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Research paper

Protective role of the ginsenoside Rg1 against methimazole-induced gestational hypothyroidism on reflexive behaviors, conditioned fear and cortical antioxidant levels in mice offspring

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ABSTRACT

Ginsenoside Rg1(Rg1), a monomer of a tetracyclic triterpenoid derivative, possesses diverse medicinal properties attributed to its unique chemical structure and may have beneficial effects on fetal development. This study aimed to investigate the protective effects of prenatal exposure to Rg1 against Methimazole-induced gestational hypothyroidism on reflexive behaviors, conditioned fear, and cortical antioxidant levels in mouse offspring.40 female virgin mice and 12 male NMRI mice were assigned to four groups: group 1 served as the control, group 2 received Methimazole(MMI) at a concentration of 0.02% in their drinking water, group 3 received Rg1(150 mg/kg), and group 4 received both MMI and Rg1.Groups of 2–4 were administered the substances from days 1–9 of gestation. After delivery, pups were selected, and reflexive motor behaviors and conditioned fear were assessed. Additionally, levels of brain tissue catalase(CAT), malondialdehyde(MDA), superoxide dismutase(SOD), and glutathione peroxidase(GPx) levels were measured. Furthermore, postpartum immobility time in the forced swimming test (FST), tail suspension test (TST), and the number of squares crossed in the open field test (OFT) were determined. The results demonstrated that maternal exposure to Rg1 improved ambulation score, hind-limb suspension score, grip strength, front-limb suspension, hind-limb foot angle, negative geotaxis, surface righting, and conditioned fear in hypothyroidism-induced offspring($P<0.05$). Rg1 decreased immobility time in the FST, and TST, and increased the number of squares crossed in the OFT in postpartum hypothyroidism-induced mice ($P<0.05$). Moreover, Rg1 reduced brain tissue MDA levels and increased brain tissue CAT, SOD, and GPx levels in mice and their offspring($P<0.05$). These findings indicate that Rg1 mitigated postpartum depression in mice and improved reflexive motor behaviors in their pups.

Introduction

Prenatal central nervous system development is crucial for postnatal life (Moreno and de Brugada, 2021). Medications or maternal nutrition during pregnancy can cross the placenta and penetrate the fetal blood-brain barrier, leading to both beneficial and detrimental effects on cognitive function in adulthood (Tachibana et al., 2021), and reflexive motor behaviors (Khodadadeh et al., 2020). Neurodevelopmental reflexes are involuntary and repetitive movements observed in infants, which reflect the functioning of brainstem and spinal cord reflexes. These reflexes serve as a rapid assessment tool for evaluating neurological development in offspring. Abnormal synaptogenesis, functioning, and myelination resulting from neural and brain disorganization can cause delays or absence of neurodevelopmental reflexes. Abnormal reflexes are predictive of developmental disorders in both humans and animals (Nguyen et al., 2017). Due to the similarities in developmental patterns among rodents, primates, and humans (Andersen, 2003), rodents are well-suited models for studying motor skills and assessing neurodevelopmental disabilities and reflex delays using reflex batteries (Semple et al., 2013).

Thyroid hormones play a critical role in brain development, and gestational hypothyroidism disrupts fetal brain development (Ahmed, 2015), leading to deafness, delays in preweaning motor activity, malformations, and developmental defects in the cerebellar and cerebral

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cortex of offspring (El-bakry et al., 2010; GoLDEY et al., 1995). Hypothyroidism can also contribute to postpartum depression (McCoy, 2011). Therefore, Methimazole-induced gestational hypothyroidism can negatively impact the developmental milestones of offspring (Hipólito et al., 2023). Maternal thyroid hormones need to be transported across the placenta during the early stages of gestation since the fetal thyroid gland remains non-functional until mid-gestation (Melancia et al., 2017).

Guidelines recommend higher initial levothyroxine doses for children with congenital hypothyroidism based on studies linking treatment levels with developmental outcomes. However, there are inconsistent reports regarding improved outcomes, and concerns exist about potential negative effects of high dosages (Heyerdahl and Oerbeck, 2003). Recent studies have explored the use of herbal medicines like Withania somnifera to mitigate the harms of hypothyroidism (Jatwa and Kar, 2009). Additionally, Panax ginseng has shown the potential to ameliorate neural impairments induced by hypothyroidism (Wahman and Elgoly). Postpartum depression is typically treated with selective serotonin reuptake inhibitors (SSRIs) (sertraline being the preferred choice for lactating women), serotonin-norepinephrine reuptake inhibitors (SNRIs), adrenergic antidepressants, or GABA receptor modulators (Ahmed, 2018; McCoy, 2011; Melón et al., 2018). On the other hand, neuroprotective agents like Rg1 do not necessarily need to penetrate the brain to exert their effects. Since designing central nervous system drugs with good brain distribution and minimal cerebral side effects is challenging, targeting peripheral immune responses may hold promise for future investigations (Zheng et al., 2014).

Rg1 is a steroid glycoside derived from the Chinese herbal medicine Panax ginseng Meyer, known for its neurotrophic and neuroprotective properties (Gao et al., 2017; Lee et al., 2019). It promotes hippocampal neurogenesis, neuroplasticity, learning, memory, anti-aging effects, anti-fatigue effects, immune modulation, and antitumor activity through various mechanisms, including antioxidant activation, immune stimulation, anti-inflammatory and anti-apoptotic activities, effects on nerve growth factors, inhibition of excitotoxicity, preservation of neuronal structural integrity, and maintenance of cellular adenosine triphosphate (ATP) levels (Xie et al., 2018). Rg1 also reduces intracellular reactive oxygen species (ROS) levels and increases levels of total superoxide dismutase (T-SOD), catalase (CAT), and glutathione (GSH) (Zhu et al., 2009). Studies have demonstrated that Panax ginseng injection in patients with chronic heart failure increased levels of triiodothyronine (T3) and thyroxine (T4) while decreasing reverse triiodothyronine (rT3) (Dai et al., 2000).

Based on the existing literature, Rg1 has been shown to have positive effects on neurodevelopment, fetal growth, and the antioxidant defense system. However, it remains unclear whether Rg1 plays a role in reflexive motor behavior in offspring prenatally exposed to MMI-induced hypothyroidism. Thus, the primary objective of the present study was to investigate the impact of prenatal exposure to Rg1 on reflexive motor behaviors and antioxidant activity (MDA, SOD, GPx, and CAT) in the offspring of mice. The study also aims to explore the potential effects of Rg1 on postpartum depression in hypothyroid dams induced by MMI.

Materials and methods

Animals

The experimental subjects consisted of 12 male NMRI mice and 40 female virgin mice aged 8–10 weeks with a weight range of 28–30 g. These mice were obtained from the Pasture Institute (Tehran, Iran). They were housed in standard plastic enclosures under laboratory conditions with a regulated temperature of 22 ± 2°C and a 12-hour light/dark cycle. The mice had ad libitum access to standard chow pellets and fresh water. Prior to the commencement of the study, the mice underwent a one-week adaptation period. The study protocol was approved by the Animal Ethics Committee of the Science and Research Branch of Islamic Azad University, Tehran, Iran (IR.IAU.SRB.REC.1402.030; 2023–06–10).

