in random order. To prevent any bias due to olfactory cues, the apparatus was cleaned with 70% ethanol and water with superoxidized hypochlorous acid after testing. Behavioral tests were performed in accordance with the order described below. The aqueous and ethanol extracts of the aerial parts of *R. gallica officinalis* were administered by oral route 30 min before the behavioral test. The control mice received a similar volume of vehicle.

2.6. General health

The body weight and rectal temperature of the mice were recorded.

2.7. Light/dark transition test

The light/dark transition test developed by Crawley and Goodwin (1980) was performed (Crawley and Goodwin, 1980). The apparatus consisted of a cage (60 × 60 × 40 cm) divided into two sections of equal size with a small rectangular opening (5 × 3 cm) allowing access into each chamber. One chamber was brightly illuminated (400 lux) and the other chamber was dark (10 lux). Mice were placed into the dark chamber and allowed to move freely between the two chambers for 10 min. The distance traveled in the light chamber (cm), the total number of transitions, latency to enter the light chamber(s), and the time spent in the light chamber(s) were recorded on video and analyzed by using video tracking software (TopScan, CleverSys Inc., Reston, VA, USA).

2.8. Elevated plus maze test

The elevated plus maze test was used to evaluate anxiety-like behavior (Komada et al., 2008). The apparatus consisted of two open arms (8 × 25 cm) and two closed arms of the same size with 30 cm high transparent walls. The arms were made of white plastic plates and were elevated to a height of 40 cm above the floor, with arms of the same type located opposite each other. Each mouse was placed in the central square of the maze, facing one of the closed arms, and allowed to move freely between the two arms for 10 min. The number of arm entries, distance traveled (cm), latency to enter the open arms (s), and time spent in open arms were recorded on video and analyzed by using video tracking software (TopScan).
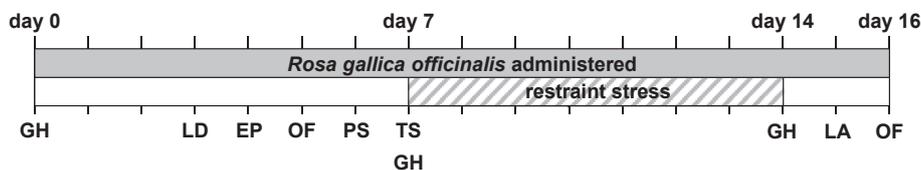
Fig. 1. Schematic illustration of the experimental design. Experimental scheme. GH, general health; LD, light/dark transition; EPM, elevated plus maze; OF, open field; PS, Porsolt forced swim; TS, tail suspension; LA, locomotor activity.

2.9. Open field test

Each subject was placed in the center of an open field apparatus (60 × 60 × 40 cm). The total distance traveled (m) and time spent in the center area(s) were recorded. The center area was defined as the 30 × 30 cm area located at the center of the field. The data were collected over a 30 min period. The data analysis was performed automatically by using video tracking software (TopScan).

2.10. Porsolt forced-swim test

The Porsolt forced swim test, developed by Porsolt and colleagues was performed (Porsolt et al., 1977; Petit-Demouliere et al., 2005). The apparatus for the Porsolt forced swim test consisted of four Plexiglas cylinders (20 cm height × 10 cm diameter). The cylinders were filled with water (23 °C) up to a height of 7.5 cm. The mice were placed into the cylinders, and their behavior was recorded over a 6 min test period. Images were captured using a video camera, and immobility time was measured from the captured images. In this test, the "immobile period" was defined as the period when the animals stopped struggling for ≥1 s. Data acquisition and analysis were performed automatically by using video tracking software (ANY-MAZE, Stoelting Co., Wood Dale, IL, USA).

2.11. Tail suspension test

The tail suspension test was used to evaluate depression-like behavior (Steru et al., 1985). Each mouse was suspended by the tail at 60 cm above the floor, in a white plastic chamber, using adhesive tape placed <1 cm from the tip of the tail. The behavior was recorded for 6 min. Similar to the Porsolt forced-swim test, the immobility time was evaluated using the ANY-MAZE software.

