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Effects of Eight Weeks of Interval Swimming Training and Motor-Enriched Environment Activity Combined with Artemisia Extract on Serotonin and Dopamine Levels in the Brain Tissue of Rats with Parkinson's disease

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ABSTRACT

Introduction: Parkinson's disease (PD) is the second most common cause of death among neurodegenerative diseases. The aim of the present study was to investigate and compare the effect of eight weeks of interval swimming training (IST) and motor enriched environment activity (MEEA) along with Artemisia (Ar) extract on serotonin and dopamine levels in the brain tissue of rats with PD.

Methods: In this experimental study, 42 male Sprague-Dawley rats (250–270 grams, 14–16 months old) were used, with PD induced using 2 mg/kg reserpine. They were divided into six groups: (1) Parkinson's disease control (Res), (2) IST, (3) MEEA, (4) Ar extract only, (5) IST+Ar, and (6) MEEA+Ar. In addition, in order to investigate the effect of Parkinson's disease induction on research variables, 7 healthy rats were selected as a healthy control group (HC).

Results: The serotonin and dopamine levels were significantly higher in the IST, MEEA, Ar, IST+Ar, and MEEA+Ar groups compared to the Res group (P=0.001). Additionally, serotonin and dopamine levels were higher in the IST, MEEA, IST+Ar, and MEEA+Ar groups compared to the Ar alone group (P=0.001). In the IST+Ar and MEEA+Ar groups, dopamine levels were also significantly higher compared to the IST and MEEA groups (P=0.001).

Conclusion: IST, MEEA, and Ar extract, individually and in combination, appear to improve neurotransmitter levels. However, the combination of training and Ar, particularly with overload training principles, may exert more favorable effects on neurotransmitter levels under neurodegenerative conditions.

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Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, marked by motor impairments, balance issues, bradykinesia, and a reduced quality of life (1). PD is a progressive neurological disease characterized by symptoms such as slowed movement, tremors, muscle rigidity, and postural instability (2). Dysfunction in oxidative stress, immune system regulation, and neuroprotective mechanisms contribute to neurotransmitter imbalances, particularly in serotonin and dopamine, ultimately leading to the breakdown of the extrapyramidal system and apoptosis of dopaminergic neurons in the midbrain (3). Dopamine and serotonin deficiencies disrupt central body control centers, with dopamine deficits specifically causing movement, posture, and functional disabilities (3,4). Studies have shown that depressive symptoms are prevalent among PD patients, often worsening with disease progression and linked to dopaminergic dysfunction (5). Cognitive impairments in PD are similarly associated with disturbances in neurotransmitters such as serotonin and dopamine, monoamine oxidase imbalance, and other neurochemical factors (6). Given the need

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for innovative, less invasive treatment methods, research has increasingly focused on the benefits of regular exercise. Physical activity has been shown to improve general health, protect against neurodegenerative disorders, and enhance quality of life (7). Exercise appears to benefit PD by enhancing neurotrophic mechanisms, improving antioxidant defense and cognitive function, and strengthening muscles, potentially alleviating behavioral symptoms of PD (7). However, motivation for physical activity can decline in neurodegenerative conditions, prompting interest in activities conducted in motivating environments. "Motor-enriched environment activity" (MEEA) includes diverse and stimulating physical activities and has recently attracted attention for its potential to boost engagement in movement-based therapies (8). Research suggests that physical activity in such environments can enhance cognitive function and certain neurotransmitters (9). Studies have also demonstrated that MEEA can improve motor and cognitive functions in animal models of PD (10). In general, exercise increases neurotransmitter concentrations through sympathetic nervous system activation, impacting both dopaminergic and serotonergic systems (11). Ghanbari et al. found that exercise raises serotonin and dopamine levels and alleviates depression and anxiety in diabetic rat models (12). Given dopamine's critical role in motor control, its synthesis may also affect motivation for physical activity (13). Additionally, combining exercise with natural antioxidants and medicinal plants could amplify neuroprotective effects in neurodegenerative diseases (14). Among these medicinal plants, Artemisia (Ar) plant belongs to the Asteraceae family (15). The herb extract also improves cognitive function in brain tissue with antioxidant mechanisms, improving neurotrophin function and similar transcriptional pathway of antioxidants (16). The antioxidant components in Ar may activate sirtuin via deacetylase mechanisms, subsequently triggering phosphatidylinositol 3-kinase (PI3K). PI3K can then activate nuclear factor erythroid-2-related factor 2 (Nrf2), leading to the transcription of antioxidants and neurotrophins, which improve cognitive function in animal models of neurodegenerative diseases (16). While exercise has been studied for its role in improving motor function in PD, comparing various exercise types to determine the most effective approach is critical. Furthermore, the combined impact of neurotrophic and behavior-improving medicinal plants like Ar with physical activities has not been thoroughly investigated. Therefore, this study aimed to examine the effects of eight weeks of interval swimming training (IST) and MEEA, combined with Ar extract, on dopamine and serotonin levels in the brain tissue of rats with PD.

