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Original Article

An Experimental Study on Phytochemical Composition and Memory Enhancing Effect of *Ginkgo Biloba* Seed Extract

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Abstract

Introduction: The *Ginkgo biloba L.* tree is considered as one of the oldest species on Earth. It is known as a "living fossil" dating back approximately 200 million years. Both the leaves and seeds of this tree have been used for millennia in traditional Chinese medicine.

Aim: To study the phytochemical profile of Gingko biloba seed extract (GBSE) and its memory enhancing effects.

Materials and methods: Liquid chromatography with mass detection (LC-MS) was performed for phytochemical analyses of the extracts. For the *in vivo* experiments, male Wistar rats were divided randomly into 5 groups (n=8): saline; piracetam; GBSE 50; 100, and 200 mg/kg b.w. Y-maze, T-maze, step-down passive avoidance and novel object recognition test (NORT) were performed. The observed parameters were: percentage of spontaneous alternations (% SA), working memory index, latency of reaction and recognition index, respectively. Statistical analysis was done using SPSS 19.

Results: LC-MS analysis showed the presence of the flavonoids quercetin, kaempferol and isorhamnetin (as aglycones), the ginkgolides A, B, C, J, and bilobalide. In Y-maze task, the groups treated with 50 and 100 mg/kg of GBSE significantly increased the % SA during the memory test compared to saline ($p<0.05$). In T-maze test, the three experimental groups with GBSE significantly increased the working memory index in comparison with that of the control group ($p<0.05$). In step-down test, the animals receiving 100 mg/kg b.w. GBSE, notably increased the latency during both retention tests ($p<0.05$ and $p<0.01$, respectively). In NORT, only the animals with the middle dose of GBSE ameliorated the recognition index when compared to saline ($p<0.05$).

Conclusions: GBSE enhances spatial working memory, recognition memory, and short- and long-term recall in naïve rats due to the synergic effects of detected flavonoids and terpene lactones on brain functions. The brain structures involved are probably the hippocampus and prefrontal cortex.

Keywords

flavonoids, memory, phytopreparation, terpene lactones

Abbreviations in text	
b.w.: body weight	LC-MS: liquid chromatography with mass-detector
CNS: central nervous system	MeCN: acetonitrile
EGb 761®: Ginkgo biloba standardized leaf extract	mPFC: medial prefrontal cortex
FA: formic acid	ORT: object recognition test
GBLE: Ginkgo biloba leaf extract	PCM: piracetam
GBSE: Ginkgo biloba seed extract	RI: recognition index
HESI: heated electrospray ionization	SA: spontaneous alterations

INTRODUCTION

Ginkgo biloba L. tree is one of the oldest species on Earth. It is known as a "living fossil" because it has been living on Earth for more than 200 million years. Both the leaves and seeds of this tree have been used for millennia in traditional Chinese medicine. The most used parts of the Ginkgo tree are fresh or dried leaves. The standardized leaf extract of *Ginkgo biloba* (EGb 761®) has become a popular remedy for treatment of cerebral vascular disease, memory problems and even for Alzheimer's disease. It has a wide range of other biological activities, including anti-inflammatory, antioxidant, anticancer, photoprotective effects.[1] In Bulgaria, different pharmaceutical forms of *Ginkgo biloba* leaves are available either as food supplement or as medicinal product and they are among the top selling phytopharmaceuticals.[2]

The commercially available *Ginkgo biloba* products have usually been standardized based on the contents of terpene lactones and flavonoids.[3] According to the European Pharmacopoeia 8.0 *Ginkgo biloba* leaf extract (GBLE) should contain flavonoids expressed as flavone glycosides (22 to 27%), bilobalide (2.6 to 3.2%), ginkgolides A, B, and C (2.8 to 3.4%), ginkgolic acids – maximum 5 ppm. Ginkgolides are specific to this tree species and are found only in it.

