ORIGINAL ARTICLE

Clinical study of *Pfaffia glomerata* (Spreng.) Pedersen on healthy middle-aged and elderly men: assessment of the effects on cognition

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ABSTRACT

Pfaffia glomerata (Spreng.) Pedersen (PG) has potential effects on cognitive function, yet it has not been studied in humans. This randomized, double-blinded, placebo-controlled study investigated the effect of PG roots standardized extract (1.0% of β-ecdysone) on cognition in healthy middle-aged and elderly men. Thirty-eight men (aged 50-73) were randomized into Placebo and PG groups and later reorganized into Placebo and PG athlete and non-athlete subgroups; each subject took one capsule of PG extract (300 mg) or brown sugar as placebo (500 mg) twice daily for 6 months. Cognitive function was assessed at baseline and at in the 6th-month using neuropsychological tests. The PG extract was well tolerated, with only one dropout case in the placebo group. Overall, there were no significant differences between the groups from initial to final evaluation. However, subgroup analysis by physical activity profile showed significant improvements in WMS-digit span forward (Athletes' PG group) and WMS-logical memory (Athletes and Non-athletes' PG groups); conversely, negative interference was found in Toulouse-Pierón Q and WMS-orientation subtests on Non-athletes' PG group. These findings suggest that PG extract has both negative and positive effects on cognition in healthy middle-aged and elderly men. But more research is needed to better understand its effects.

Keywords: *Pfaffia glomerata*; Brazilian ginseng; suma; memory enhancement

INTRODUCTION

The development of modern societies has led to an increase in life expectancy worldwide, significantly impacting health services (Karacan et al. 2020). Given that the elderly present their own physiological characteristics, there has been a push for research in this area to develop methodologies and products that assist this significant portion of the population and contribute to the planning and preparation of healthcare services (Passos et al. 2020). In this context, medicinal plants have garnered attention, being widely utilized globally for their diverse effects, including antioxidative, vasodilatory, neuroprotective, anti-inflammatory properties, among others. Notable examples include species such as *Ginkgo biloba* L., *Curcuma longa* L., *Hypericum perforatum* L., *Huperzia serrata* (Thunb.) Trevis., among others (Perry and Howes 2011; Babich et al. 2022).

In this context, adaptogens emerge as noteworthy, being substances that enhance a state of nonspecific resistance in organisms following exposure to various stressors (Wagner et al. 1994). Typically, they are herbal preparations with low toxicity, lacking acute effects and necessitating prolonged administration to aid the body in maintaining homeostatic balance or recovering from instances of imbalance (Brekhman and Dardymo 1969).

Received: May 20th, 2024
Accepted after revision: October 14th, 2024
Published on line: October 18th, 2024
ISSN 1983-084X

https://doi.org/10.70151/k4d79p39

In Brazil, the population has adopted the practice of using native plants for similar therapeutic purposes, employing them as general tonics to enhance both physical and mental well-being, including sexual health. Notable species in this regard include guarana (*Paullinia cupana* Kunth.), catuabas (*Anemopaegma arvense* [Vell.] Stellfeld ex de Souza; *Trichilia catigua* A.Juss.), nó-de-cachorro (*Heteropterys tomentosa* A.Juss.), and Brazilian ginsengs (*Pfaffia glomerata* [Spreng.] Pedersen; *Hebanthe erianthos* [Poir.] Pedersen) (Carlini 1995; Mendes 2011).

Among these native species, *Pfaffia glomerata* (Spreng.) Pedersen (PG) has garnered attention due to its abundance of raw material, wide geographical distribution, ecdysteroid content, and presence in the Brazilian market. These factors have spurred research in both chemical and pharmacological fields, positioning it as a potential economic asset (Prudente et al. 2024; Ribeiro et al. 2024). PG is predominantly found along the border between the states of Paraná and Mato Grosso do Sul, and its commercialization constitutes a significant economic activity. Approximately 300 tons of roots were cultivated and sold between 2019 and 2020, with the majority destined for export (Faep/Senar PR 2021).

PG demonstrates notable anti-inflammatory, analgesic, antioxidant, antidepressant, aphrodisiac, gastroprotective, and prebiotic actions (Ribeiro et al. 2024). In the central nervous system field, Paris et al. (2000) conducted an evaluation using an alcoholic extract obtained through hot reflux, administered acutely in animal models of behavior and memory; the findings revealed a depressant profile, characterized by decreased memory retention and, when administered intraperitoneally, a dose-dependent amnesic effect. Conversely, Oliveira et al. (2004) conducted acute treatments on male mice using a hydroalcoholic extract in the elevated T-maze model. The results indicated an enhancement in the acquisition of inhibitory avoidance behavior with some of the tested doses.

Additionally, Marques et al. (2004) assessed a standardized extract (containing 1.1% β-ecdysone) derived from PG roots obtained via cold turbolysis in animal models; their findings demonstrated a reversal of memory deficits in elderly rats following chronic treatment, as evidenced in models of passive avoidance and left-right discrimination tests. Furthermore, Franco et al. (2021; 2024) conducted extensive evaluations on extracts from both roots and aerial parts of PG, focusing on fractions enriched in the active compound β-ecdysone (20-hydroxyecdysone). They discovered antioxidant and anticholinesterase properties, along with the ability to mitigate behaviors associated with stress, anxiety, and depression, as well as improvements in the brain's antioxidant system; collectively, these effects were deemed neuroprotective. In terms of safety, a toxicological study involving acute treatment of male and female rats for 90 days determined that the standardized extract of PG roots exhibited an LD_{50} above 5 g/kg, indicating a low toxicity profile compatible with human use (Marques et al. 1998).

Given these promising preclinical findings and the observed low toxicity, it was decided to conduct a clinical trial to investigate potential cognitive effects of the standardized extract derived from PG roots.

MATERIAL AND METHODS

Extract used and its standardization

The roots were purchased in pulverized form from a wholesale supplier in São Paulo, with the supplier indicating that their origin was the region of Porto Rico in the state of Paraná, Brazil. Flowering specimens were collected from this region and exsiccates were compiled. These exsiccates were then identified as *P. glomerata* by botanist Josafá Siqueira, who serves as the curator of the Herbarium Friburguense (FCAB). A voucher specimen has been deposited at the herbarium under the number 5426.