Material and Methods

Animals

In this experimental study, 49 male Sprague Dawley rats (250-270 grams, 14-16 months old) were acquired from the laboratory animal breeding and reproduction center of the Pishtazan Institute of Higher Education. Upon arrival, they were transferred to the sports physiology laboratory at this institution, where they were allowed a seven-day acclimatization period to adapt to the new environment. During this period, the rats were housed under standard conditions, including a controlled temperature of 22-24°C, relative humidity of 55-60%, and a 12-hour light-dark cycle. Ethical guidelines were strictly followed in accordance with the Helsinki Declaration.

Induction of Parkinson's disease and Grouping

Next, after a 12-hour fasting period, 42 rats were anesthetized with an intraperitoneal injection of ketamine and xylazine. To induce Parkinson's disease (PD), each rat received an intraperitoneal injection of 2 mg/kg reserpine dissolved in normal saline. Fourteen days post-injection, clinical examinations were conducted to confirm PD induction. Indicators such as periorbital bleeding, aggression, anxiety, tail twisting, impaired gait, and rotation tests were assessed (17). After confirming PD induction, the rats were allocated into six groups: 1) PD control (Res), 2) Interval Swimming Training (IST), 3) Motor Enriched Environment Activity (MEEA), 4) Artemisia (Ar), 5) IST+Ar, and 6) MEEA+Ar. Additionally, seven healthy rats were selected as a Healthy Control (HC) group to examine baseline levels of the research variables.

Interval Swimming Training Protocol

The IST was conducted over eight weeks, with three sessions per week. Training took place in a specially designed animal swimming pool with dimensions of 150 × 70 × 70 cm. Initially, the

rats underwent a one-week acclimatization period, swimming in the water for 5 minutes daily to become familiar with the environment. During the main training sessions, each session consisted of 14 intervals of 20 seconds of swimming, followed by 10-second rest periods. To adhere to the principle of progressive overload, the rats carried a weight equivalent to 9% of their body weight, attached to their tails, during the 20-second swimming intervals in the first and second weeks. Each subsequent week, an additional 1% of body weight was added, reaching a final load of 16% of the rats' body weight by the eighth week (18).

Motor Enriched Environment Activity

The MEEA program was conducted over an eight-week period. To create this enriched environment, rats were placed in a cage measuring 40 × 60 × 90 cm equipped with various interactive elements, including a small house, balls, toys, ropes, climbing rings in various shapes, a spinning wheel, and a ladder. This setup provided ample opportunity for play and engagement, enhancing the animals' physical and cognitive stimulation. Additionally, rats in this group had free access to water and food throughout the program (9).

Artemisia Extract

The Artemisia (Ar) extract was prepared through water distillation using a Clonger machine. In this process, 50g of plant powder and 500ml of water were placed in the distillation flask and heated until the distillation rate reached 2–3ml per minute. After 4 hours, the essential oil of the plant was collected, dried over anhydrous sodium sulfate for 24 hours to remove any residual moisture, and stored. Following preparation, the Ar groups received a daily oral dose of 50mg/kg of the Ar extract (19).