2.12. Stress

Animals in the experimental groups were subjected to restraint stress once per day. To assess the anti-stress effects, *R. gallica officinalis* was administered to experimental groups at 5 mL/kg, 30 min prior to the restraint stress. The mice were kept in a tightly fitted ventilated plastic tube for 6 h without access to food or water. This procedure was performed for 7 days (Fig. 1). The control mice were housed in a separate room, with no contact with the stressed mice, and were administered the same volume of vehicle every day for 7 days.

2.13. Locomotor activity test

To measure the locomotor activity, the mice were acclimated to the single housing environment for 2 h. Locomotor activity data were measured by using a photobeam activity system (ACTIMO-100; BRC Co., Nagoya, Aichi, Japan), and activity counts were recorded at 10 min intervals.

2.14. Statistical analysis of behavioral tests

The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test, two-way repeated measures ANOVA followed by Fisher's LSD test, Student's *t*-test, or paired *t*-test. A p-value of <0.05

was regarded as statistically significant. All data were presented as the mean ± SEM.

3. Results

3.1. Effects of R. gallica consumption on changes in body weight and temperature

We compared the general health of the *R. gallica*-treated and control groups. There were no significant differences in body weight between *R. gallica*-treated and control mice (Fig. 2A, $F_{1,36} = 0.051$, p = 0.823). There were no significant differences in body temperature between *R. gallica*-treated and control mice (Fig. 2B, $F_{1,36} = 0.074$, p = 0.787).

3.2. Effects of R. gallica consumption on the light/dark transition test and the elevated plus maze test

We evaluated anxiety-like behavior in *R. gallica*-treated mice. In the light/dark transition test, there were no significant differences in the number of total transitions, time spent in the light chamber, latency to enter the light chamber, and the distance traveled in the light chamber between the *R. gallica*-treated and control group mice (Fig. 2C, $t = 0.893$, p = 0.194; Fig. 2D, $t = 0.064$, p = 0.475; Fig. 2E, $t = 0.84$, p = 0.207; Fig. 2F, $t = 0.062$, p = 0.476). In the elevated plus maze test, there were no significant differences in the number of open arm entries, time spent in the open arms, latency to enter the open arms, and the distance traveled between the *R. gallica*-treated and control group mice (Fig. 2G, $t = 0.062$, p = 0.476; Fig. 2H, $t = 0.378$, p = 0.356; Fig. 2I, $t = 0.938$, p = 0.186; Fig. 2J, $t = 0.081$, p = 0.468).

### 3.3. Effects of R. gallica consumption on the open-field test

In the open field test, we observed no significant difference between groups in the total distance traveled (Fig. 3A, $F_{1,18} = 0.105$, p = 0.749) or time spent in the center area (Fig. 3B, $F_{1,18} = 0.851$, p = 0.369).

3.4. Effects of R. gallica consumption on the Porsolt forced swim test and tail suspension test

Next, we evaluated depressive-like behavior in *R. gallica*-treated mice. In the Porsolt forced swim test, there were no significant differences in the percentage of immobility time in each 1 min period during the 6 min test period between the *R. gallica*-treated and control mice (Fig. 3C, $F_{1,18} = 1.789$, p = 0.198). In the tail suspension test, there were no significant differences in the percentage of immobility time in each 1 min period during the 6 min test period between the *R. gallica*-treated and control mice (Fig. 3D, $F_{1,18} = 0.539$, p = 0.473).

3.5. Effects of R. gallica consumption on restraint stress-induced behavioral alteration

The mice were subjected to physical stressed for 7 days. In this study, we aimed to clarify the effect of *R. gallica* consumption on restraint stress. There was no significant difference in the body weight between *R. gallica*-treated and control mice (Fig. 4A, $F_{1,36} = 0.001$, p = 0.9702). We observed a significant increase in the locomotor activity in the *R. gallica*-
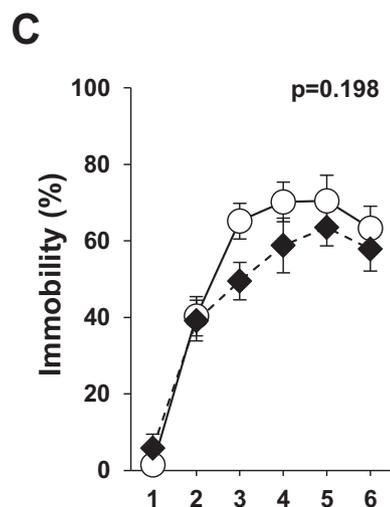
General Health