The roots underwent maceration at a concentration of 10% (w/v) in a hydroalcoholic solvent (50% v/v) for approximately 4 h, followed by application of the turbolysis technique at 3500 rpm for 30 min, with intermittent cooling. Subsequently, the solution obtained was filtered, concentrated under vacuum, and dried using corn starch as an excipient in an industrial atomizer. The resulting PG dry extract, along with brown sugar utilized in the placebo, were encapsulated into opaque hard gelatin capsules of size "0" and wine color. The capsules were then packaged into opaque PVC plastic containers, each containing 120 units, and labeled with the name of the institution. To maintain the double-blind format of the research, the containers were randomly coded.

The PG extract was standardized based on the marker β-ecdysone Sigma using a high-performance liquid chromatography (HPLC) system, specifically a Hewlett Packard model 1050, equipped with a Merck Lichrosphere 100 (RP-18) 5 µm column sized 250 mmx4 mm. The chromatographic conditions included a temperature of 27 ºC, a mobile phase consisting of methanol-water (40:60, v/v), a flow rate of 1 ml/min, and a total running time of 18 min. The detection was performed at a wavelength of 235 nm.

Inclusion criteria

Healthy middle-aged and elderly male volunteers between 50 and 75 years were recruited and, due to parallel assessment in the field of exercise physiology, with different patterns of physical activity (athletes: activity above 3 h per week and participation in sports competitions; and non-athletes: sedentary or with less than 3 h of activity per week). The results of this parallel evaluation were published separately (Danucalov 2000; Marques et al. 2002).

Exclusion criteria

Volunteers diagnosed with dementia, heart problems, uncontrolled hypertension or other serious pathological conditions, or using adaptogen and/or nootropic medications were excluded.

Ethical aspects

The project underwent review and approval by the Ethics and Research Committee of the Federal University of São Paulo (in response to letter PSICOB/EAC/072/97). Volunteers were provided with a declaration of consent, which they read and signed.

Screening of volunteers

The screening process began with an anamnesis and a rapid assessment of mental state using the Mini Mental Test (Schaefer et al. 2024). Subsequently, blood and urine samples were collected for laboratory tests to assess hematological and biochemical profiles. This was followed by a cardiological evaluation and an exercise stress test on a stationary bike. Upon receiving laboratory and cardiological reports indicating suitability for participation in the study, the baseline cognitive tests were applied. Typically, this occurred approximately one week after the screening phase.

Neuropsychological assessment

The instruments of this assessment were the following:

a) Toulouse-Pierón Test (Concentrated Attention): This test is designed to evaluate concentrated attention, particularly visual attention, as well as the ability to focus, react quickly, perform tasks accurately, and discriminate and localize stimuli. The speed of performance is measured by the productivity achieved within a set time (R subtest), while the quality is assessed by the number of errors made (Q subtest) (Araújo 2011).

b) Wechsler Memory Scale (Revised): This scale comprises six subtests (information, orientation, mental control, logical memory, numbers, and associated pairs). It assesses various aspects of memory function, including general knowledge, temporal-spatial orientation, remote memory, immediate memory, working memory, exact reasoning, and logical memory. Tasks within this scale involve answering general questions, numerical counting, verbal repetition of the alphabet, and recall of story content immediately and after a delay period (Elwood 1991).

c) Progressive Matrices Test: This test is used to assess cognitive abilities such as mental level, intellectual development, and learning capacity, particularly in older individuals. It consists of questions organized into five modules, each with progressively increasing difficulty levels (Raven 1993).

Study protocol

The volunteers were randomly assigned to either the control (placebo) or experimental (PG) groups, with randomization based on age parameters, Mini-Mental Test scores, and self-reported level of physical capacity.

The product provided was a standardized PG dry extract encapsulated at a dose of 300 mg, to be taken twice daily (equivalent to 8.6 mg/kg for a subject weighing 70 kg). This dosage was determined based on the customary use of approximately 2 g of the roots per day (Brasmédica n.d.), and the extract's yield in the form of a drug-derivative ratio (DDR) of 2-3:1, resulting in a daily dose of approximately 600 mg. The placebo consisted of 500 mg brown sugar capsules, also to be taken twice daily. The treatment duration was six months, aligning with studies on other species used in the age range associated with the onset of memory impairment (Rai et al. 1991; Wiklund et al. 1994; Neri et al. 1995).

Volunteers were instructed to note any effects they experienced. Additionally, they were advised not to alter their behavior or lifestyle, especially regarding medication use and physical activity patterns. They were requested to return for follow-up every 1-2 months, during which adherence to the protocol, general condition, and any potential negative or positive effects were assessed and recorded in their medical records.

Neuropsychological tests were performed initially (pre-treatment) and repeated only at the end of the study period (post-treatment). This time gap between assessments aimed to mitigate the influence of learning aspects during the tests, which could impact the final results (Figure 1).