Sampling

A total of 48 hours after the final training session, following a 12-hour fasting period, the rats were anesthetized with doses of ketamine (75mg/kg) and xylazine (25mg/kg). Upon confirming full anesthesia, trained laboratory personnel used sterile surgical instruments to carefully extract the brain tissue. The hippocampus was then precisely isolated, placed in a specialized tissue preservation microtube, and immediately transferred to storage at -70°C for preservation.

The Method of Measuring Variables

In this study, serotonin (5-hydroxytryptamine) levels were measured using an ELISA kit (Catalog No. E-EL-0033) from Elabscience (China), with a sensitivity of 9.38ng/ml. Dopamine levels were also quantified with an ELISA kit (Catalog No. E-EL-0046) from the same manufacturer, featuring a sensitivity of 18.75pg/ml. This research was approved under ethical code IR.UM.REC.1402.227.

Statistical Analysis Method

Data are presented as mean ± standard deviation. The Shapiro-Wilk test was used to assess the normality of data distribution. Given the normal distribution of the data, a one-way analysis of variance (ANOVA) with Tukey's post-hoc test was performed to evaluate differences between groups using SPSS software (version 22). Statistical significance was set at $P \leq 0.05$.

Results

The Shapiro-Wilk test confirmed a normal distribution of data. One-way ANOVA results indicated a significant difference in serotonin levels ($F=75.84$, $P=0.001$) and dopamine levels ($F=209.41$, $P=0.001$) across the research groups. Tukey's post-hoc test results showed that serotonin levels in the Res group were significantly lower than in the HC group ($P=0.001$). In contrast, serotonin levels were significantly higher in the Ar ($P=0.001$), IST ($P=0.001$), MEEA ($P=0.001$), IST+Ar ($P=0.001$), and MEEA+Ar ($P=0.001$) groups compared to the Res group. Additionally, serotonin levels in the IST ($P=0.001$), MEEA ($P=0.001$), IST+Ar ($P=0.001$), and MEEA+Ar ($P=0.001$) groups were significantly higher than in the Ar group (Figure 1).

The results indicated that dopamine levels in the Res group were significantly lower than in the HC group ($P=0.001$). In contrast, dopamine levels were significantly higher in the Ar ($P=0.001$), IST ($P=0.001$), MEEA ($P=0.001$), IST+Ar ($P=0.001$), and MEEA+Ar ($P=0.001$) groups compared to the Res group. Additionally, dopamine levels in the MEEA+Ar group were significantly higher than those in the IST ($P=0.001$), MEEA ($P=0.001$), and Ar ($P=0.001$) groups, and the levels in the IST+Ar group were also significantly higher than in the MEEA+Ar group ($P=0.001$).