Light / Dark Transition Test

Elevated Plus Maze Test
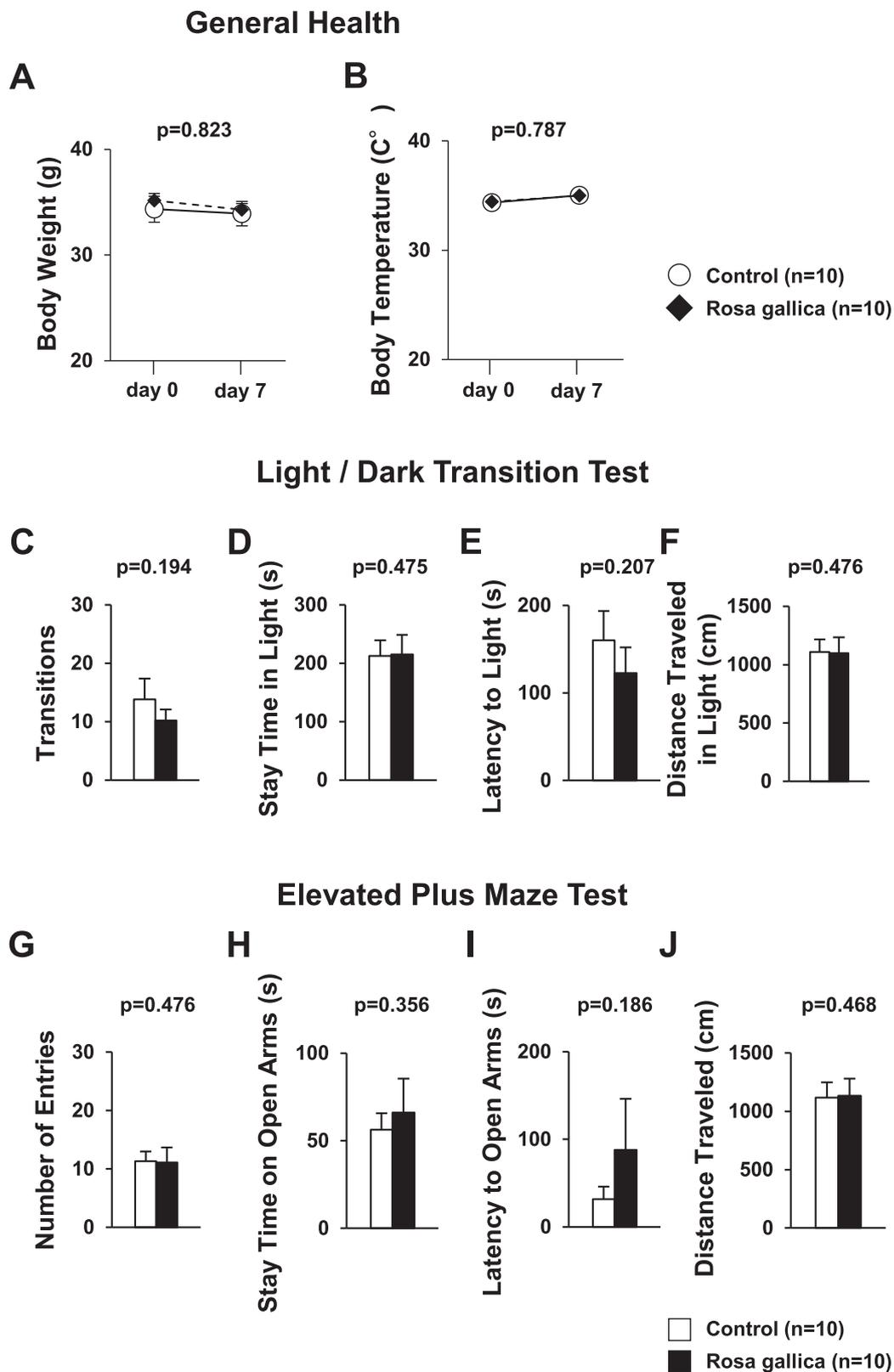
Fig. 2. General health, light/dark transition test, and elevated plus maze test. General health: body weight (**A**), body temperature (**B**), light/dark transition test: number of transitions (**C**), time spent in the light chamber (**D**), latency to enter the light chamber (**E**), and distance traveled in the light chamber (**F**). Elevated plus maze test: number of open arm entries (**G**), time spent in open arms (**H**), latency to enter the open arm (**I**), and distance traveled (**J**). All data are presented as the mean $\pm$ SEM. The p values indicate the administration effect in two-way repeated measures ANOVA (**A**, **B**) and Student's t-test (**C–F**).
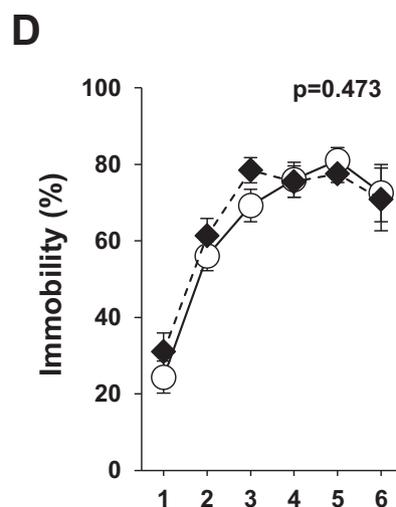
Open Field Test