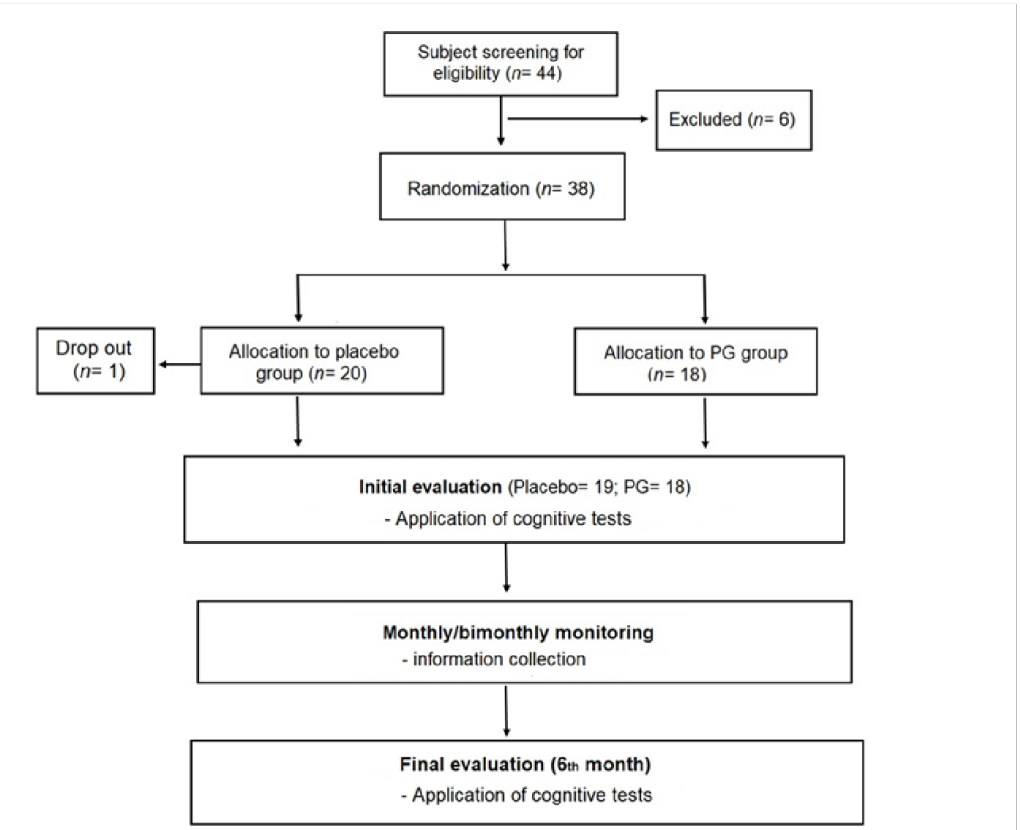

Figure 1. Flow-chart of clinical protocol.

Statistical analysis

The data from the neuropsychological tests were subjected to analysis using two-way Analysis of Variance (ANOVA) with education level as a covariate; post-hoc comparisons were conducted using Duncan's Test. Additionally, Fisher's Exact Test was utilized to assess the subjective effects reported by volunteers during the treatment period. A significance level of $p \leq 0.05$ was considered for all statistical analyses.

RESULTS AND DISCUSSION

Dry extract stardardization

The high-performance liquid chromatography (HPLC) system was employed for the standardization of the dry extract. The marker exhibited retention times of 12.084 min (for the isolated standard) and 12.673 min in the dry extract. Based on these retention times, the content of β-ecdysone in the dry extract was calculated to be 1.0%.

Selection and characteristics of volunteers

Out of the initially evaluated 44 volunteers, 6 were excluded from the study (4 cases of severe hypertension, 1 case of an exercise stress test suggestive of coronary obstruction, and 1 case with various abnormalities in laboratory tests). However, during the course of the study, one volunteer from the placebo group withdrew after experiencing a severe episode of chest pain, prompting the discontinuation of treatment. As a result, the research concluded with 37 healthy volunteers, aged between 50 and 73 years (Table 1).

Global neuropsychological assessment

The data from all the evaluations are presented in table 2. The overall assessment indicates that no significant differences were observed in any of the subtests, suggesting that the PG extract did not have a significant effect on the cognitive parameters of the volunteers at the tested doses and groups.

These findings suggest a lack of adaptive effects from the standardized PG extract in both adult and elderly volunteers, at the evaluated dose and treatment duration, in disagreement with popular and commercial recommendations (Mendes 2011; Brasmédica s.d.). It's worth noting that studies with adaptogens usually involve models and conditions under stress, with negative results occurring in the absence of these parameters (Bhattacharya and Muruganandam 2003). In the present study, healthy middle-aged and elderly individuals were

Table 1. Characteristics of the clinical study volunteer groups.

Characteristics	Placebo group (N= 19)	PG group (N= 18)
Age (mean ± SD)	59.7 ± 6.6	61.7 ± 7.3
3rd degree	11	10
2nd degree	3	4
1st degree	5	4
Caucasian	16	16
Black	0	1
Asian	3	1
Athlete	8	8
Non-athlete	11	10

Two-way ANOVA evaluation; non-significant data

Table 2. Overall results of cognitive tests applied to volunteers in the initial and final phases of treatment.

Cognitive tests	Placebo (N= 19) (mean±s.d.)		PG (N= 18) (mean±s.d.)	
	Early phase	Final phase	Early phase	Final phase
Toulouse-Pierón				
R subtest (speed)	112.7 ± 32.1	123.2 ± 38.6	105.8 ± 42.0	109.1 ± 44.6
Q subtest (quality)	8.4 ± 10.1	7.8 ± 9.7	8.1 ± 7.3	12.9 ± 20.1
Wechsler Memory Scale				
Information and orientation	10.5 ± 0,7	10.3 ± 0.6	10.6 ± 0.6	10.3 ± 0.7
Mental control	5.6 ± 2,3	6.7 ± 2.1	6.3 ± 2.5	6.7 ± 2.1
Verbal paired associates				
Pair 1	5.6 ± 1.7	6.2 ± 1.2	5.1 ± 1.3	5.8 ± 1.5
Pair 2	6.7 ± 1.6	7.2 ± 1.1	6.3 ± 1.8	6.7 ± 1.7
Pair 3	7.2 ± 1.5	7.8 ± 1.5	7.1 ± 1.7	7.5 ± 1.7
Total	19.5 ± 4.2	21.2 ± 3.2	18.6 ± 4.2	20.0 ± 4.3
Digit span				
Digits forward	5.9 ± 1.2	6.5 ± 1.2	5.3 ± 2.0	5.8 ± 1.4
Digits backwards	3.8 ± 1.2	3.7 ± 1.9	3.6 ± 1.9	3.7 ± 1.9
Logical memory I (immediate)				
Story A	12.4 ± 4.2	13.9 ± 4.0	10.5 ± 5.1	12.4 ± 4.3
Story B	14.2 ± 4.1	13.6 ± 4.0	11.9 ± 5.8	13.2 ± 3.8
Logical memory II (post-delay)				
Story A	10.3 ± 4.0	12.2 ± 4.9	8.1 ± 4.1	10.9 ± 4.2
Story B	12.6 ± 4.1	12.5 ± 4.3	10.3 ± 5.3	11.5 ± 4.6
Progressive matrices	41.7 ± 9.6	41.3 ± 10.5	36.7 ± 15.3	37.6 ± 15.7

Two-way ANOVA evaluation; non-significant data

evaluated, not subjected to any specific stressful condition, only under the normal decline of aging and its representation in terms of cellular stress and maintenance of homeostasis.

Future studies should explore alternative methodological models, expand the spectrum of extracts, doses, and include a larger number of volunteers and patients to comprehensively understand its actions.