Study protocol

Following the acclimatization period, the female mice were paired with fertile male mice and daily examinations were conducted to detect the presence of sperm or vaginal plugs as indicators of pregnancy. The pregnant mice were then randomly divided into four groups, each consisting of ten individuals. All groups had ad libitum access to food and water. The control group received water as a placebo, while the second group received MMI (Iran Hormone Pharmaceutical Co.) at a concentration of 0.02% in their drinking water until gestational Day 9 (Hipólito et al., 2023). In the third group, Rg1 (>99.9% purity, Merck KGaA, Darmstadt, Germany) was injected intraperitoneally at a dosage of 150 mg/kg until gestational Day 9 (Xie et al., 2015; Zeng et al., 2014). The fourth group received both MMI at a concentration of 0.02% in their drinking water and Rg1 (150 mg/kg) intraperitoneally until gestational Day 9. After parturition, 20 male pups were selected from each litter based on the criterion of anogenital distance using a digital caliper by measuring the distance(mm). Measurements were made from the base of the genital papilla to the center of the anus. Mice were held by the skin of their neck and turned on their back so that the anogenital region was visible(Cantoni et al., 1999; Hotchkiss and Vandenbergh, 2005). These pups then underwent neonatal assessments of fear and motor skills behavior (Fig. 1). To minimize complications arising from body temperature or hunger/separation, the young mice were separated from their mother for a maximum of 15 minutes.

Offspring experiments

Pups were temporarily removed from their dams between 12:00 and 13:00 for daily monitoring and promptly returned to their primary habitat. Physical, behavioral, and conditioned fear parameters were evaluated (Hipólito et al., 2023).

Ambulation

The crawling behavior is observed in juvenile mice until postnatal day 5, after which they begin walking between 5 and 10 days of age (Williams and Scott, 1953). The ambulation test was performed on 5-day-old offspring to take advantage of this transitional period. The mice were placed in a transparent chamber that allowed visibility from the top and all four sides. To encourage walking, a gentle prodding of the tail was applied. The ambulation test utilized a grading system with four categories: a score of 0 indicated no movement, a score of 1 denoted crawling with asymmetrical limb movement, a score of 2 indicated slow crawling with symmetrical limb movement, and a score of 3 indicated rapid crawling or walking. To minimize the influence of learning on the test results, the trial was repeated three times within a three-minute interval (Feather-Schussler and Ferguson, 2016).

Hind-limb foot angle

The study aimed to analyze the hind-limb foot angle in 8-day-old offspring (PD 8) to investigate changes in hind-limb posture during the developmental transition from crawling to ambulation. As the pups matured and started walking, their hind limbs shifted position to be located beneath their bodies. Consequently, the angle formed by the hind limbs during walking was observed to be smaller compared to that during crawling. To observe the motion of the pups, a simple enclosure with an unobstructed view, equipped with a video recorder, was used. The pups were encouraged to walk by gently touching their tails. The measurement of foot angle was performed using recorded videos, where a line was drawn from the end of the heel/shin to the tip of the middle digit. Foot angle measurements were limited to pups that executed a

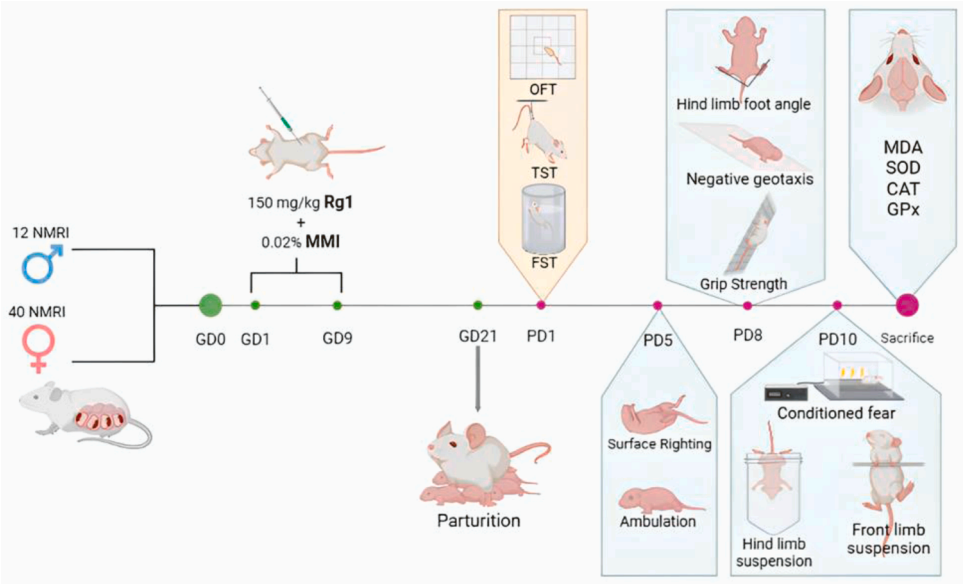

Fig. 1. The flow chart of study.

complete stride in a linear trajectory with their feet in a flat position on the ground. To minimize potential experimental errors, foot angle averages were calculated from three to five sets of measurements for each pup. The test results did not show any evidence of learning-related effects (Feather-Schussler and Ferguson, 2016).

Front-limb suspension

The front-limb suspension test is typically performed on pups no younger than 10 days of age (Heyser, 2003). However, to establish a consistent testing protocol, we conducted this examination on postnatal day 10. The pups were allowed to grip a wire using their front limbs bilaterally. Subsequently, they were released, and the duration of their descent was carefully recorded in seconds (Corti et al., 2008). To minimize the influence of the subjects' learning ability, the experiment was conducted three times within a three-minute time frame (Feather-Schussler and Ferguson, 2016).

Hind-limb suspension

The hind-limb suspension test was conducted to assess the strength and neuromuscular function of young pups up to PD 14, with testing initiated on PD 2 (El-Khodor et al., 2008). To minimize errors, all pups were examined on PD 10. The procedure involved placing the offspring in a standard 50 mL conical laboratory tube with their hind limbs suspended over the edge of the tube and then releasing them. The hind-limb posture was evaluated using a scoring system, where a score of 0 indicated uninterrupted hind-limb clasping, holding onto the tube. A score of 1 indicated nearly clasped hind-limbs with an elevated tail, indicating weakness. A score of 2 indicated proximate hind-limbs that were frequently touched. A score of 3 described noticeable hind-limb weakness with the limbs closer together and infrequently touching each other. Lastly, a score of 4 depicted normal hind-limb separation with an elevated tail. The testing was performed three times within a three-minute interval to minimize the influence of learning on the outcomes (Feather-Schussler and Ferguson, 2016).

Surface righting

The surface righting reflex, which involves the ability of offspring to rotate onto their feet from a supine posture, is observed in rodents aged between postnatal days 1–10, as documented in scientific literature (Heyser, 2003). The surface righting test is commonly used to measure this reflex. In this particular study, PD 5-day-old offspring were placed on their dorsal position on a cotton fabric and maintained in that position for 5 seconds. The time required for the pups to revert to their ventral stance after being released from the fixed position was carefully recorded (Anjos et al., 2022). Due to the absence of reported instances of learning effects, the examination was conducted three times within a three-minute time frame (Feather-Schussler and Ferguson, 2016).

Grip strength

This test assessed grip strength in pups within the PD 5–15 age range. However, to ensure precision and consistency, the experiment was conducted on PD 8 offspring. The objective of the study was to determine the ability of animals to maintain their grip on a screen and produce a measure of grip force. To evaluate the grasping ability of all four limbs, a 16 × 18 fiberglass screen was gradually rotated from a horizontal to a vertical position (Corti, 2017). The hanging impulse, which indicates the amount of force required to withstand gravity, was calculated using the following formula: [latency to fall (s) × weight (g)] (Venerosi et al., 2009).