Ginkgo biloba seed grows with a soft shell that ferments during ripening and has a strong, unpleasant odor. After removing this soft shell, a hard, very thin shell appears under which the actual seed (nut, kernel) is. Although there is a lot of information regarding the chemical composition and biological activity of GBLE, phytochemistry and potential pharmacological properties of *Ginkgo biloba* seeds remain in the shadow.[4] They have been used in China as a traditional food after fermentation or cooking, and for treating pulmonary diseases such as asthma, coughs, and enuresis for several thousand years.[5]

Zhou et al. reported that the active substances with potentially pharmacological effects found in leaves are also presented in the nuts.[6]

Ginkgo biloba seed extracts (GBSE) contain compounds from two pharmacologically important chemical groups: flavonol glycosides (with quercetin, kaempferol, and isorhamnetin as the principal aglycones) and terpene lactones (bilobalide and ginkgolides A, B, C, and J). Apart from this, the kernels also contain polyphenolic organic acids, carbohydrates, lipids, vitamins, inorganic salts, and amino acids. Many of these have been shown to be beneficial in treating neurodegenerative diseases, cancer, cardiovascular diseases, stress responses, and mood, and memory disorders.[7]

GBLE can improve cognitive functions in rats with experimental models of memory impairment[8] and in healthy subjects or patients with different types of dementia (Alzheimer's disease, vascular, Lewy body and frontotemporal dementia).[7] Since the chemical composition of GBSE seems to be comparable with that of leaf extracts we can assume similar biological activity.

AIM

The aim of our study was phytochemical analyses of GBSE and experimental research on the possible memory enhancing effect *in vivo*.

MATERIALS AND METHODS

Animals

Adult male Wistar rats weighting 150±20 g were used in our experiments. They were housed under standard laboratory conditions (08:00-20:00 light-dark cycle, temperature 22±2°C, food and water *ad libitum*). The experiments were approved by the Animal Health and Welfare' Directorate of Bulgarian Food Safety Agency, licence No 249/20.11.2019.

Drugs and substances

Piracetam (PCM) amp. 3 g/5 ml purchased from UCB Farchim.

Preparation of *Ginkgo biloba* seed extract

Mature seeds of *Ginkgo biloba* obtained from a tree located on the campus of Medical College in Plovdiv, Bulgaria were harvested in November 2018. The seeds were shelled and the kernel (endosperm) was homogenized in a high-speed tissue homogenizer. Dry matter was determined

gravimetrically. The obtained paste was dispersed in 70% methanol (1:10 w/v, dry matter base) and stirred for 12 hours at room temperature in a light-protected flask. The extract was centrifuged at 6000 g for 10 min and the supernatant was collected. The extraction and centrifugation were repeated twice on the residue. The extracts were combined, and solvent was evaporated at 30°C under vacuum to a dry residue. For animal testing the dry extract was dissolved in distilled water.

Chromatographic analysis of GBSE

Liquid chromatography with mass detection (LC-MS/MS) of analytes was performed using chromatographic system Thermo Dionex Ultimate 3000 and triple quadrupole mass spectrometer Thermo TSQ Quantum Access MAX, with HESI (Heated Electrospray Ionization). The chromatographic system includes a quaternary two-piston pump, autosampler and column thermostat. Separation was performed on a chromatographic column Syncronis C18 150 mm×4.6 mm, particle size 5 μm.

Analytical conditions for quantification of flavonoids

LC conditions: Mobile phase A consisted of 0.1% formic acid (FA)/H_2O (1/1000, v/v) in acetonitrile (MeCN)/H_2O (90:10, v/v). Mobile phase B was composed of 0.1% FA/H_2O (1/1000, v/v) in MeCN/H_2O (10:90, v/v) and the flow rate was 0.7 mL min^{-1}. The gradient conditions were 0-15 min 3% A, 15-19 min 63% A, 19-23 min 63% A, and 23-25 min 3% A. *MS conditions:* HESI was used in negative mode, the capillary voltage was set to 3000 V, vaporizing temperature 350°C, Sheath gas – 50 AU, the capillary temperature – 300°C, Aux gas – 5 AU.

Analytical conditions for quantification of terpenes

LC conditions: Isocratic elution was applied, using mobile phase consisted of 0.1% FA/H_2O (1/1000, v/v) in MeCN/H_2O (50:50, v/v) at flow rate 0.5 mL min^{-1}. *MS conditions:* HESI was used in negative mode, the capillary voltage was set to 3000 V, vaporizing temperature 300°C, Sheath gas – 30 AU, the capillary temperature – 300°C, Aux gas – 10 AU.

Experimental design

The animals used to evaluate the effect of GBSE on learning and memory were divided randomly into 5 groups (n=8) as follows: group 1 – control group: saline 0.1 ml/100 g body weight (b.w.); group 2 – positive control group with standard memory enhancer piracetam (PCM) 600 mg/kg b.w.; group 3 – GBSE 50 mg/kg b.w.; group 4 – GBSE 100 mg/kg b.w.; group 5 – GBSE 200 mg/kg b.w. GBSA, PCM and saline were administered by oral gavage. The animals were pretreated for two weeks prior to the tests.