Neuropsychological assessment by physical activity profiles

In an exploratory statistical evaluation (Table 3), we aimed to assess the impact of PG extract on cognitive tests in relation to different patterns of physical activity in volunteers distinguishing between athletes and non-athletes. This evaluation was conducted in consideration of the well-established relationship between cognition and physical activity, with regular exercise being recognized as a potential means to mitigate cognitive decline in adults (Xu et al. 2023).

A similar exploratory evaluation format was utilized by Perry et al. (2018) in a double-blind, placebo-controlled study investigating the effects of a combination of sage (*Salvia officinalis* L.), rosemary (*Rosmarinus officinalis* L.) and lemon balm (*Melissa officinalis* L.) on memory in normal volunteers. Despite initially finding no significance in the overall data, further evaluation in subgroups revealed improvement specifically in the delayed word recall test.

The results from certain tests indicate statistically significant differences between Athletes and Non-athletes' subgroups when using PG extract. There was, however, no confluence for one group in particular with significant results with Non-athletes PG (Toulouse-Pierón Q; WMS-orientation), one with Athletes PG (WMS-digits forward) and three others involving both treated subgroups (WMS-logical memory I and II). The reasons for these non-confluent results are unclear and require further investigation.

The Non-athletes PG subgroup exhibited two significant findings (Toulouse-Pierón Q and WMS-orientation subtests) that could be interpreted as negative in cognitive terms. In the first case, there was an increase in the number of errors in

Table 3. Significant results of the cognitive subtests applied to volunteers in the initial and final phases of treatment, evaluated according to physical activity patterns.

Cognitive tests - Athletes (mean ± s.d.)				
	Placebo (N= 8)		**PG (N= 8)**	
	initial	final	initial	final
Toulouse-Pierón (Q)	6.5 ± 7.5	5.0 ± 4.9	6.6 ± 4.4	7.1 ± 6.3
WMS - Orientação	4.6 ± 0.5	4.6 ± 0.5	5.0 ± 0.0	4.9 ± 0.4
WMS-digit span forward	5.6 ± 1.5	6.6 ± 1.2	4.6 ± 2.6	6.0 ± 1.6**
WMS-logical memory I	12.0 ± 2.4	13.1 ± 3.7	12.9 ± 3.6	13.7 ± 3.4
WMS- logical memory II	9.6 ± 2.1	12.0 ± 5.4	9.6 ± 3.4	12.7 ± 2.5**

Cognitive tests - Non-athletes (mean ± s.d.)				
	Placebo (N= 11)		**PG (N= 10)**	
	initial	final	initial	final
Toulouse-Pierón (Q)	10.5 ± 12.2	10.4 ± 12.5	7.3 ± 6.7	18.2 ± 25.8*
WMS - Orientação	4.9 ± 0.3	4.5 ± 0.5	4.8 ± 0.4	4.3 ± 0.7*
WMS-digit span forward	6.0 ± 1.0	6.5 ± 1.3	5.8 ± 1.5	5.6 ± 1.4
WMS-logical memory I	12.7 ± 5.5	14.4 ± 4.5	9.2 ± 5.7	12.5 ± 3.9*
WMS- logical memory II	11.2 ± 5.1	12.3 ± 5.0	7.3 ± 4.6	10.5 ± 4.3*

*Differs from Non-athletes PG group, initial; p≤0.05 – Duncan test; **Differs from Athletes PG group; initial; p≤0.05 – Duncan test.

the post-treatment phase with the extract (initial: 7.3±6.7; final: 18.2±25.8; p≤0.05) indicating a decline in attention and psychomotor performance among volunteers. The second finding indicates a negative impact of the PG extract on general cognitive functions such as spatial orientation, knowledge of the month, and day of the year (initial: 4.8±0.4; final: 4.3±0.7; p≤0.05). These effects were not observed in the other groups, although the Non-athletes placebo group did show a slight decrease in performance during the post-treatment stage in the Orientation subtest (initial: 4.9±03; final: 4.5±0.5; p≥ 0.05).

Such declines could potentially be associed with the depressant effects on central nervous system (CNS) mentioned by Paris et al. (2000) and also attributed to the substance β-ecdysone (Fenner et al. 2008); CNS depressant drugs, such as benzodiazepines, are known to impair attention and psychomotor performance, as assessed by the Toulouse-Pierón Concentrated Attention test (Bonazzi et al. 1994), and it's plausible that a similar effect occurred in this study. Additionally, the Non-athletes PG group consisted of volunteers with an average age 8 years older than the other treated group (Non-athletes PG: 63.9±5.5 years; Athletes PG: 55.9±6.1 years; p≤0.05). This significant age difference may have contributed to the observed negative performance.

In the WMS-digits forward subtest, it was observed that the Athletes PG group exhibited, after treatment, a greater capacity to memorize and repeat the sets of numbers presented by the evaluator after treatment (initial: 4.6±2.6; final: 6.0 ±1.6; p≤0.05), indicating a significant improvement; conversely, the Athletes placebo group also showed positive progress, albeit without statistical significance (Figure 2A).

This test evaluates short-term memory, also known as working memory or verbal working memory, which integrates attention and memory for a given task (Oliveira and Bueno 1993). This cognitive system has a limited retention and temporary storage capacity, typically lasting a few seconds, with a storage limit of approximately 5 to 9 pieces of information (Miller 1956; Oliveira and Bueno 1993). The improvement observed in the Athletes PG subgroup post-treatment reflects an enhancement in this score, which initially was at a lower numerical level compared to the Non-athletes