A   **B**
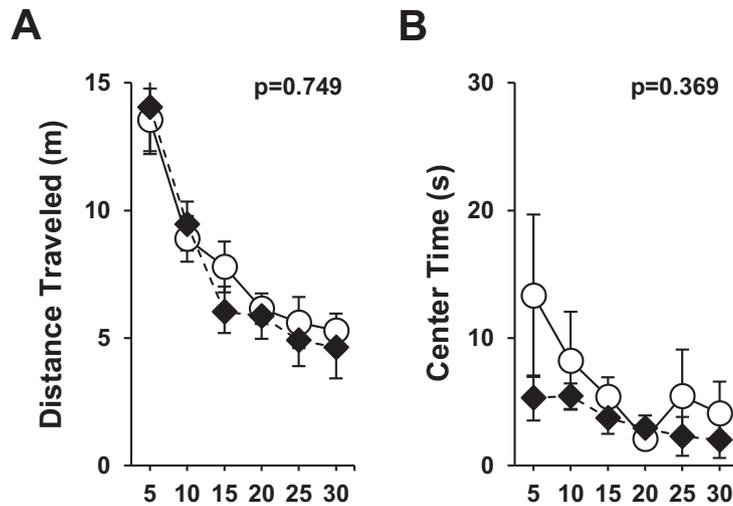
Porsolt forced swim test   Tail suspension test

C   **D**

☐ Control (n=10)
■ Rosa gallica (n=10)

Fig. 3. Open field test, Porsolt forced swim test and tail suspension test. Open field test: distance traveled in each area (**A**), and time spent in the center (**B**) in each 5 min period. Porsolt forced swim test: the proportion of time spent immobile in each 1 min period (**C**). Tail suspension test: the proportion of time spent immobile in each 1 min period (**D**). All data are presented as the mean ± SEM. The *p* values indicate the administration effect analyzed by two-way repeated measures ANOVA (**A–D**).

treated group compared with the control mice (Fig. 4B, $F_{1,25} = 0.1.851$, p = 0.023).

In the open field test, we found no significant differences in the distance traveled between *R. gallica*-treated and control mice (Fig. 4C, $F_{1,18} = 1.171$, p = 0.293). The *R. gallica*-treated mice spent significantly more time in the center area during the 30 min test period than the control mice (Fig. 4D, $F_{1,18} = 4.738$, p = 0.044).

4. Discussion

In this study, we concluded that the administration of *R. gallica* did

not affect anxiety-like behavior, depression-like behavior, and locomotor activity in mice, but it exerted anti-stress activity when chronic stress was applied. Although *R. gallica* has been thought to exert psychological effects since ancient times, no scientific basis for this existed. In this study, we have demonstrated the biological activity of the water-soluble extracts derived from *R. gallica* on the nerves.