Behavioural tests

Elevated Y-maze test

The test was conducted in two consecutive days – training and memory retention. The Y-maze consists of three identical arms – A, B, C. The protocol includes 2 phases: habituation and test. During the test phase the number of arm entries and triads were recorded, for a period of 5 minutes. The spontaneous alternations (% SA) were calculated according to the formula:

$$\% \text{ SA} = \frac{\text{Number of alternations}}{\text{Total number of entries} - 2} \times 100$$

Elevated T-maze test

This experiment was conducted to evaluate spatial working memory in rats. The test depends on either spontaneous or rewarded alternation. In this study we used the second option. Food intake was ceased 24 hours prior to the experiments. Learning sessions consisted of 11 trials – an initial forced trial followed by 10 choice trials. During the initial forced trial one arm was closed and reward pallets were placed in the other arm, therefore the animal was forced to step in the baited arm. During choice trails both arms were accessible, but the reward was available at the same arm as before. The animal was placed at the base of the T-shape and arm entries were counted. The inter-trial interval was 5 minutes. A working memory index was calculated as the number of correct choices out of the total number of trials.

One-way step-down inhibitory "passive" avoidance test

A two-compartment apparatus (Ugo Basile, Italy) was used. The training session lasted 2 days. Short-memory test was performed at day 3, but the long-term memory test was conducted at day 8. Both, learning and retention sessions consisted of 2 trials with the following parameters of the apparatus - electrical foot shock for 10 sec with intensity of 0.4 mA. Memory retention tests were performed without electrical stimulation. The latency of reaction was accepted as a criterion for learning and retention. The animals that remained on the platform 60 sec were considered as trained.

Novel object recognition test (NORT)

The test was carried out in two consecutive days. The protocol consists of three phases: habituation, training/recognition and test. During the habituation the animal explores the environment without the presence of any other objects. Training generally involves exploration of two identical objects whereas the test requires replacement of one of the familiar objects with a novel one. The time spent for exploration of the two objects was recorded and a recognition index (RI) was calculated:

$$\text{RI} = \frac{\text{N}}{\text{N} + \text{F}} \times 100$$

where N is the time spent on the new object and F is the time spent on the familiar object.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 19.0. All data are expressed as mean ± SEM (standard errors of the mean). Data were analyzed by one-way ANOVA, followed by Tukey's post hoc test for comparisons between the groups. A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Phytochemical analyses

In order to provide the required amount of extract for treatment of animals during the experiment (1 month), 12 batches of GBSE with average content of dry matter 6-8% were obtained. Quantitative LC-MS analysis showed presence of the flavonoids quercetin, kaempferol and isorhamnetin (as aglycones), the ginkgolides A, B, C, J and bilobalide. The mean levels of these bioactive constituents of the extracts are shown in **Table 1**.

Table 1. Levels of bioactive compounds in GBSE (dry matter base)

Compound	Amount (μg/g)
Quercetin	14.4±1.56
Kaempferol	20.2±1.83
Isorhamnetin	30.3±2.04
Ginkgolide A	222.0±17.6
Ginkgolide B + J	367.0±12.9
Ginkgolide C	147.0±10.3
Bilobalide	118.0±8.1

Data are presented as means ± standard deviation (n=12). Ginkgolides A and J are isomers and cannot be resolved under the chromatographic conditions used

Effect of GBSE on % SA in Y-maze test

Animals treated with PCM significantly increased % SA in the memory test compared to saline ($p<0.05$) **(Fig. 1)**. The groups with GBSE in doses of 50 and 100 mg/kg significantly increased the % SA during the memory test ($p<0.05$) whereas the highest dose of GBSE did not reach significance **(Fig. 1)**.

Effect of GBSE on working memory index in T-maze test

The rats treated with PCM (600 mg/kg b.w.) notably increased the working memory index in comparison to the control group ($p<0.05$) **(Fig. 2)**. The three experimental groups treated with GBSE also significantly increased the working memory index compared to saline ($p<0.05$) **(Fig. 2)**.