Negative geotaxis

The negative geotaxis test, which measures the vestibular response to gravity, was conducted on postnatal day 8 mice, although it is applicable to postnatal days 3–15 (Heyser, 2003). The mice were placed on a 45-degree wooden slope and released, and the time taken for them to orient themselves upwards was recorded and documented (Feather-Schussler and Ferguson, 2016; Ruhela et al., 2019).

Conditioned fear

The foot shock chamber used in this study consisted of a plexiglass chamber measuring 25 × 22 × 22 cm, equipped with an 18-rod stainless steel grid floor, with each rod spaced 1.5 cm apart and connected to a shock generator. Mice offspring were assigned to the habituation, conditioning, and testing phases. Following each trial, a 5% alcohol solution was used to sanitize the chamber before the next rat. During the habituation phase, the mice were exposed to the foot shock chamber for ten minutes without the delivery of any shocks. After a 24-hour interval from the habituation phase, the conditioning shock session was

conducted on postnatal day 10. The mice were returned to the experimental chamber, where six electric foot shocks (1.5 mA, 3 s, with intervals of 20 s to 1 min) were administered (Moraes Resstel et al., 2008). The experimental protocol included a test phase, which involved a 10-minute period of re-exposure to the foot shock chamber without the administration of shocks. This phase was conducted 24 hours after the conditioning phase. Freezing behavior, defined as the complete absence of any movement other than respiration, while the animal displayed a characteristic tense posture, was assessed by a trained observer positioned 30 cm away from the foot shock chamber. Freezing behavior was then converted into percentage of freezing according to the following formula: % of freezing = (total freezing time/total test time) × 100 (Fanselow, 1980).

Maternal tests

Postpartum depression is a common disorder that affects both mothers and their offspring (Fernandez et al., 2014). Behavioral tests, such as the OFT, FST, and TST, were conducted during the postpartum period of 72 hours (Tan et al., 2018). In the FST, postpartum rats exhibited less swimming time and more immobility duration compared to virgin rats (Craft et al., 2010).

Open field test

The OFT is a widely used procedure for examining the behavioral effects of drugs and anxiety (Choleris et al., 2001; Walsh and Cummins, 1976). The primary outcome of interest is typically "movement," although this can be influenced by the dams' motion abilities, freezing response, or other fear-related behaviors (Gould et al., 2009). A 42 × 42 × 42 cm polyvinyl chloride box were divided into 9 squares and a camera was used to measure the movement of the mice in the arena or in the box (Kraeuter et al., 2019). Each individual test lasted six minutes, and the number of line crossed were recorded (Hipólito et al., 2023). The resulting video recordings were meticulously analyzed using SMART software (Seibenhener and Wooten, 2015).

Tail suspension test

The TST is widely used to assess antidepressant-like efficacy in mice. The test is based on the observation that animals subjected to short-term, inescapable stress by being suspended by their tail will adopt an immobile posture. Various antidepressant medications reverse immobility and promote escape-related behavior (Cryan et al., 2005). The total duration of the six-minute test can be divided into periods of agitation and immobility. For this experiment, the recording apparatus consisted of metallic gallows attached to a nylon catheter with a diameter of 1.5 mm and a length of 350 mm. A hook was securely fastened to the end of the catheter, with a distance of 350 mm between the floor of the device and the hook. The mouse was hung on the hook by adhesive tape placed 20 mm from the end of its tail. The immobility time was recorded in seconds (Steru et al., 1985).

Forced swimming test

Mice were individually placed into glass cylinders measuring 25 cm in height and 10 cm in diameter, filled with water to a depth of 10–13.5 cm and maintained at a temperature of 23–25°C. In this situation, from which they could not escape, animals rapidly became immobile, floating in an upright position and making only small movements to keep their heads above water (Bourin et al., 2004; Porsolt et al., 1977a,b). The duration of immobility was recorded for the last four minutes of the six-minute testing period in mice (Can et al., 2012). NMRI mice can be used to differentiate the mechanisms of action of drugs in the FST (Petit-Demouliere et al., 2005).

Antioxidant activity

After completing all behavioral assessments, cranium dissection was performed to collect brain samples from both mothers and offspring. The levels of GPx, SOD, MDA, and CAT in the brains of both mothers and their offspring were determined using assay kits provided by Zell Bio GmbH, a German-based company.

Statistical analysis

The obtained data were analyzed using one-way analysis of variance (ANOVA) and presented as mean ± standard error (SE). For treatments showing significant differences, mean values were compared using the Tukey HSD test. Treatments with P-values less than 0.05 were considered to have significant differences.

Results

Offspring tests

According to our findings, prenatal exposure to MMI significantly decreased the ambulation score in the offspring of mice compared to the control group [$F(3, 20) = 5.14$, $P<0.05$]. Prenatal exposure to Rg1 had no significant effect on the ambulation score in mice offspring compared to the MMI group ($P>0.05$). Combined prenatal exposure to MMI and Rg1 significantly improved the ambulation score in the mice offspring compared to the MMI group [$F(3, 20) = 5.68$, $P<0.05$] (Fig. 2).

Furthermore, prenatal exposure to MMI significantly increased the hindlimb foot angle in the offspring of mice compared to the control group [$F(3, 20) = 8.71$, $P<0.05$]. Prenatal exposure to Rg1 decreased the hindlimb foot angle in mice offspring compared to the MMI group [$F(3, 20) = 7.12$, $P<0.05$]. Combined prenatal exposure to MMI and Rg1 significantly improved the hindlimb foot angle in the mice offspring compared to the MMI group [$F(3, 20) = 6.55$, $P<0.05$]. (Fig. 3).

Prenatal exposure to MMI significantly decreased the hindlimb suspension parameters (score × falling time) in the offspring of mice compared to the control group [$F(3, 20) = 9.11$, $P<0.05$]. Prenatal exposure to Rg1 increased the hindlimb suspension parameters (score × falling time) in mice offspring compared to the MMI group [$F(3, 20) = 11.27$, $P<0.05$]. Combined prenatal exposure to MMI and Rg1 significantly improved the hindlimb suspension parameters (score × falling time) in the mice offspring compared to the MMI group [$F(3, 20) = 7.51$, $P<0.05$]. (Fig. 4).

According to Fig. 5, prenatal exposure to MMI significantly increased surface righting duration in the offspring of mice compared to the control group [$F(3, 20) = 2.73$, $P<0.05$]. Prenatal exposure to Rg1 decreased surface righting duration in mice offspring compared to the MMI group [$F(3, 20) = 3.45$, $P<0.05$]. Combined prenatal exposure to MMI and Rg1 significantly improved surface righting duration in the mice offspring compared to the MMI group [$F(3, 20) = 2.99$, $P<0.05$].

Based on Fig. 6, prenatal exposure to MMI significantly decreased grip strength in the offspring of mice compared to the control group [$F(3, 20) = 6.84$, $P<0.05$]. Prenatal exposure to Rg1 increased grip strength in mice offspring compared to the MMI group [$F(3, 20) = 8.32$, $P<0.05$]. Combined prenatal exposure to MMI and Rg1 significantly improved grip strength in the mice offspring compared to the MMI group [$F(3, 20) = 5.98$, $P<0.05$].