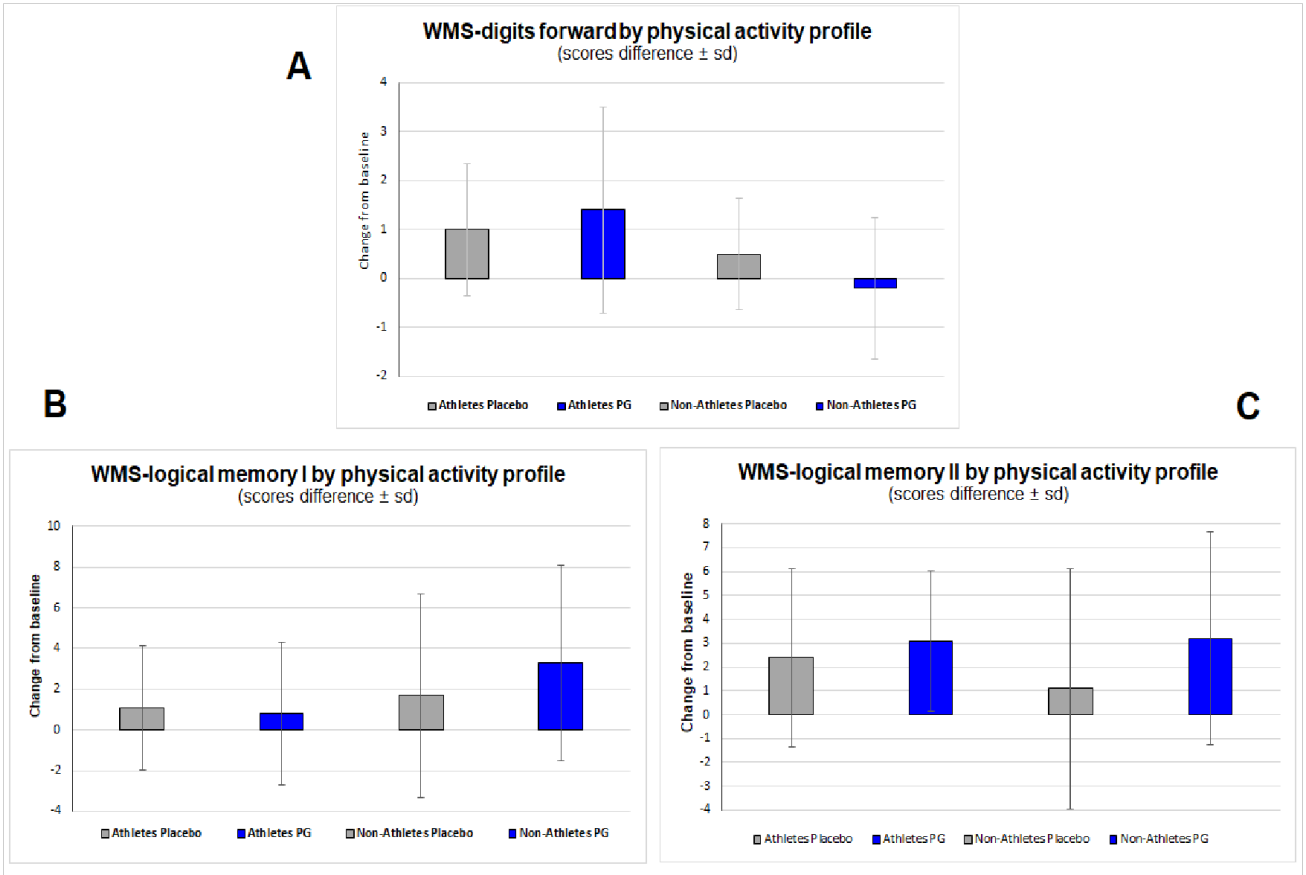

Figure 2. Results of the positive WMS subtests (A - digits forward; B- logical memory I; C- logical memory II) expressed as a difference between initial and final scores of the volunteers' groups subdivided by physical activity profile.

placebo and PG subgroups (Table 2). However, the association between physical activity and improved performance in the WMS digits subtest remains unclear, as indicated by other studies (Freitas et al. 2023), which did not demonstrate improvements compared to placebo group, albeit showing differences in relation to the group that underwent cognitive stimulation. Further evaluations are warranted to gain a comprehensive understanding of this result.

Finally, data from the WMS-logical memory I and II subtests revealed three effects related to the ability to memorize and retrieve information, both immediately after its presentation (1st call – immediate memory) and after approximately 30 min of delay (2nd call – late memory) (Figure 2B and C). In this instance, significant results were observed in the Athletes PG group (logical memory I) and in the Non-athletes PG group (logical memory I and II) during the post-treatment phase; these findings appear to be independent of the pattern of physical activity and suggest a facilitative effect of PG on memory retention capacity, particularly noteworthy in the instances involving delayed memory recall after a 30-min interval, which poses challenges for retaining information (Oliveira and Bueno 1993).

Comparing these positive results with those obtained in the Toulouse-Pierón Q subtest, it can be inferred that the impairment detected in the Non-athletes PG group appears to be more closely associated with the psychomotor component rather than with attention and memories, which have been demonstrated to be preserved and even strengthened in certain subtests of the WMS scale.

Effects on memory are commonly associated with the cholinergic system, and its decline is a prominent indicator of dementia (Ferreira-Vieira et al. 2016). One therapeutic approach for dementia involves anticholinesterase drugs, which inhibit the enzyme responsible for breaking down the neurotransmitter acetylcholine, thereby enhancing the overall functioning of the memory system (Sharma 2019). Several medicinal species are well-known for their potential to improve dementia symptoms or at least to slow down its progression, including *Melissa officinalis* L.*, Huperzia serrata* (Thunb.) Trevis.*, Salvia officinalis* L.*, Bacopa monnieri* (L.) Wettst.*, Ginkgo biloba* L., among others (Ahmed et al. 2013).

In this context, Seidl (2010) conducted an evaluation of several plant species and identified a positive acetylcholinesterase inhibitory effect for Brazilian ginseng, with the highest percentage of inhibition among the various extracts tested (85% at a concentration of 0.5 mg/ml). Subsequently, Franco (2021) replicated this effect for PG fractions rich in β-ecdysone, attributing the pharmacological activity to the known action of this substance on the enzyme acetylcholinesterase, among others (Yang et al. 2004; Xia et al. 2014). Therefore, these studies corroborate, at least in part, that the anticholinesterase effect is one of the mechanisms through which PG may impact human memory.

The Progressive Matrices Test did not reveal significant differences between the groups, either in the overall analysis or in the subgroups categorized by physical activity patterns. This suggests that there was no notable cognitive decline among the volunteers during the six months of treatment, and furthermore, that the PG extract did not exhibit any discernible impact, positive or negative, on the assessment conducted in the test.

Subjective data from the clinical study

Throughout the clinical study, volunteers were closely monitored until the final evaluation. Subjective reports were gathered on planned aspects such as memory and other cognitive functions, as well as on other areas highlighted by the volunteers, with no restrictions on the scope of topics discussed (Table 4).