Effect of GBSE on step-down passive avoidance test

The group treated with PCM notably increased the latency during the two training days ($p<0.05$ and $p<0.01$, respectively) as well as the memory tests ($p<0.05$ and $p<0.01$, respectively) **(Fig. 3)** compared to saline. The animals treated with the lowest dose of GBSE (50 mg/kg b.w.) significantly prolonged the reaction time during the long-term memory test compared to the control group ($p<0.01$) **(Fig. 3)**. The rats treated with GBSE at a dose of 100 mg/kg b.w. notably increased the latent time during short- and long-term memory tests ($p<0.05$ and $p<0.01$, respectively) **(Fig. 3)**. The highest dose of GBSE did not reach significance during the retention tests in comparison to the saline.

Effect of GBSE on recognition index in NORT

PCM-treated animals significantly improved the recognition index when compared to the control group ($p<0.05$) **(Fig. 4)**. The analysis of the three experimental groups showed that only the animals with the middle dose of GBSE ameliorated the recognition index in comparison to saline ($p<0.05$) **(Fig. 4)**.

DISCUSSION

The results of the present study showed that GBSE contains the major bioactive compounds found in GBLE albeit in different proportions. We detected the presence of the flavonoids quercetin, kaempferol and isorhamnetin, and the terpene lactones ginkgolides A, B, C, J and bilobalide. GBSE improved memory in four different behavioral tests that depend on different brain structures. Additionally, its memory improving effect was similar or greater of that observed with piracetam – a standard drug for treatment of dementia.

Animals treated with a medium dose of GBSE showed significant improvement in short- and long-term memory in step-down passive avoidance task. This was evaluated by their ability to remember the foot shock that was received in the learning session and to recall the memory traces on day 3 (short-term memory) and day 8 (long-term memory). The magnitude of the effect was identical with that reached with PCM. Rats treated with the highest dose of the extract failed to improve memory functions. Behavioral responses in passive avoidance tasks are mediated by

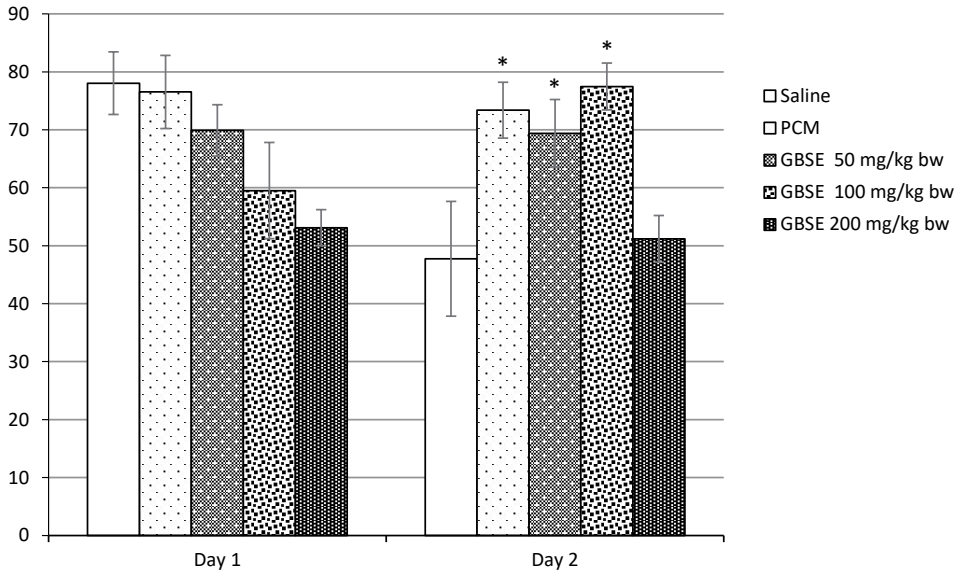

Figure 1. Effect of GBSE on % SA in Y-maze test; *p<0.05 compared to the control group treated with saline.