As seen in Fig. 7, prenatal exposure to MMI significantly decreased front-limb suspension in the offspring of mice compared to the control group [$F(3, 20) = 8.11$, $P<0.05$]. Prenatal exposure to ginsenoside increased front-limb suspension in mice offspring compared to the MMI group [$F(3, 20) = 7.43$, $P<0.05$]. Combined prenatal exposure to MMI and Rg1 significantly improved front-limb suspension in the mice offspring compared to the MMI group [$F(3, 20) = 5.29$, $P<0.05$].

As shown in Fig. 8, prenatal exposure to MMI significantly increased negative geotaxis in the offspring of mice compared to the control group

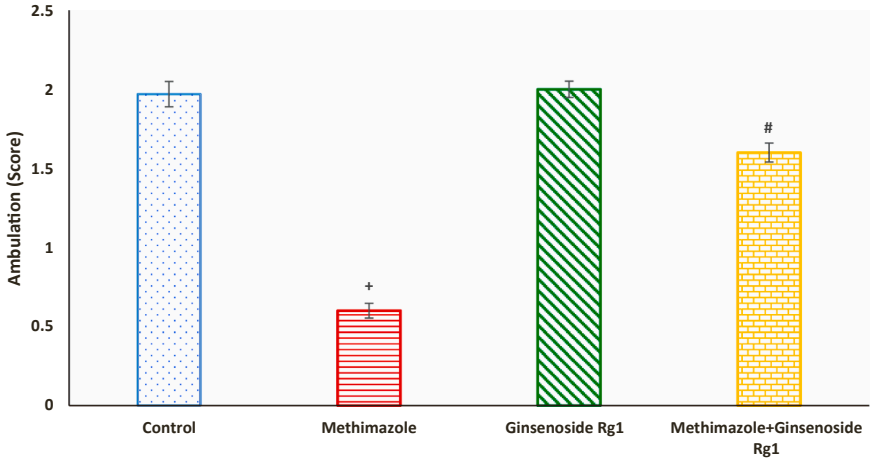

Fig. 2. The effects of prenatal exposure to methimazole, ginsenoside, and their combined administration during gestation on the ambulation score in mice offspring. Data are expressed as mean ±SE. + Indicate a significant difference compared to control group ($P < 0.05$). # Indicate a significant difference compared to methomazole group ($P < 0.05$).

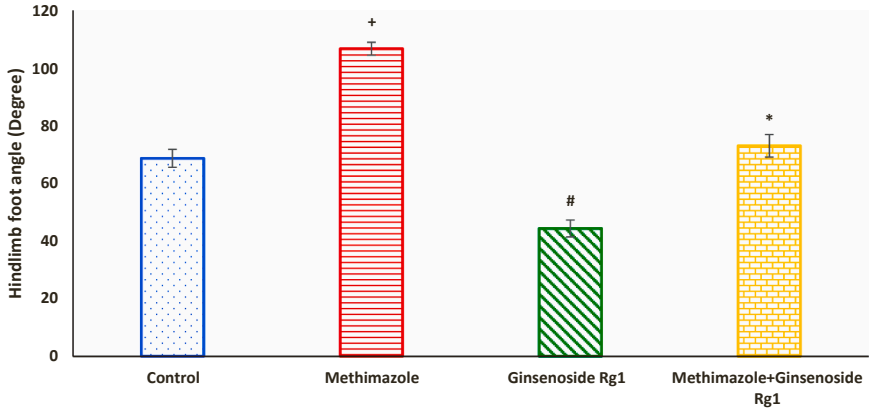

Fig. 3. The effects of prenatal exposure to methimazole, ginsenoside, and their combined administration during gestation on the hindlimb foot angle in mice offspring. Data are expressed as mean ±SE. + Indicate a significant difference compared to control group ($P < 0.05$). # Indicate a significant difference compared to methimazole group ($P < 0.05$). * Indicate a significant difference compared to methimazole group ($P < 0.05$).

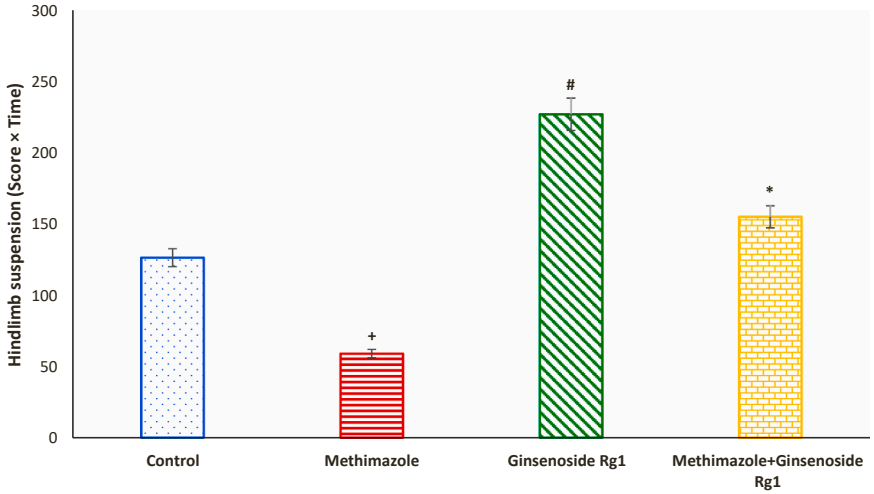

Fig. 4. The effects of prenatal exposure to methimazole, ginsenoside, and their combined administration during gestation on the hindlimb suspension (score x time) in mice offspring. Data are expressed as mean ±SE. + Indicate a significant difference compared to control group ($P < 0.05$). # Indicate a significant difference compared to methimazole group ($P < 0.05$). * Indicate a significant difference compared to methimazole group ($P < 0.05$).

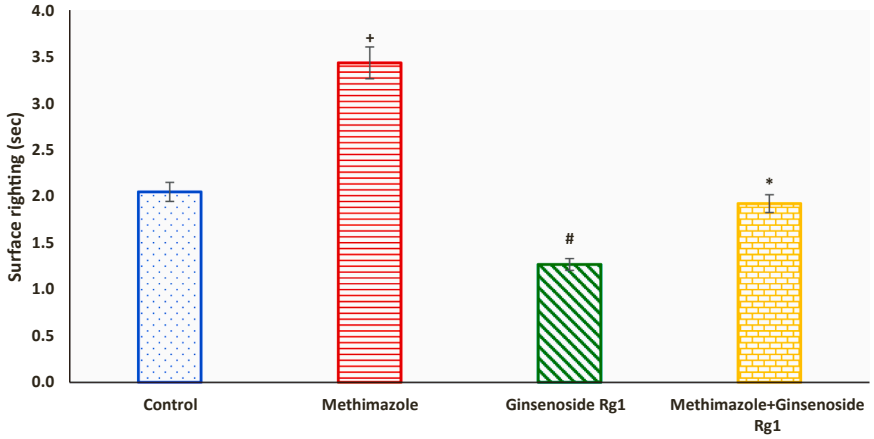

Fig. 5. The effects of prenatal exposure to methimazole, ginsenoside, and their combined administration during gestation on the surface righting duration in mice offspring. Data are expressed as mean ±SE. + Indicate a significant difference compared to control group ($P < 0.05$). # Indicate a significant difference compared to methimazole group ($P < 0.05$). * Indicate a significant difference compared to methimazole group ($P < 0.05$).