Non-significant citations of improvement in

Table 4. Reports from participants of subjective positive effects (placebo N= 19; PG N= 18).

Subjective effects	Placebo	PG	Statistical analysis
Cognitive and mental aspects	3	7	NS
Physical aspects	4	6	NS
Sleep	1	4	NS
Dreams	1	3	NS
Sexual activities	1	3	NS
Other general aspects	1	7	p≤0.05
Total	11	30	

* Differs from the Placebo group; p £ 0,05 – Fisher's test; NS - not significant

mental and cognitive aspects varied between the placebo and PG groups. In the placebo group, two specific references highlighted improvements in memory and overall mental activity. In the PG group, three references focused on improvements in short-term memory, while four references emphasized general mental and cognitive enhancements, such as increased lucidity, stable thinking, improved mood, and reduced anxiety in anxiety-provoking situations (e.g., travel).

In terms of physical aspects, although both volunteers from the placebo and the PG groups report increased overall disposition or in sports activities, in the dose and time evaluated, PG did not express significant benefits in this area (Marques et al. 2002).

The third aspect mentioned concerns the sleep characteristics of the volunteers. In the placebo group, there was one instance of a volunteer using midazolam. In contrast, in the PG group, there were four mentions regarding a potential effect of the treatment in inducing a shorter latency, longer duration, deeper, and more restful sleep, typically observed in the initial days of treatment (non-significant data). PG effects on sleep duration in barbiturate sleep models in animals have previously been documented by Paris et al. (2000) The effects of sleep-facilitating substances are particularly relevant for elderly patients, who commonly experience insomnia as a negative aspect of the aging process, impacting cognition as well (Dzierzewski et al. 2018).

Another aspect evaluated, although not statistically significant, was related to the occurrence of dreams, a topic spontaneously raised by some volunteers. One volunteer from the PG group, in particular, mentioned several times that “it is the first time I have had a dream in my life,” or even “for those who had never dreamed, it is a profound experience.” One hypothesis for this effect could be the increase in REM sleep, which is essential for maintaining cognitive functions; dreams can occur in any phase of sleep, but they are generally more common, vivid, and intense during the REM sleep phase (Fases do sono 2021). The relationship between dreams and cognitive functions also encompasses functions related to emotion regulation, learning, and even memory consolidation (Barbosa et al. 2023). Exploring studies with PG in this area could yield interesting findings.

In terms of sexual activity, we aimed to assess any changes considering both traditional use and commercial indications in this area. While there was only one report of improvement in a 52-year-old athlete volunteer in the placebo group, there were three reports in the PG group, involving two non-athlete volunteers (aged 59 and 64) and one 66-year-old athlete (non-significant data). Dias et al. (2020) evaluated the hydroalcoholic extract of PG roots in adult male mice and observed an increase in nitric oxide leading to vasodilation, as well as increases in corpora cavernosa and collagen, among other effects. Similarly, Huang et al. (2023) investigated the effects of PG on sexual dysfunction in rats induced by paroxetine; they found improvements in sexual performance, restoration of sexual hormone levels, an increase in nitric oxide and cyclic guanosine monophosphate (cGMP), increased activity of nitric oxide synthase in penile tissues, and decreased phosphodiesterase-5 activity, which enhances penile erection. Further studies in this area would be beneficial.

Regarding general effects, numerically significant between the groups were observed. One participant in the placebo group claimed that the product was increasing their appetite. In the PG group, seven different manifestations occurred: two cases involved the elimination of a mole (nevus) in the chest area and corns on the soles of the feet; two other reports referred to a decrease in desire for alcoholic beverages; and three other volunteers reported improvement in nighttime urinary elimination, with less frequent and more productive episodes.

All these effects of subjective manifestations with PG extract seem to compose a diffuse picture but one that aligns with effects reported in some articles under the umbrella of “improvements in quality of life.” For instance, Wiklund et al. (1994) evaluated 400 healthy volunteers on various general aspects (well-being, anxiety, vitality, self-control, depression, health, sleep, relaxation, appetite, and sexual interest, among others), finding significantly increased scores for several parameters in the experimental group (*Panax ginseng* with vitamins and minerals). Other authors (Neri et al. 1995), also using a product based on Korean ginseng with vitamins, assessed psychological states of well-being and perception of quality of life in 60 volunteers with an average age of 61 years, with age-related memory impairment; in the treated group, a positive correlation was found between cognitive index and social contact, mood, and factors related to self-control. The data from this study seem to show a similar relationship, with the PG group showing various improvements that, collectively, bring remarkable benefits to quality of life, including the cognitive benefits that were evidenced as well as the negatives to be considered as warnings.

Adverse events data

Citations of events with negative aspects are presented in table 5.

Table 5. Reports from participants of adverse events (placebo N= 11; PG N= 9).

Adverse events	Placebo group	PG group
Changes in stool consistency or color	1	1
Dry mouth	1	0
Headache	0	1
Arm pain/numbness	2	1
Abdominal pain	1	0
Rash	2	0
Flatulence	1	1
Salivation	1	0
Different taste sensations	2	0
Dizziness	2	2
Torpor and excessive drowsiness	1	1
Urticaria	-	1
Total	14	8

Fisher's test; non-significant data

These data show a higher number of mentions in the placebo group compared to the PG group (non-significant difference); overall, there were mild manifestations, such as those related to the intake of new substances promoting changes in feces and flatulence, reported by one volunteer from each group, which usually cease after a few days of habituation. Thus, overall, it can be stated that tolerance to the PG extract was good.

CONCLUSIONS

In summary, the data presented indicate significant cognitive effects induced by standardized PG extract in healthy male volunteers' subgroups aged 50-73 years old. Among these alterations, two were negative, affecting psychomotor performance and orientation, while four were positive, including improvements in digits and both immediate and delayed memory. Importantly, there was no evidence of generating adverse risk events over the six-month treatment period. Further assessments with other extracts, doses, and profiles of volunteers, including patients, are necessary for a comprehensive evaluation and to contribute to the understanding of the effects of *Pfaffia glomerata*, also known as Brazilian ginseng.