In this study, several behavioral experiments were conducted to investigate the neuropsychological effects of *R. gallica*. Compared with control mice, mice that received the extract of *R. gallica* showed no clear behavioral change in the behavioral experiment in this study. Given that there are almost no papers on clinical trials and animal experiments using

After restraint stress

Locomotor Activity Test

Open Field Test
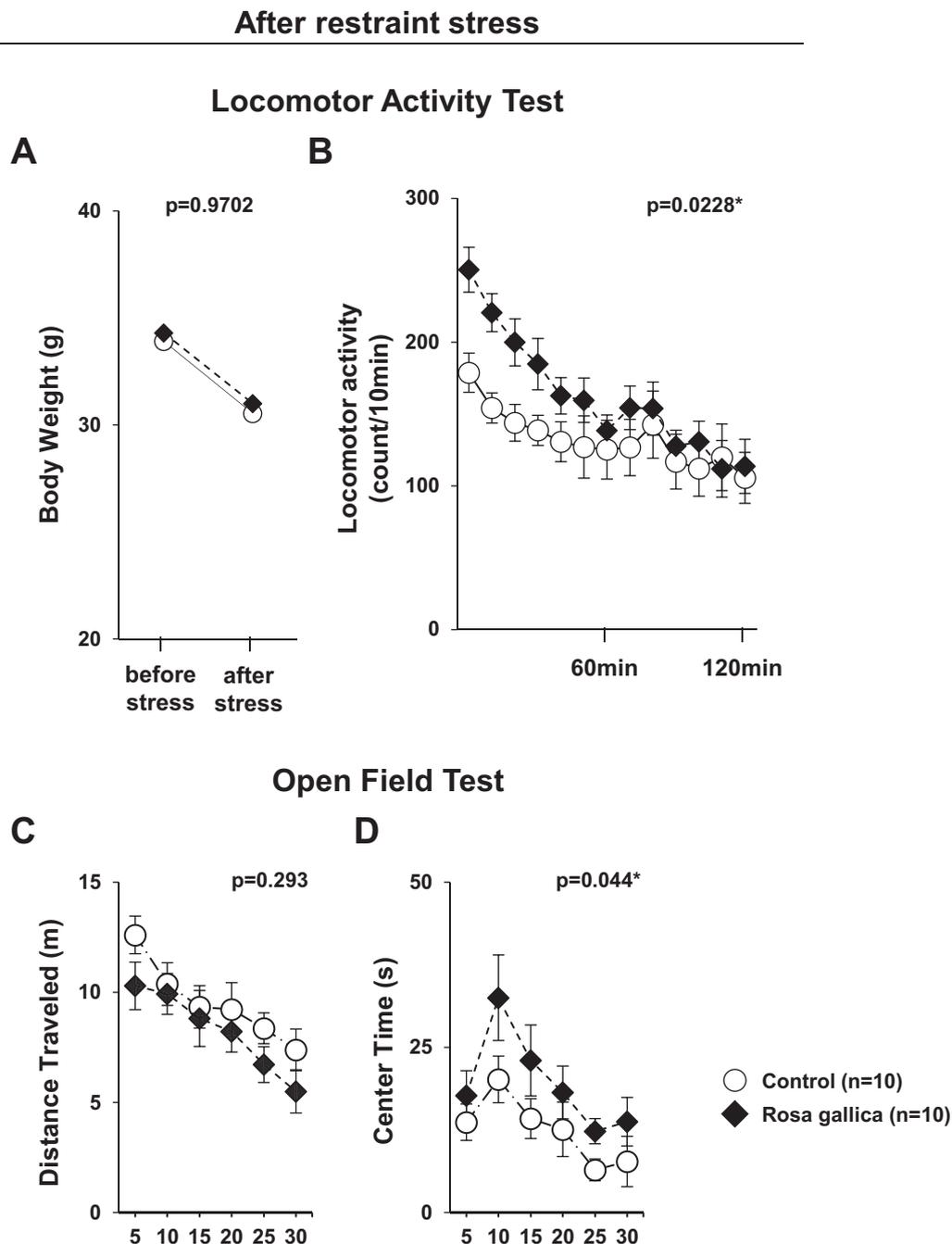
Fig. 4. Effect of *Rosa gallica officinalis* on locomotor activity of stressed mice. Body weight after 7 days of repeated restraint stress (**A**). Locomotor activity test in new home cage after 7 days of repeated restraint stress (**B**). Each dot indicates the mean of 10 min. Open field test: distance traveled in each area (**C**), and time spent in the center (**D**) in each 5 min period. All data are presented as the mean ± SEM. The *p* values indicate the administration effect analyzed by two-way repeated measures ANOVA (**A**–**D**).