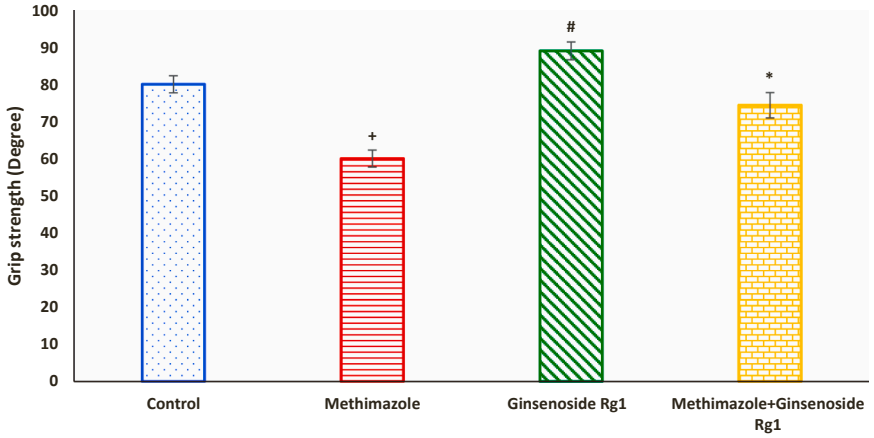

Fig. 6. The effects of prenatal exposure to methimazole, ginsenoside, and their combined administration during gestation on the grip strength in mice offspring. Data are expressed as mean ±SE. + Indicate a significant difference compared to control group ($P < 0.05$). # Indicate a significant difference compared to methimazole group ($P < 0.05$). * Indicate a significant difference compared to methimazole group ($P < 0.05$).

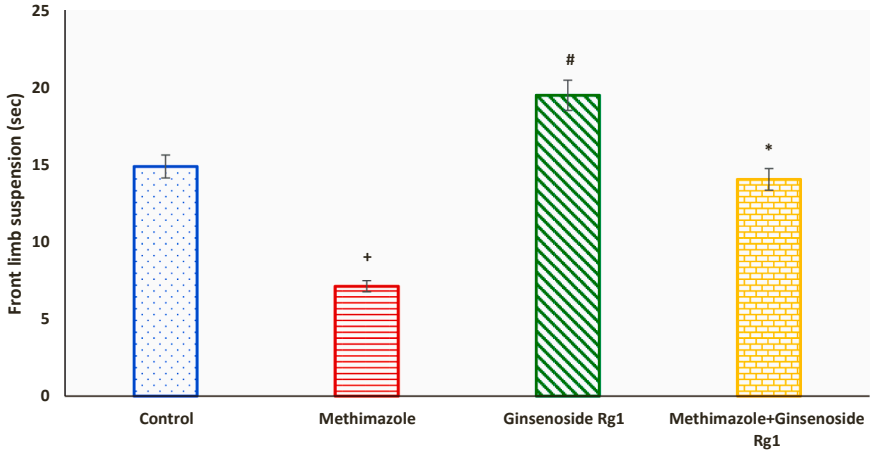

Fig. 7. The effects of prenatal exposure to methimazole, ginsenoside, and their combined administration during gestation on the front-limb suspension in mice offspring. Data are expressed as mean ±SE. + Indicate a significant difference compared to control group ($P < 0.05$). # Indicate a significant difference compared to methimazole group ($P < 0.05$). * Indicate a significant difference compared to methimazole group ($P < 0.05$).

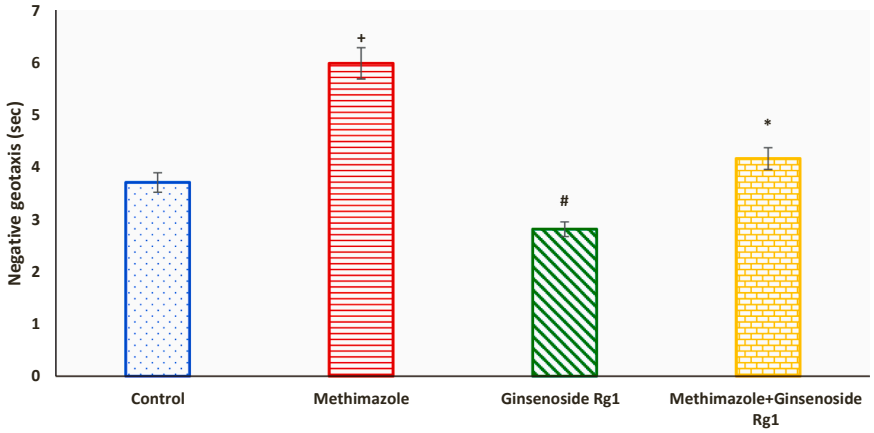

Fig. 8. The effects of prenatal exposure to methimazole, ginsenoside, and their combined administration during gestation on negative geotaxis in mice offspring. Data are expressed as mean ±SE. + Indicate a significant difference compared to control group ($P < 0.05$). # Indicate a significant difference compared to methimazole group ($P < 0.05$). * Indicate a significant difference compared to methimazole group ($P < 0.05$).

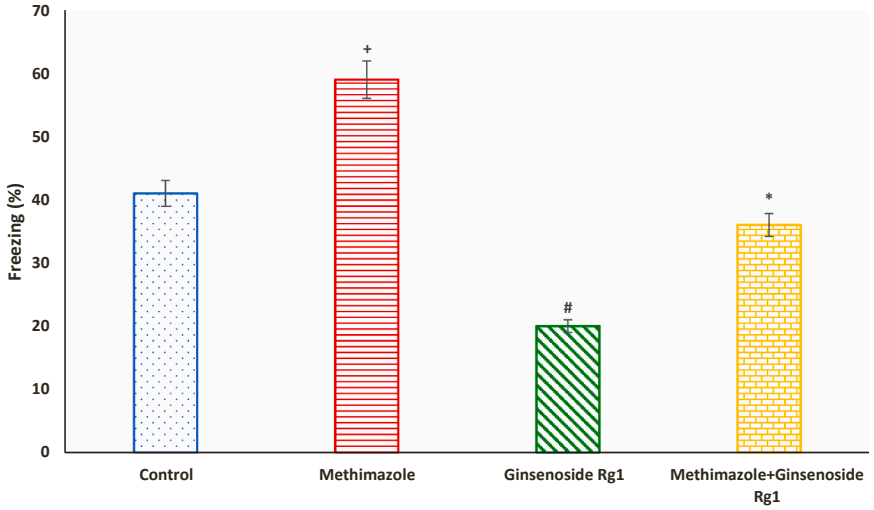

Fig. 9. The effects of prenatal exposure to methimazole, ginsenoside, and their combined administration during gestation on conditioned fear in mice offspring. Data are expressed as mean ±SE. + Indicate a significant difference compared to control group ($P < 0.05$). # Indicate a significant difference compared to methimazole group ($P < 0.05$). * Indicate a significant difference compared to methimazole group ($P < 0.05$).