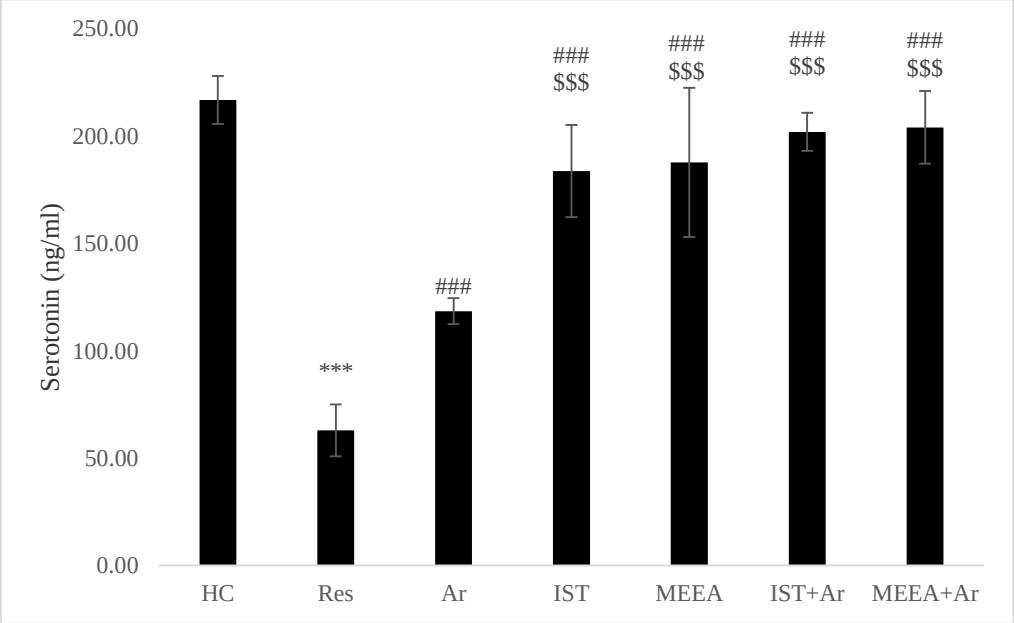

Figure 1. Serotonin levels in the brain tissue of rats in the studied groups
*** (P=0.001) significant decrease compared to HC group; ### (P=0.001) significant increase compared to the Res group; $$$ (P=0.001) significant increase compared to Ar group

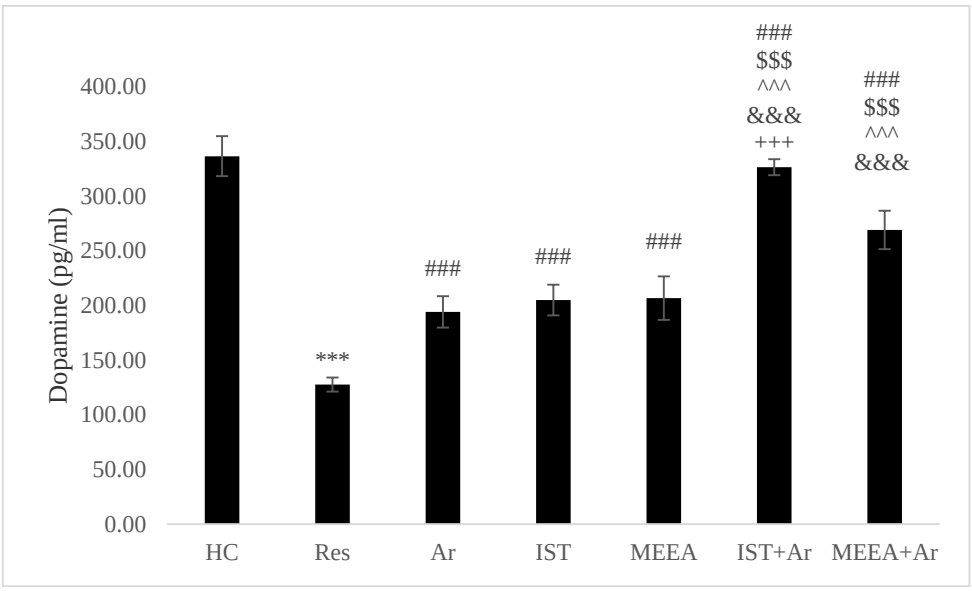

Figure 2. Dopamine levels in the brain tissue of rats in the studied groups
***(P=0.001) significant decrease compared to HC group; ### (P=0.001) significant increase compared to the Res group; $$$ (P=0.001) significant increase compared to Ar group; ^^^ (P=0.001) increase compared to the IST group; &&& (P=0.001) significant increase compared to the MEEA group; +++ (P=0.001) significant increase compared to MEEA+Ar group

Discussion

The results showed that serotonin and dopamine levels in the IST and MEEA groups were significantly higher than those in the Res group. This aligns with evidence that exercise can improve serotonin and dopamine expression and