ACKNOWLEDGMENTS

We would like to express our gratitude to the professors and staff of the Psychobiology department at the Federal University of São Paulo and a special thanks are also due to the volunteers who willingly participated in all stages of the study and consumed the extract under evaluation. Additionally, we extend our appreciation to the team at Sanrisil S/A for preparing the dry extract and conducting the chromatographic measurements, as well as to the Fontovit Laboratory for encapsulating the dry extract.

AUTHORS' CONTRIBUTION

This work is part of LCM's doctoral thesis, supervised by ELAC. LCM: methodology, investigation, acquisition and analysis of data, manuscript preparation, manuscript editing and review. ELAC: Conceptualization, methodology, funding acquisition, project administration, supervision, acquisition and analysis of raw data (*in memorian*).

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

Ahmed F, Ghalib RM, Sasikala P, Ahmed KKM (2013) Cholinesterase inhibitors from botanicals. Pharmacogn Rev 7(14):121–130. https://doi.org/10.4103/0973-7847.120511

Araujo RS (2011) Teste de atenção concentrada Toulouse-Pierón: atualização dos estudos de padronização, validade e precisão. 82p. Dissertação (Mestrado em Psicologia), Universidade de São Paulo, São Paulo, Brasil.

Babich O, Larina V, Ivanova S, Tarasov A, Povydysh M, Orlova A, Strugar J, Sukhikh S (2022) Phytotherapeutic approaches to the prevention of age-related changes and the extension of active longevity. Molecules 27(7):2276. https://doi.org/10.3390/molecules27072276

Barbosa FC, Tadine RM, Rezenda JDP, Lopes GCD, Shiozawa P, Borges WHP (2023) Dreams' neurophysiology: integration of neuroscience concepts and psychoanalysis. Rev Gest Soc Ambient 17(2):1-19. e03416. https://doi.org/10.24857/rgsa.v17n2-019

Bhattacharya SK, Muruganandam AV (2003) Adaptogenic activity of *Withania somnifera*: an experimental study using a rat model of chronic stress. Pharmacol Biochem Behav 75(3):547-55. https://doi.org/10.1016/s0091-3057(03)00110-2

Bonazzi M, Riva A, Marsicano M, Prampolini F, Speranza R, Andriolli A, Laveneziana D (1994) [Trazodone versus flunitrazepam in premedication in day-care surgery]. Clinical Trial Minerva Anestesiol 60(3):115-21.

Brasmédica S/A Indústrias Farmacêutica (s.data) Folheto do medicamento Tai Ginseng do Brasil. São Paulo.

Brekhman II, Dardymov IV (1969) New substances of plant origin which increase nonspecific resistance. Annu Rev Pharmacol 9:419-30.

Carlini EA (1995) Efeito adaptógeno ou resistógeno de algumas plantas. Medicamentos, drogas e saúde. São Paulo: Hucitec. 255p.

Dias FCR, Gomes MLM, Melo FCSA, Menezes TP, Martins AL, Cupertino MDC, Otoni WC, Matta SLPD (2020) *Pfaffia glomerata* hydroalcoholic extract stimulates penile tissue in adult Swiss mice. J Ethnopharmacol 261:113182. https://doi.org/10.1016/j.jep.2020.113182

Elwood RW (1991) The Wechsler Memory Scale-Revised: psychometric characteristics and clinical application. Neuropsychol Rev 2(2):179-201. https://doi.org/10.1007/BF01109053

Faep/Senar PR (2021) Ginseng do Paraná é disputado por compradores internacionais. Available at: https://cnabrasil.org.br/noticias/ginseng-do-parana-e-disputado-por-compradores-internacionais - Acesso em 04 de abril 2024.

Fases de sono: veja quais são e entenda a importância (2021) Disponível em https://institutodosono.com/artigos-noticias/fases-de-sono-veja-quais-sao-e-entenda-a-importancia/. Accessed on: 22 Apr 2024.

Fenner R, Zimmer AR, Neves G, Kliemann M, Gosmann G, Rates SMK (2008) Hypnotic effect of ecdysterone isolated from *Pfaffia glomerata* (Spreng.) Pedersen. Rev Bras Farmacogn 18(2):170-176. https://doi.org/10.1590/S0102-695X2008000200005

Ferreira-Vieira TH, Guimarães IM, Silva FR, Ribeiro FM (2016) Alzheimer's disease: targeting the cholinergic system. Curr Neuropharmacol 14(1):101-15. https://doi.org/10.2174/1570159x13666150716165726

Franco RR, Franco RM, Justino AB, Borges ALS, Bittar VP, Saito N, Saraiva AL, Nicolau Júnior N, Otoni WC, Espindola FS (2024) Phytochemical composition of aerial parts and roots of *Pfaffia glomerata* (Spreng.) Pedersen and anticholinesterase, antioxidant, and antiglycation activities. Protoplasma https://doi.org/10.1007/s00709-023-01916-9

Franco RR, Takata LA, Chagas K, Justino AB, Saraiva AL, Goulart LR, Ávila VMR, Otoni WC, Espindola FS, Silva CR (2021) A 20-hydroxyecdysone-enriched fraction from *Pfaffia glomerata* (Spreng.) Pedersen roots alleviate stress, anxiety, and depression in mice. J Ethnopharmacol 1:267:113599. https://doi.org/10.1016/j.jep.2020.113599

Freitas GJB, Sardinha LS, Tallo FS, Lima RY, Gehrke FS, Carvalho RG, Menezes-Rodrigues FS, Lemos VA (2023) Influence of cognitive stimulation and physical exercise on sleep quality, mood and cognition in the elderly. Res Soc Dev 12(3):e8912340442. https://doi.org/10.33448/rsd-v12i3.40442

Huang Q, Wu H, Qin X (2023) Extract of *Pfaffia glomerata* ameliorates paroxetine-induced sexual dysfunction in male mice and the characterization of its phytoconstituents by UPLC-MS. Foods 12(17):3236. https://doi.org/10.3390/foods12173236.