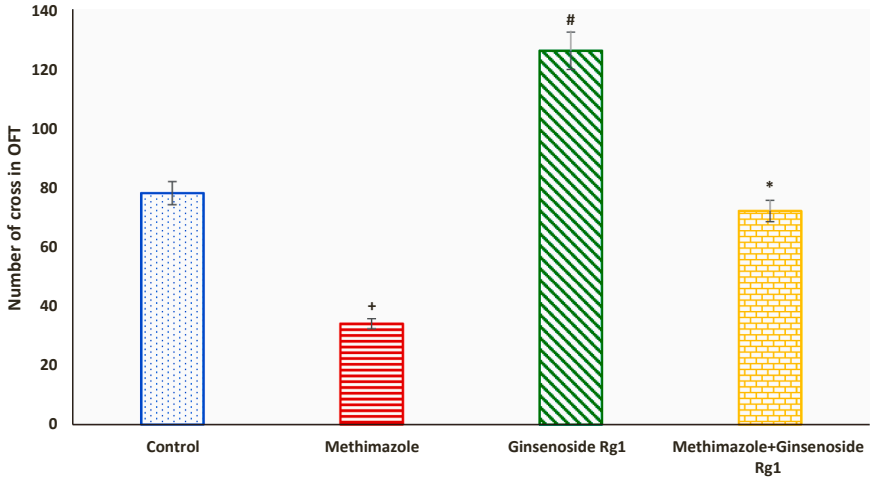

Fig. 10. The effects of administration of methimazole, ginsenoside, and their combined administration during gestation on OFT in mothers. Data are expressed as mean ±SE. + Indicate a significant difference compared to control group ($P < 0.05$). # Indicate a significant difference compared to methimazole group ($P < 0.05$). * Indicate a significant difference compared to methimazole group ($P < 0.05$).

[F (3, 20) = 14.68, P<0.05]. Prenatal exposure to ginsenoside decreased negative geotaxis in mice offspring compared to the MMI group [F (3, 20) = 9.41, P<0.05]. Combined prenatal exposure to MMI and Rg1 significantly improved negative geotaxis in the mice offspring compared to the MMI group [F (3, 20) = 6.57, P<0.05].

Based on Fig. 9, prenatal exposure to MMI significantly decreased fear conditioning in the offspring of mice compared to the control group [F (3, 20) = 13.67, P<0.05]. Prenatal exposure to ginsenoside increased fear conditioning in mice offspring compared to the MMI group [F (3, 20) = 8.65, P<0.05]. Combined prenatal exposure to MMI and Rg1 significantly improved fear conditioning in the mice offspring compared to the MMI group [F (3, 20) = 10.54, P<0.05].

Maternal tests

According to our findings, the administration of MMI during gestation in mothers significantly decreased moving frequency in the OFT compared to the control group [F (3, 20) = 15.87, P<0.05]. The administration of Rg1 during pregnancy in mothers increased moving frequency in the OFT compared to the MMI group [F (3, 20) = 14.35, P<0.05]. Combined administration of MMI and Rg1 significantly improved moving frequency in the OFT compared to the MMI group [F (3, 20) = 10.58, P<0.05]. (Fig. 10).

Furthermore, the administration of MMI during gestation in mothers significantly increased immobility time in the TST compared to the control group [F (3, 20) = 12.36, P<0.05]. The administration of Rg1 during pregnancy in mothers decreased immobility time in the TST compared to the MMI group [F (3, 20) = 14.65, P<0.05]. Combined administration of MMI and Rg1 significantly decreased and improved immobility time in the TST compared to the MMI group [F (3, 20) = 15.68, P<0.05]. (Fig. 11).

As indicated, the administration of MMI during gestation in mothers significantly increased immobility time in the FST compared to the control group [F (3, 20) = 16.35, P<0.05]. The administration of Rg1 during pregnancy in mothers decreased immobility time in the FST compared to the MMI group [F (3, 20) = 10.56, P<0.05]. Combined administration of MMI and Rg1 significantly decreased and improved immobility time in the FST compared to the MMI group [F (3, 20) = 13.25, P<0.05]. (Fig. 12).

Antioxidant activities

According to our findings, the administration of MMI during gestation in mothers significantly increased MDA [F (3, 20) = 2.36, P<0.05] and reduced SOD [F (3, 20) = 3.56, P<0.05], CAT [F (3, 20) = 4.36, P<0.05], and GPx in mothers' brain tissue compared to the control group [F (3, 20) = 3.65, P<0.05]. The administration of Rg1 during pregnancy in mothers decreased MDA [F (3, 20) = 3.36, P<0.05] and increased SOD [F (3, 20) = 4.69, P<0.05], CAT [F (3, 20) = 6.57, P<0.05], and GPx [F (3, 20) = 5.11, P<0.05] in mothers' brain tissue compared to MMI group. Combined administration of MMI and Rg1 significantly decreased MDA production F (3, 20) = 4.12, P<0.05] and increased SOD F (3, 20) = 5.68, P<0.05], CAT F (3, 20) = 7.14, P<0.05], and GPx F (3, 20) = 6.78, P<0.05] in mothers' brain tissue compared to the MMI group (Table 1).

According to our findings, prenatal exposure to MMI during gestation in mothers significantly increased MDA [F (3, 20) = 2.74, P<0.05] and reduced SOD [F (3, 20) = 4.25, P<0.05], CAT [F (3, 20) = 5.12, P<0.05], and GPx [F (3, 20) = 4.36, P<0.05] in the offspring's brain tissue compared to the control group (P < 0.05). Prenatal exposure to Rg1 during pregnancy in mothers decreased MDA [F (3, 20) = 2.36, P<0.05] and increased SOD [F (3, 20) = 4.12, P<0.05], CAT [F (3, 20) = 3.85, P<0.05], and GPx [F (3, 20) = 4.13, P<0.05] in the offspring's brain tissue compared to the MMI group (P < 0.05). Combined administration of MMI and Rg1 significantly decreased MDA production F (3, 20) = 3.56, P<0.05] and increased SOD F (3, 20) = 5.39, P<0.05], CAT F (3, 20) = 5.41, P<0.05], and GPx F (3, 20) = 5.57, P<0.05] in offspring's brain tissue compared to the MMI group (Table 2).

Discussion

The findings of our study have demonstrated a significant improvement in the overall motor development milestones of offspring whose mothers were treated with both MMI and Rg1. In contrast, offspring whose mothers were treated with MMI alone exhibited less motor and physical development. Our findings have indicated that the administration of Rg1 has compensated for the decrease in motor evolution in mice that were solely treated with MMI. It is a well-known fact that administering MMI to pregnant rats can induce hypothyroidism (Santos et al., 2012); However, an unexpected outcome of our study was the improvement in the motor development aspect of offspring injected with Rg1, compared to the control group that received no medication. The optimal development of offspring depends on the supply of maternal iodine and thyroid hormone, and the deficit observed may be associated with delayed neural processes in correlated areas (Hipólito et al., 2023). Our findings, which were based on motor and sensory tests such as

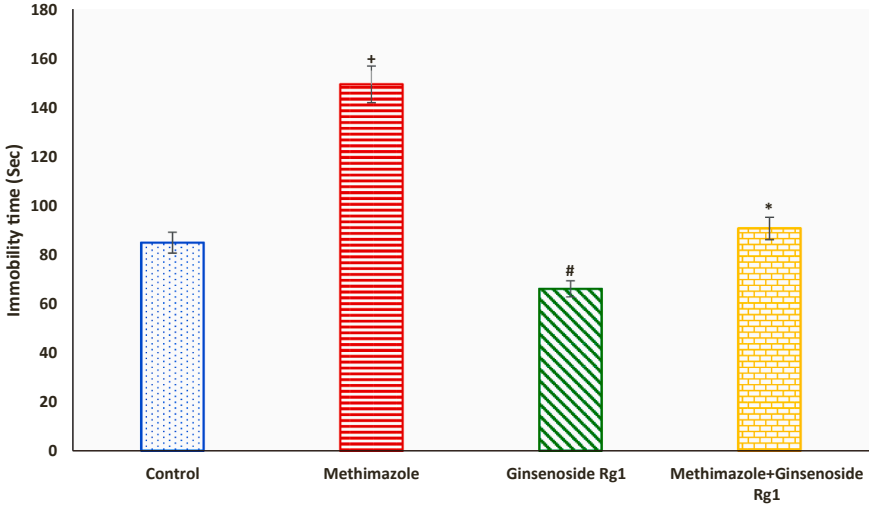

Fig. 11. The effects of administration of methimazole, ginsenoside, and their combined administration during gestation on TST in mothers. Data are expressed as mean ±SE. + Indicate a significant difference compared to control group (P < 0.05). # Indicate a significant difference compared to methimazole group (P < 0.05). * Indicate a significant difference compared to methimazole group (P < 0.05).