regulation through various mechanisms. A primary pathway appears to involve activation of the cyclic AMP (cAMP)/protein kinase A (PKA)/CREB pathway, which promotes transcription of 5-hydroxytryptamine-1 (5HT1A) and subsequently increases synthesis of insulin-like growth factor-1 (IGF-1). This process is associated with neurogenesis and may help alleviate depressive and anxiety-like behaviors (20). Physical activities also appear to reduce pro-inflammatory markers—like interferon-gamma and tumor necrosis factor-alpha (TNF-α)—through mechanisms including activation of anti-inflammatory macrophages, increased beta-endorphin and epinephrine release, and myokine interactions, particularly irisin in the brain. These effects promote the expression of tyrosine kinase B (TrkB) and brain-derived neurotrophic factor (BDNF), ultimately enhancing dopamine expression (20). Further, one study found that high-intensity interval training (HIIT) on a treadmill and MEEA both increased neurotrophins, enhanced brain metabolic function, and reduced depression in neurodegenerative animal models by improving blood flow, increasing tyrosine kinase activity, reducing oxidative stress, and enhancing antioxidant transcription pathways (9). However, the researchers noted that exercise type, overload principle, and intensity can influence the degree of benefit for neurotrophin enhancement (8). In another study, MEEA was shown to increase ephedrine, brain glutamate receptors, blood flow, dopamine type 1 receptor, and serotonin expression (21). Additional research demonstrated that MEEA significantly boosted BDNF expression in the frontal cortex and improved working memory in elderly rats (22), which has also been reported that MEEA reduced anxiety-like behaviors (23).

In the present study, serotonin and dopamine levels in the Ar group were significantly higher than those in the Res group. Although there is limited information on the specific effects of Artemisia (Ar) on serotonin and dopamine, it appears that the isoflavones in Ar may activate acetylases, thereby initiating the PI3K/protein kinase B (Akt)/β-catenin pathway. This activation could increase Bcl-2 expression, inhibit metalloproteinases, reduce oxidative stress, and regulate calcium channels and TRPML1. Collectively, these processes may lead to an increased expression of neurotransmitters (24). Supporting this, one study reported that Ar reduced amyloid-beta, caspase-3, and reactive oxygen species in the brain tissue of rats with Alzheimer's disease (25). Another study found that administering 400mg/kg of *Stachys lavandulifolia* reduced depressive symptoms in rats (26). Similarly, Kim et al. demonstrated that Ar extract enhances sirtuin 1, Nrf2, and antioxidant enzyme expression, which collectively improved cognitive function in an animal model of dementia (16). Though evidence on Ar's direct effects on serotonin and dopamine remains limited, the existing data suggest that Ar enhances antioxidant mechanisms, neurotrophin levels, and cognitive function. This study is notable for its innovative exploration of Ar's impact on serotonin and dopamine levels.

The results further showed that serotonin and dopamine levels in the IST+Ar and MEEA+Ar groups were significantly higher than those in the Res group, with the effects of IST, MEEA, IST+Ar, and MEEA+Ar on serotonin being greater than that of Ar alone. Additionally, dopamine levels in the IST+Ar and MEEA+Ar groups were elevated more than in the IST and MEEA groups. These findings suggest that training activities promote nuclear transcription of 5HT1A and IGF-1 through the cAMP/PKA/CREB pathway. This pathway, alongside increases in beta-endorphin, epinephrine, myokine release, and improved TrKB and BDNF expression, contributes to dopamine production (20). Concurrently, Ar seems to influence serotonin and dopamine expression via the PI3K/Akt/β-catenin mechanism, inhibiting apoptosis, enhancing Nrf2 expression, increasing antioxidant activity, and improving calcium channel and TRPML1 function (24). The data indicate that the effects of regular physical activity and Ar intake may be mutually reinforcing in regulating serotonin and dopamine. However, to achieve optimal results, physical activity intensity may need to reach levels that stimulate adaptive responses, such as increased antioxidant expression and gene transcription related to PI3K (9). Given that PI3K, Nrf2, CREB, and BDNF are common elements in both physical activity and Ar pathways, the absence of their measurement in this study is a limitation. Future studies are recommended to include these variables to gain a more comprehensive understanding of these mechanisms.

Conclusion

IST, MEEA, and Ar extract, both individually and in combination, appear to enhance certain neurotransmitter levels. Notably, the combined effect of training with Ar, particularly when incorporating the principle of overload, shows even more favorable outcomes for neurotransmitter improvement under neurodegenerative conditions. A limitation of the present study was the inability to measure gene expression levels of serotonin and dopamine directly. Therefore, future studies are encouraged to investigate these gene expression levels to provide deeper insights.

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Conflict of Interest

The authors declare no conflict of interest regarding publication of this article.

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