R. gallica, the results of this study are considered valid.

In this study, mice subjected to chronic stress for 1 week and administered *R. gallica* showed a significant weight reduction when compared with the control mice (Jiang et al., 2017). Restraint stress causes a decrease in activity and anxiety in mice (Jiang et al., 2017; Chu et al., 2016). Interestingly, in the mice receiving *R. gallica,* the reduction in activity after chronic stress was decreased compared with control mice. The chronic stress-loading method is a typical method for the induction of depression in rodents and is similar to the occurrence of stress in humans (Abo-youssef, 2016). Restraint stress increases reactive oxygen species in mice (Moretti et al., 2013) and causes depression-like behavior (Chu et al., 2016). The characteristics of depression are increased immobility and anxiety (Katz et al., 1981). Oxidative stress plays an important role in mental disorders such as depression and is suggested to be an important factor in the etiology of depression (Maes et al., 2011). Increased oxidative stress has been observed in patients with depression (Sarandol et al., 2007). Indeed, antioxidants have been tested as new treatments for the treatment of depression, and promising results have been obtained (Sarandol et al., 2007). In this study, it is presumed that oxidative stress is caused by restraint stress. Further research is necessary to clarify to what extent the oxidative stress actually occurs.

Many studies have reported that *Rosa* species have antioxidant functions (Masek et al., 2017). As rose species contains various terpenes (glycosides), flavonoids, and anthocyanins, it is thought to exhibit various biological activities (Kumar et al., 2009). *Rosa gallica* also contains similar ingredients. However, there are no reports on the antioxidant and anti-stress effects of *R. gallica*. Further research to investigate the antioxidant action of *R. gallica* is necessary. Although the details of the mechanism of action of *R. gallica* are unknown, this study shows that substances with anti-stress activity may be present in the water-soluble extract of *R. gallica*.

The pharmacological activity of rose oil has been evaluated by several in vitro and in vivo tests (Boskabady et al., 2006). The effects of rose oil on the central nervous system have been reported including hypnosis, anticonvulsant, antidepressant, anxiolytic, analgesic, and morphine withdrawal symptoms (Abbasi Maleki et al., 2013). In humans, it has also been shown that olfactory stimulation by rose oil causes physiological and psychological relaxation effects (Igarashi et al., 2014). The inhalation of rose oil reduces the level of salivary cortisol and testosterone in healthy participants (Fukui et al., 2007). Furthermore, it is known that the antioxidant effect is also present in 2-phenylethanol, which is a component of the rose scent (Ueno et al., 2018). However, the molecular mechanism of the antidepressant effect of rose oil has not yet been elucidated (Zarghami et al., 2002). Flavonoids and kaempferol compounds contained in rose varieties are said to have antidepressant properties (Moallem et al., 2007). Anthocyanins have also been reported to have beneficial biological activity (Meiers et al., 2001). Rose oil may have antidepressant effects owing to its antioxidant activity (Nazıroğlu et al., 2013). In this study, a water-soluble extract from *R. gallica* was used. The water-soluble extract from the rose seed contains 10%–50% rose oil (Mohamadi et al., 2013). Therefore, it is thought that it exerted the antidepressant effect by the same molecular mechanism as rose oil. However, to elucidate the type of mechanism involved, further research is needed.

5. Conclusions

In this study, we showed that the water-soluble extract from *R. gallica* did not affect normal behavior in mice, but exerted anti-stress effects under conditions of chronic stress. The present research has shown part of the scientific basis that the water-soluble extract derived from *R. gallica* acted on the central nervous system of mice, suggesting that *R. gallica* may be developed as a prophylactic drug. Further research to investigate the cellular-molecular effects of *R. gallica* based on the anti-stress effects in this study is necessary.

Declarations

Author contribution statement

Hiroshi Ueno: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Atsumi Shimada: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Shunsuke Suemitsu: Performed the experiments; Analyzed and interpreted the data; Shinji Murakami, Naoya Kitamura, Kenta Wani, Yosuke Matsumoto, Takeshi Ishihara: Contributed reagents, materials, analysis tools or data.

Motoi Okamoto: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Yuko Fujiwara: Analyzed and interpreted the data; Wrote the paper.

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