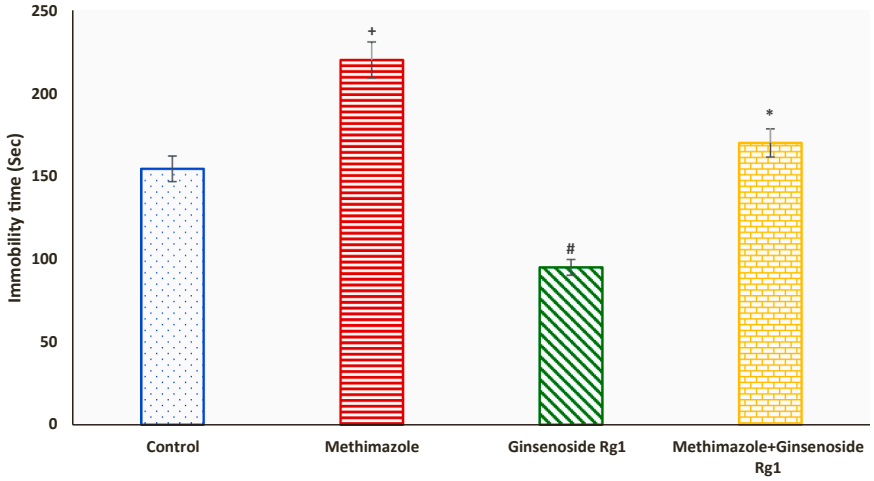

Fig. 12. The effects of administration of methimazole, ginsenoside, and their combined administration during gestation on FST in mothers. Data are expressed as mean ±SE. + Indicate a significant difference compared to control group ($P < 0.05$). # Indicate a significant difference compared to methimazole group ($P < 0.05$). * Indicate a significant difference compared to methimazole group ($P < 0.05$).

Table 1
Effect of Methimazole, Ginsenoside Rg1 and their co-administration on the antioxidant levels of cerebral cortex tissue of mother in mice.

	MDA (µg/mg protein)	SOD (% inhibition)	GPx (U/mg protein)	CAT(U/mg protein)
Control	0.052± 0.002	55.79± 3.54	148.31± 10.12	97.23± 8.87
Methimazole	0.23 ±0.001^{+}	32.63± 2.56^{+}	54.12 ± 4.54^{+}	52.14± 5.87^{+}
Ginsenoside Rg1	0.033 ±0.001$^{\#}$	87.86 ±4.34$^{\#}$	181.12± 11.12$^{\#}$	131.3 ±13.23$^{\#}$
Methimazole+Ginsenoside Rg1	0.045 ±0.002*	66.55 ±3.67*	134 ±8.34*	112.73± 6.38*

Note: + Indicate a significant difference compared to control group ($P < 0.05$). # Indicate a significant difference compared to methimazole group ($P < 0.05$). * Indicate a significant difference compared to methimazole group ($P < 0.05$). GPx, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase.

Table 2
Effect of Methimazole, Ginsenoside Rg1, and their co-administration on the antioxidant levels of cerebral cortex tissue of mice offspring.

	MDA (µg/mg protein)	SOD (% inhibition)	GPx (U/mg protein)	CAT(U/mg protein)
Control	0.022± 0.001	23.23± 2.78	93.52± 4.45	64.11± 4.11
Methimazole	0.44 ±0.003^{+}	15.12± 1.99^{+}	71.12± 2.98^{+}	46.78± 3.72^{+}
Ginsenoside Rg1	0.021 ±0.002$^{\#}$	31.13 ±3.17$^{\#}$	130.65± 8.44$^{\#}$	87.32 ±5.23$^{\#}$
Methimazole+Ginsenoside Rg1	0.018 ±0.001*	24.23 ±2.84*	121.36 ±7.21*	76.21± 4.76*

Note: + Indicate a significant difference compared to control group ($P < 0.05$). # Indicate a significant difference compared to methimazole group ($P < 0.05$). * Indicate a significant difference compared to methimazole group ($P < 0.05$). GPx, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase.

ambulation, hindlimb foot angle, front limb suspension, hind limb suspension, surface righting, negative geotaxis, and grip strength (Feather-Schussler and Ferguson, 2016; Nguyen et al., 2017), have shown a decrease in development in MMI-treated mice.

Neurological reflexes demonstrate brain stem and spinal cord reflexes evolving through migration, myelination, and synaptogenesis. Alterations in central nervous system development result in abnormal cortical wiring, functioning, and myelination, causing neurodevelopmental reflex delays or absences. These alterations often manifest as abnormal early reflexes in human infants and can predict developmental disabilities. While myelination commences in utero in humans, it appears in the forebrain of rat pups around PND7–10, which is why the majority of offspring tests were conducted during this period. However, it should be noted that reflex testing is not a direct measure of cognitive or behavioral abnormalities, and the persistence of any observed abnormalities is uncertain (Nguyen et al., 2017). Nevertheless, a study demonstrated a strong inter-rater reliability of over 95% for hindlimb suspension scores (El-Khodor et al., 2008; Feather-Schussler and Ferguson, 2016).

Although the active principles of ginseng can cross the placenta and reach the fetus (Wong, 1979). Ginsenosides has shown low transplacental transfer (Belanger et al., 2016). Additionally, Rg1 has been shown to have a limited ability to penetrate both the blood-brain barrier (BBB) and the placenta. Nevertheless, Rg1 has demonstrated the capacity to protect the integrity of the BBB (Zhai et al., 2021), athwart Rg1 nanoparticle could penetrate the BBB (Shen et al., 2017). During the GD 6–8 in mice the incomplete differentiation of trophoblastic cells results in an incomplete placental barrier. Therefore, maternal exposure to drugs or medications during this period may have teratogenic effects (Costa et al., 2016). This is precisely why we utilized Rg1 during PND 1–9. Our findings indicate that Rg1 not only mitigates the effects of gestational hypothyroidism induced by MMI on offspring but also enhances the motor developmental profile of mice offspring when administered during gestation (GD_{1-9}) in a euthyroid state without MMI, which could be attributed to Rg1 promoting glutamate release via a calcium/calmodulin-dependent protein kinase II-dependent signaling pathway (Liu et al., 2010). However, Mother's aggression postpartum is a negative aspect of Rg1 administration that should be considered (Yoshimura et al., 1988). Also Some people could have an allergic reaction to ginseng, which could impede its use in pregnancy.