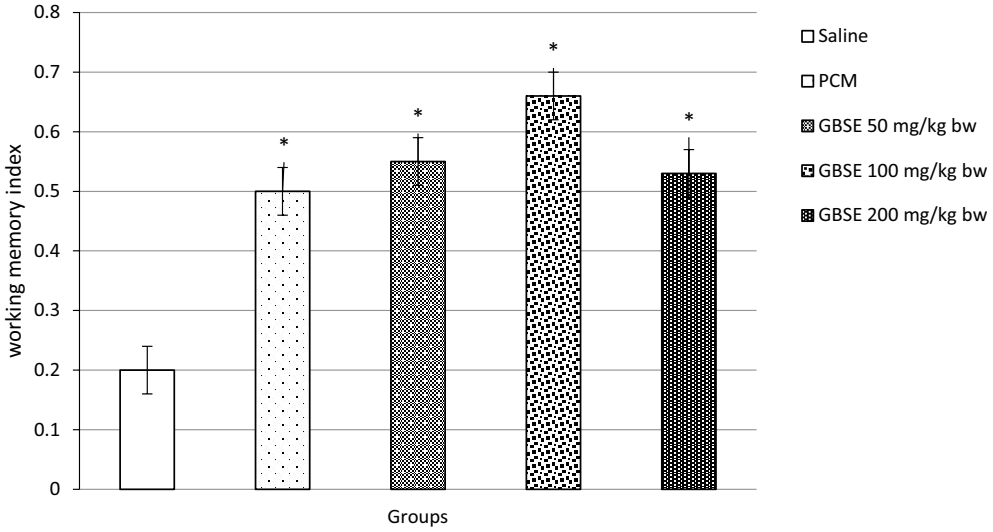

Figure 2. Effect of GBSE on working memory index in T-maze test; *p<0.05 compared to saline.

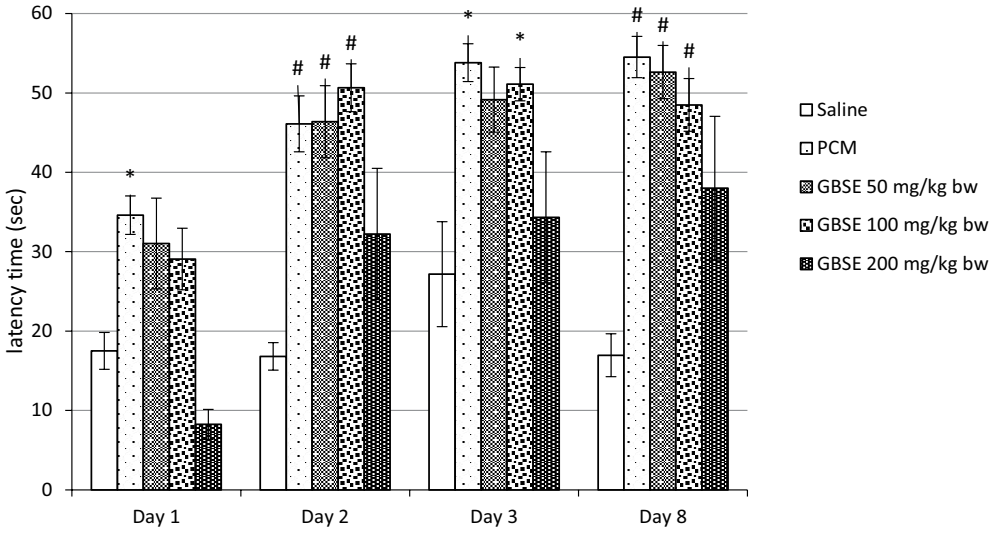

Figure 3. Effect of GBSE on step-down passive avoidance test; *p<0.05 compared to saline; # p<0.01 compared to saline.

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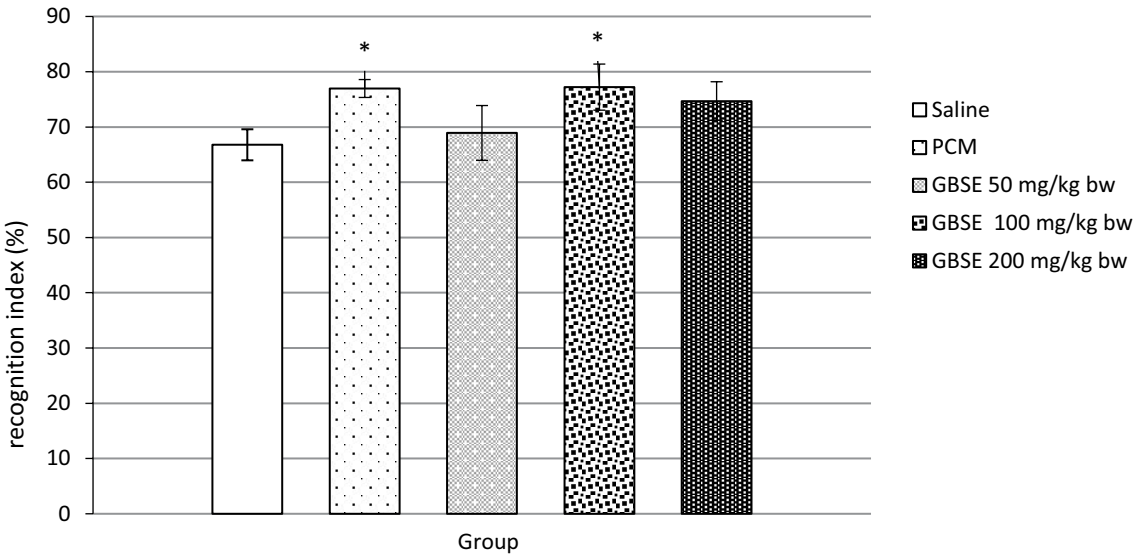