Karacan I, Sennaroglu B, Vayvay O (2020) Analysis of life expectancy across countries using decision tree. East Mediterr Health J 26(2):143–151. https://doi.org/10.26719/2020.26.2.143

Marques LC, Danucalov MA, Torres F, Galduróz JCF, Carlini ELA, Silva AC (2002) Estudo clínico duplo-cego de extrato padronizado (BNT-08) das raízes de *Pfaffia glomerata* (Spreng.) Pedersen: avaliação do efeito tônico em atividade física. Rev Bras Farmacogn 12(suppl 1):44-47. https://doi.org/10.1590/S0102-695X2002000300021

Marques LC, Galvão SMP, Espínola E, Dias RF, Mattei R, Oliveira MGM, Carlini ELA (2004) Psychopharmacological assessment of *Pfaffia glomerata* roots (extract BNT-08) in rodents. Phytother Res18:566–572. https://doi.org/10.1002/ptr.1500

Marques LC, Galvão SMP, Sandoval HK, Carlini EA (1998) Estudo toxicológico pré-clínico de *Pfaffia glomerata* (Spreng.) Pedersen. In: 15º Simpósio de Plantas Medicinais do Brasil. Programa e Resumos. Águas de Lindóia, 14 a 17 de outubro.

Mendes FR (2011) Tonic, fortifier and aphrodisiac: adaptogens in the Brazilian folk medicine. Rev Bras Farmacogn 21(4):754-763. https://doi.org/10.1590/S0102-695X2011005000097

Miller GA (1956) The magic number 7, plus or minus two: some limits on our capacity for processing information. Psychol Rev 63:81-93.

Neri M, Andermarcher E, Pradelli JM, Salvioli G (1995) Influence of a double-blind pharmacological trial on two domains of well-being in subjects with age associated memory impairment. Arch Gerontol Geriatr 21(3):241-52. https://doi.org/10.1016/0167-4943(95)00659-9

Oliveira CP, Mendes J, Costa P, Soares L, Silva-Santos

JE (2004) Effects of a hydroalcoholic extract from roots of *Pfaffia glomerata* (Spreng.) on the elevated T-maze in mice. In: 36º Congresso Brasileiro de Farmacologia e Terapêutica Experimental. Águas de Lindóia, 17 a 20 de outubro.

Oliveira MGM, Bueno OFA (1993) Neuropsicologia da memória humana. Psicologia USP 4(1-2):117-138.

Paris F, Neves G, Salgueiro JB, Quevedo J, Izquierdo I, Rates SMK (2000) Psychopharmacological screening of *Pfaffia glomerata* Spreng. (Amaranthaceae) in rodents. J Ethnopharmacol 73:261–269. https://doi.org/10.1016/s0378-8741(00)00329-9

Passos VMA, Champs APS, Teixeira R, Lima-Costa MFF, Kirkwood R, Veras R, Nascimento BR, Nogales AM, Schmidt MI, Duncan BB, Cousin E, Naghavi M, Souza FM (2020) The burden of disease among Brazilian older adults and the challenge for health policies: results of the Global Burden of Disease Study 2017. Popul Health Metr 18(Suppl 1):1-15 https://doi.org/10.1186/s12963-020-00206-3

Perry E, Howes M-JR (2011) Medicinal plants and dementia therapy: herbal hopes for brain aging? CNS Neurosci Ther 17(6):683–698. https://doi.org/10.1111/j.1755-5949.2010.00202.x

Perry NSL, Menzies R, Hodgson F, Wedgewood P, Howes M-JR, Brooker HJ, Wesnes KA, Perry EK (2018) A randomized double-blind placebo-controlled pilot trial of a combined extract of sage, rosemary and melissa, traditional herbal medicines, on the enhancement of memory in normal healthy subjects, including influence of age. Phytomedicine 5:39:42-48. https://doi.org/10.1016/j.phymed.2017.08.015

Prudente CM, Ramos CS, Rodrigues DS, Oliveira Júnior CJF (2024) *Pfaffia glomerata* (Spreng.) Pedersen: Brazilian ginseng, potential for Brazilian socio-biodiversity. DELOS 17(53):1-19. https://doi.org/10.55905/rdelosv17.n53-022

Rai GS, Shovlin C, Wesnes KA (1991) A double-blind, placebo-controlled study of *Ginkgo biloba* extract ('Tanakan') in elderly out-patients with mild to moderate memory impairment. Curr Med Res Opin 12(6):350-5. https://doi.org/10.1185/03007999109111504

Raven JC (1993) Testes das matrizes progressivas – escala geral. In: Manual de psicologia aplicada. São Paulo: Cepa. 254p.

Ribeiro STC, Gancedo NC, Oliveira AJB, Gonçalves RAC (2024) A comprehensive review of *Pfaffia glomerata* botany, ethnopharmacology, phytochemistry, biological activities, and biotechnology. J Ethnopharmacol 328:118003. https://doi.org/10.1016/j.jep.2024.118003

Seidl C (2010) Pesquisa de substâncias naturais inibidoras da acetilcolinesterase. 86p. Dissertação (Mestrado em Ciências Farmacêuticas), Universidade Federal do Paraná, Curitiba, Brasil.

Sharma K (2019) Cholinesterase inhibitors as Alzheimer's therapeutics (Review). Rev Mol Med Rep 20(2):1479-1487. https://doi.org/10.3892/mmr.2019.10374

Wagner H, Norr H, Winterhoff H (1994) Plant adaptogens. Phytomedicine 1:63-76. https://doi.org/10.1016/S0944-7113(11)80025-5

Wiklund I, Karberg J, Lund B (1994) A double-blind comparison of the effect on quality of life of a combination of vital substances including standardized ginseng G115 and placebo. Curr Ther Res 55(1): 32-42. https://doi.org/10.1016/S0011-393X(05)80075-X

Xia X, Zhang Q, Liu R, Wang Z, Tang N, Liu F, Huang G, Jiang X, Gui G, Wang L, Sun X (2014) Effects of 20-hydroxyecdysone on improving memory deficits in streptozotocin-induced type 1 diabetes mellitus in rat. Eur J Pharmacol 740:45-52. https://doi.org/10.1016/j.ejphar.2014.06.026

Xu L, Gu H, Cai X, Zhang Y, Hou X, Yu J, Sun T (2023) The effects of exercise for cognitive function in older adults: a systematic review and meta-analysis of randomized controlled trials. Int J Environ Res Public Health 20(2):1088. https://doi.org/10.3390/ijerph20021088

Yang SF, Yang ZQ, Zhou QX, Wu Q, Huang XN, Shi JS (2004) Effect of ecdysterone on the expression of c-fos in the brain of rats induced by microinjection beta-AP25-35 into the hippocampus. Yao Xue Xue Bao 39:241-244.