Prenatal administration of MMI in mice resulted in increased levels of freezing behavior, indicating higher anxiety-like behavior in their offspring. However, co-administration of MMI and Rg1 led to the recovery of this behavior to levels comparable to the control group. This can be attributed to the protective effects of Rg1 against PTSD-like behaviors in mice, which reduces freezing duration in conditioned fear tests through the promotion of synaptic proteins such as PSD95, Arc, and GluA1, and the reduction of Kir4.1 and TNF-α in the hippocampus (Zhang et al., 2021). MMI increases $GABA_A$ and dopamine type 2

(specifically D4) receptor expression in amygdala. However, serotonin receptor expression remains unchanged. Furthermore, a decrease in parvalbumin-positive neurons may result in impaired fear control in certain brain areas, leading to increased freezing behavior. Interestingly, a study has shown that male offspring treated with MMI 0.02% exhibited enhanced responsiveness to conditioned fear-like behavior through unknown mechanisms. However, the animal model used in this study involved adult Wistar nulliparous female and male rats, which distinguishes it from our study. Consistently, thyroidectomized rats showed an increase in the freezing behavior under hypothyroidism, due to increase of corticosterone release after fear conditioning with higher expression of glucocorticoid and mineralocorticoid receptors in the lateral amygdala (Hipólito et al., 2023).

Limbic brain regions (prefrontal cortex, hippocampus, and amygdala) play a role in mood disorders (Mayberg, 2009) Within the brain of patients with major depressive disorders, there are reported occurrences of atrophy, overactivation of oxidative stress, neuro-inflammation, and reduced hippocampal volume (Li et al., 2020; Savitz et al., 2015). The various etiologies of postpartum depression (PPD) include changing plasma levels of estrogen and progesterone, postpartum hypothyroidism, sleep deprivation, or difficult life circumstances (McCoy, 2011). Hypothyroidism may affect postpartum mood through its association with diminished central 5-HT (serotonin) activity (Upadhyaya et al., 1992), and elevated levels of prolactin and cortisol, which are linked to anxiety, depression, and hostility in hypothyroidism due to the reduction of central 5-HT activity (Cleare et al., 1995). Women with antenatal hypothyroidism symptoms (weight gain, cold intolerance, lethargy), may be at greater risk of developing postpartum depressive symptoms (Hendrick et al., 1998; Pedersen et al., 2007). Furthermore, hypothyroidism can impair the neurotransmission activity in the hippocampus, delay cellular proliferation and migration, and cause neurological and behavioral deficits, as well as irreversible motor dysfunctions. Hypothyroidism may also prompt depressive symptoms (Ahmed, 2018). Although the association between hypothyroidism and clinical depression was considerably lower than previously assumed, there is a modest association between overt hypothyroidism in female individuals and depression (Bode et al., 2021). However, our research indicates that MMI has the potential to cause postpartum depression as assessed by OFT, TST, and FST compared to the control group.

Based on our findings, Rg1 exhibited a significant reduction in depression-like behaviors as assessed in the FST, OFT, and TST, indicating remarkable anti-depressant and anti-inflammatory effects based on these tests. Rg1 also attenuates neuroinflammation and maintain the integrity of BBB by upregulation of MMP-2 and ICAM-1 (Zheng et al., 2014). Moreover, prenatal ginseng administered to rats exposed to prenatal stress showed an improvement in FST and OFT in offspring (Kim et al., 2015). Furthermore, an interesting study showed Postnatal Korean red ginseng, inhibited hyperactivity induced by valproic acid in rat offspring in OFT (Kim et al., 2013). It is well known that postpartum depression (PPD) can lead to adverse outcomes such as increased risk of marital disruption and divorce, child abuse and neglect, and even maternal suicide or infanticide. Children of depressed mothers may experience insecurity, low self-esteem, and even decreased intellectual skills or language development (Cuijpers et al., 2008).

Our study demonstrates that Rg1 could attenuate PPD and related adverse effects induced by MMI. Likewise, our findings suggest that Rg1 could improve mood-related behavioral measures during the postpartum period in euthyroid mice. Reduced crossings in OFT results from depression but not changes in motor activity in mice (Katz et al., 1981; Nikseresht et al., 2012; Wang et al., 2021). The FST was performed to assess despair behavior (Porsolt et al., 1977a,b), depression, and anxiety-like behavior (Nikseresht et al., 2012), and inclusively, maternal behavior, in this case, PPD (Melón et al., 2018). However, the tests' selectivity for monoamine mechanisms may limit their ability to detect novel mechanisms (Chatterjee et al., 2012). The FST shows negative symptoms of psychosis and is based on dopamine function in mice. but, The TST is more sensitive to acute neurochemical changes involving both serotonergic and dopaminergic systems in the model (Chatterjee et al., 2012).

The findings of our study have demonstrated improvement in the brain's antioxidant levels in both mothers and offspring whose mothers were treated with both MMI and Rg1. In contrast, mothers and offspring whose mothers were treated with MMI alone exhibited a decrease in the brain's antioxidant levels. The CNS is particularly susceptible to oxidative damage due to its high rate of oxygen utilization (Sayre et al., 2008). Rg1 attenuates oxidative stress in the hippocampal CA1 region of depressed rats by decreasing levels of MDA and reactive oxygen species (ROS), and increasing GPx and SOD levels (Xu et al., 2019). These results can be attributed to the antioxidant properties of Rg1, which may also contribute to alleviating cognitive impairment in mice by reducing oxidative stress and downregulating the Akt/mTOR signaling pathway (Chen et al., 2018). Another study showed that Rg1 increased the antioxidant activity of SOD, CAT, and GPx, and reduced levels of the oxidation products of MDA in tree shrews by regulating the Wnt/GSK-3β/β-catenin signaling pathway, Rg1 could thereby alleviate oxidative stress damage, improve neuroinflammation, and protect neurons (Yang et al., 2022). Moreover, in a mouse model exposed to cumulative cadmium, Rg1 administration was found to increase the activity of SOD, GPx, and CAT while decreasing MDA levels in both the blood and brain, via the BDNF-TrkB/Akt and Notch/HES-1 signaling axes (Ren et al., 2021). Similarly, the use of KRG fraction extract also resulted in decreased MDA levels and increased GPx activity in paraquat-treated mice (Lee, 2000). In a separate study, Rg1 (20 mg/kg; IP) enhanced total activity of SOD and GSH levels in the substantia nigra of Parkinson's disease mouse model, albeit not as effectively as N-acetylcysteine administration at 300 mg/kg. Use of a higher Rg1 dosage (150 mg/kg) may, likewise in our study, result in better improvement (Chen et al., 2005). Additionally, oral administration of KRG at 100 and 200 mg/kg in rats lowered MDA activity and increased myeloperoxidase levels in rat brains.(Iqbal et al., 2020). In conclusion, these findings indicate that Rg1 mitigated postpartum depression in mice and improved reflexive motor behaviors in their pups and these effects mediates by its antioxidant properties.

Ethical approval

All experimental procedures were approved at the Animal Ethics Committee of the Science and Research Branch of Islamic Azad University, Tehran, Iran (IR.IAU.SRB.REC.1402.030; 2023–06–10).

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CRediT authorship contribution statement

Ali Sanaiee: DVM, Experimental procedure, Draft of paper. Shahin Hassanpour: Thesis supervisor, Study design, Proofing the paper, Revise the paper. Bita Vazir: Thesis advisor.

Declaration of Competing Interest

The authors have no conflicts of interest to declare regarding the study described in this article and preparation of the article.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

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Consent to publish

All authors reviewed and approved the manuscript.

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