Figure 4. Effect of GBSE on recognition index in NORT; *p<0.05 compared to saline.

different brain structures but the hippocampus plays a central role. Its morphological and functional integrity is important for memory retention in passive avoidance tests.[9] In the Y-maze task the memory enhancing effect of the investigated extract was assessed by calculating the % SA. An increase in this index shows improvement in spatial working memory. It is a well-established behavioral assay for testing hippocampal integrity and hippocampal dependent memory.[10] In our study, the lowest and the middle dose of GBSE significantly increased the % SA compared with the negative control. The observed effect did not differ significantly from that of PCM. The highest dose of the extract again failed to enhance memory. Based on the results from step-down passive avoidance and Y-maze tests we may speculate that the hippocampus plays an important role in the observed memory enhancing effect of GBSE.

Medial prefrontal cortex (mPFC) neurons are important for encoding spatial working memory in the T-maze task.[11] It is based on the natural exploratory behavior of rats and their willingness to visit a new arm of the maze rather than the one they visited before. To study spatial memory in T-maze, spontaneous or rewarded alterations are used.[12] In our research, we used the second option in which rats were rewarded with food pellets if they chose the correct arm of the maze. In the T-maze, the memory enhancing effect of GBSE was registered in all experimental groups indicating the significant role of the mPFC since its neuronal activity encodes the choice and outcome in this task.[13] Obviously, this effect is not strongly dependent on the hippocampal integrity and function but also involves cortical structures.

The novel ORT is widely used to investigate the effect of different substances on memory functions. It is based on rats' natural preference to the novelty when exploring their environment. It does not require long habituation, reward, and electric stimulation and is not sensitive to changes in the motor functions of the animals. Another advantage is that it consists only of one learning trial.[14] The improvement in memory is assessed by increasing the recognition index which shows that the rat spends more time exploring the novel object than the familiar one. This indicates that in the rat memory there is information about the familiar object. The results from the novel ORT depend on the hippocampus and the perirhinal cortex. The latter is necessary for obtaining basic information about familiarity or novelty of an object whereas the hippocampus is responsible for object memorization. Its neurons encode and keep object's information during longer retention intervals.[15] In our study the duration of this interval was 24 hours enabling us to distinguish real hippocampal dependent memory improvement from false positive results due to very short retention interval. Rats with hippocampal lesions do not show memory impairment in ORT after a very short delay.[16] The obtained results demonstrate that GBSE at a dose of 100 mg/kg b.w. has a memory enhancing effect in the novel ORT and this effect was comparable with that elicited by the standard drug PCM. The other two doses did not reach statistical significance. These results confirmed the role of the hippocampus in the observed memory enhancing effect of GBSE.

The determined phytochemical composition provides probable explanation of this effect. Gingkolides and bilobalide have numerous beneficial effects in the central nervous system (CNS). Studies showed that they have neuroprotective effect in different settings such as cerebral ischemia, seizures and peripheral nerve injury. Their neuroprotective properties are achieved through different mechanisms – antioxidant activity, anti-inflammatory effect, inhibition of apoptosis, decreased permeability of the blood-brain barrier, improved neuronal metabolism, etc.[17] The results from *in vivo* studies with bilobalide showed that it enhances memory functions in experimental

animals. Li et al. demonstrated that in a rat model of vascular dementia, bilobalide improves learning and memory abilities in the Morris water maze test. This effect was achieved due to its ability to inhibit apoptosis in brain cortex and the hippocampal CA1 region by decreasing the oxidative stress and decreasing the expression of the pro-inflammatory cytokine TNF-α.[18] Bilobalide was effective in preventing memory impairment in a rat model of Alzheimer's disease with an intra-hippocampal injection of Aβ25-35 (beta-amyloid) by decreasing apoptosis, oxidation, Aβ1-40 and TNF-alfa expression in the frontal cortex and CA1 region of the hippocampus.[19] This could explain the memory improving effect of GBSE in both hippocampal and frontal cortex-dependent memory tasks. Other possible mechanism by which bilobalide exerts its neuroprotective effect is its ability to antagonize the effect of γ-aminobutyric acid (GABA), which is well-known inhibitory neurotransmitter in the CNS.[20] Bilobalide also causes upregulation of hippocampal glucocorticoid receptors expression which may also be responsible for its memory enhancing effect.[21] Chen A et al. showed that ginkgolide B, another terpene trilactone found in *Ginkgo biloba* extracts, was able to suppress microglial activation in brain cortex which is responsible for the production of proinflammatory cytokines and neuronal death.[22] Based on our results and aforementioned data about neuroprotective effects of bilobalide and ginkgolides, which are present in significant quantities in GBSE, we may speculate that these trilactones are at least partially responsible for the memory enhancing effect of the investigated extract.

Flavonoids found in GBSE also exhibit various pharmacological effects including anti-inflammatory, antioxidant and memory improving properties.[23] Flavonoid content of the tested GBSE could also provide possible explanation of the observed memory enhancing and improving effect. Quercetin belongs to the flavonol group and possesses anti-inflammatory and antioxidant properties. After oral administration, it crosses the blood-brain barrier and accumulates in different brain regions including the hippocampus. Its neuroprotective effect is due to its ability to reduce oxidative stress and inhibit production of nitric oxide and pro-inflammatory cytokines from activated microglia.[24] Experimental data showed that quercetin protects the hippocampus from injury and improves memory in hippocampal-dependent behavioral tasks. In a rat model of Alzheimer's disease, Vargas-Restrepo F et al. demonstrated that treatment with this flavonoid has an anti-inflammatory effect in the CA1 region of the hippocampus and reduces aggregation of β-amyloid plaques.[25] Quercetin improves long-term potentiation in the CA1 (cornu ammonium 1) hippocampal region of rats with bilateral occlusion of carotid arteries.[26] Mehta V et al. found that quercetin improves impaired memory in step-down passive avoidance task. The results of their study also confirmed that this flavonoid could improve hippocampal neurogenesis by modulating insulin signalling pathway in this brain structure.[27]

Isorhamnetin, a 3-O-methylated metabolite of quercetin, possesses a wide variety of biological effects, including neuroprotective, anti-inflammatory, antioxidant, antiapoptotic, etc.[28] Ishola I et al. demonstrated that isorhamnetin improves recognition and spatial memory in mice with scopolamine-induced amnesia. Moreover, they showed that this flavonoid enhanced antioxidant defense, reduced acetylcholinesterase activity and increased brain-derived neurotrophic factor (BDNF) levels in the hippocampus and the prefrontal cortex in mice with this model of memory impairment.[29]

Kaempferol is another flavonoid found in GBSE in significant quantities. Like other flavonoids, it has a broad spectrum of pharmacological activities – anticancer, anti-inflammatory, antioxidant, antimicrobial, antidiabetic, neuroprotective, etc. The latter is associated with its ability to inhibit apoptosis, metalloproteinases, nitrotyrosines, and β-amyloid accumulation.[30] Anti-inflammatory and antioxidant properties also contribute to keampferol's neuroprotective effect. Hussein RM et al. showed that kaempferol increases the activity of antioxidant enzymes in brain, inhibits the activity of the glycogen synthase kinase 3β-erythroid 2-related factor 2 (GSK3β-Nrf2) pathway which is involved in oxidative stress and prevents cholinesterase inhibition in rats with chlorpyrifos-induced toxicity. This was associated with neuroprotective effect in the hippocampus and improvement of memory functions in Y-maze and ORT.[31] Other explanation of the kaempferol neuroprotective properties include inhibition of Akt/mTOR signalling pathway in the hippocampus.[32] The memory improving effect of kaempferol was demonstrated *in vivo* in different models of memory impairment using different behavioral tasks. Kouhestani S et al. showed that in the Morris water maze this flavonoid alleviates streptozotocin-induced memory impairment in ovariectomized rats.[33] Zarei M et al. found that kaempferol improves memory in passive avoidance task in animals with scopolamine-induce amnesia.[34] Since in our study GBSE demonstrated memory improving properties in the same behavioral task we may conclude that this effect is at least partially due to the kaempferol content of the extract.

All of the above mentioned studies have used individual constituents of GBSE in doses ranging up to 10 mg/kg bilobalide[18,19,21], 5-30 mg/kg quercetin[25-27], 10-50 mg/kg isorhamnetin[28,29], and 10-50 mg/kg kaempferol.[31-34] At the same time, in our experiments even in the highest dose of GBSE used (200 mg/kg) the level of flavonoids was around 5 orders of magnitude lower and those of ginkgolides and bilodalides were 3 orders of magnitude lower. The data obtained showed that memory and learning enhancing effects of GBSE were even more pronounced in the lower doses (50 and 100 mg/b.w.). These results argue that the constituents of GBSE may demonstrate additive and/or synergic effects on brain functions.

Another possible explanation of the demonstrated activity of GBSE in such low concentrations of the known bioactive constituents may be sought in the fact that the bulk

amounts of flavonoids in plants are glycosylated. Most likely, the hydrophilic carbohydrate moiety affects bioavailability of flavonoids but comparative studies are scarce[35] and this assumption needs more detailed research.

CONCLUSIONS

Phytochemical analyses revealed that the investigated GBSE, similarly to GBLE, contains two bioactive groups of compounds: flavonoids (quercetin, kaempferol and isorhamnetin) and terpenoids (ginkgolides A, B, C, J and bilobalide). GBSE enhances spatial working memory, recognition memory, and short- and long-term recall in naïve rats due to neuroprotective properties of the detected substances and may be as prospective as GBLE in phytopharmacy. The brain structures involved in the observed effect are probably the hippocampus and mPFC.

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Экспериментальное исследование фитохимического состава и эффекта улучшения памяти экстракта семян гинкго билоба

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Резюме

Введение: Дерево гинкго билоба считается одним из древнейших видов на Земле. Оно известно как «живое ископаемое», возраст которого составляет 200 миллионов лет. И листья, и семена этого дерева тысячелетиями использовались в традиционной китайской медицине.

Цель: Изучить фитохимический профиль экстракта семян гинкго билоба (ЭСГБ) и его влияние на улучшение памяти.

Материалы и методы: Для анализа экстрактов проводили жидкостную хроматографию с масс-спектрометрией (ЖХ-МС). Для экспериментов in vivo самцов крыс линии Wistar случайным образом разделили на 5 групп (n=8): физиологический раствор, пирацетам, ЭСГБ 50, 100 и 200 mg/kg массы тела. Были выполнены тесты Т-образный лабиринт, Y-образный лабиринт (Y-maze, T-maze), тест на пассивное избегание (step-down passive avoidance test) и тест распознавания нового объекта (novel object recognition test (NORT). Наблюдаемые параметры включали: скорость спонтанных изменений (% SA), индекс рабочей памяти, индекс познания и индекс задержки реакции. Статистический анализ был выполнен с использованием SPSS 19.

Результаты: ЖХ-МС анализ показал присутствие флавоноидов кверцетина, кемпферола, изорамнетина (в виде агликонов), гинкголидов A, B, C, J и билобалида. В задаче Y-лабиринта группы, получавшие 50 и 100 mg/kg ЭСГБ, значительно увеличили % SA во время теста памяти по сравнению с группой, получавшей физиологический раствор (p<0.05). В Т-образном лабиринте три экспериментальные группы, получавшие ЭСГБ, значительно улучшили индекс рабочей памяти по сравнению с контрольной группой (p<0.05). При тесте на пассивное избегание животные, получавшие 100 mg/kg массы тела ЭСГБ, значительно увеличили задержку ответа во время обоих тестов удержания (p<0.05 и p<0.01, соответственно). При NORT только животные со средней дозой ЭСГБ ухудшили когнитивный индекс по сравнению с группой, получавшей физиологический раствор (p<0.05).

Заключение: ЭСГБ улучшил пространственную рабочую память, когнитивную память, а также краткосрочную и долгосрочную память у исследованных крыс за счёт синергетического воздействия установленных флавоноидов и терпеновых лактонов на функцию мозга. Поражёнными структурами головного мозга, вероятно, являются гиппокамп и префронтальная кора.

Ключевые слова

флавоноиды, память, фитопрепараты, терпеновые лактоны