Middle-East Journal of Scientific Research 16 (6): 751-755, 2013
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2013.16.06.7460

The Action of *Ginkgo biloba* on Passive Avoidance Learning in Experimental Rats

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Abstract: *Ginkgo biloba* is a competitive GABA receptor antagonist which has an important role in memory modulation. This study investigates the effect of *G. biloba* on passive avoidance learning in rats. Forty male Wistar rats were divided into five equal groups. The control group received no medication. Sham group was fed with sorbitol as solvent of t he herbal medicine. The third, fourth and fifth groups received 40, 65 and 90 mg/kg of *G. biloba* for a week. They were placed in the shuttle box to determine the passive avoidance learning including three steps of habits, training and memory retention. After experiments, the animals were bled and the cortisole level was determined. The dose of 65 mg/kg of *G. biloba* was the most effective dose which significantly increased the STL suggesting that *G. biloba* could improve the induced memory impairment. *G. biloba* may be clinically beneficial in treatment of cognitive impairments. The findings support its use as a food supplement or an adjunct treatment for memory impairment.

Key words: Passive Avoidance Learning · Ginkgo Biloba · Rat

INTRODUCTION

The hypothalamus-pituitary-adrenal axis (HPA) has a direct impact on the memory and learning and cortisol plays an important role in this axix [1]. Cortisol through a negative feedback directly affects hypothalamus and posterior pituitary and reduces the cortisol level [1]. Some compounds were shown to affect this axis and be effective in treatment of neurotic disorders [2].

Herbal-derived pharmacologic agents were shown to be basically candidates in treatment of many diseases [3-6]. The reports denoted to use of *Ginkgo biloba* leaf extract for centuries in traditional Chinese medicine and nowadays as an herbal supplement is used to improve the neural function and for its antioxidant and anticancer properties. Herbal supplements were shown to have the potential to be consumed over extended periods of time but there is lack of sufficient data on long-term complication risks [7]. *G. biloba* was demonstrated to be effective in treatment of several diseases and disorders. Cognitive decline was seen in a non-demented elderly population to decrease in subjects who reported using *G. biloba* than in those who did not [8]. Oliveira *et al.* [9] found that *G. biloba* might be effective for enhancement of memory by affecting the dorsal hippocampus and amygdaloid complex An improvement in learning and memory ability of diabetic rats was seen after administration of *G. biloba*. The mechanism may be due to reduction in apoptosis in hippocampus neurons and down regulation of inflammatory mediators such as astrocytes and microglia [10]. Tian *et al.* [11] findings revealed the preventive effects of *G. biloba* extract on Cisplatin (cis-diammine-dichloroplatinum; CDDP)-induced hearing loss in rats and enhancement of antiatherogenic effects of cisplastin by inhibiting the generation of reactive oxygen species.

G. biloba can decrease the brain incompetence and amplify the memory by increasing the blood supply to brain [12]. *G. biloba* as a competitive antagonist of GABA receptor plays an important role in memory modulation [13]. The main constituents of *G. biloba* are flavonoids, triglycerides, esters coumaric acid and quercetin flavonols [14]. *G. biloba* has small amounts of glycosides isorhamnetin, myricetin and 3-methyl myricetin. Non-glycoside flavonoids, catechin and proanthocyanidins are also other constituents of *G.*

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biloba [15]. Typical compounds of diterpene lactone including ginkgolides A, B, C, J and M and bilobalide Sezkoi terpene lactones are also present in this herbal [16].

Some findings suggest the antioxidant potential of *G. biloba* extract [17]. Cheng and Liang [18] showed that *G. biloba* has antihyperglycemic, antioxidant and antihyperlipidemia activities in STZ-induced chronic diabetic rats. Its use in t reatment of peripheral vascular disease and cerebral insufficiency was successful. It increased the blood flow, platelet activating factor antagonism and prevention of membrane damage caused by free radicals which explain its mechanism of action [19]. The findings suggest that *G. biloba* can be useful for endothelial protection in the presence of high glucose such as that in diabetes mellitus (20). Hong *et al.* [21] demonstrated that *G. Biloba* extract had sufficient anti-platelet abilities without adverse events.

Tao *et al.* [22] reports showed that *G. biloba* had antibacterial activity against *Salmonella enterica*. *G. biloba* was demonstrated to have chemopreventive effects in ER-independent breast cancer through anti-proliferation and apoptosis-inducing activities [23]. Therefore, this study was undertaken to investigate the effect of *G. biloba* on passive avoidance learning in male Wistar rats.

MATERIALS AND METHODS

Forty male Sprague Dawley rats weighting 200-250 g were enrolled. The animals were provided from Shiraz University of Medical Sciences Laboratory Animal Center. Animal selection, experiments, animal care and sacrifice protocol were all similar and were adhered to the guidelines of Iran Veterinary Organization and were under the supervision of the animal care of Iran Veterinary Organization. The study received approval from the ethics committee of the institution. The animals were individually housed in a single cage under a 12 hours light and 12 hours darkness, temperature of 22°C and humidity of 30%. They were fed with a balanced diet and had free access to water. All experiments were performed in an aseptic condition.

The animals were divided into five equal groups. The control group received no medication. Sham group was fed with sorbitol as solvent of the medication (*G. biloba*). The third, fourth and fifth groups received different doses of *G. biloba* including 40, 65 and 90 mg/kg for a week using a gavage. Animals were placed in the shuttle box to determine the passive avoidance learning consisted of three steps of habits, training and memory retention. After experiments, the animals were bled and the cortisol level was determined using the Fars-Poyeshteb Company kit.

The shuttle box was composed of two parts of training box and the control compartment. The dimension of training box was 62×23×23 cm and was made of clear plexy glass sheets. It had two equal small compartments which were separated by transverse walls. All external surfaces were in black color. For the animals to pass through the transverse wall opening and closing by the guillotine door, there was a rectangular valve with a dimension of 9×7 cm. The floor was equipped with metal rods of diameter 5.2 and distance of 1 cm from each other. The controller compartment was equipped with a set of screws to establish the duration of the shock in terms of frequency, voltage and ampere.

Passive avoidance learning involved three steps of habituation, training and memory retention. In habit session, the animals were put in the bright compartment of machine and the guillotine was opened after five seconds. After arrival of the animals in the dark compartment, the guillotine was closed for 30 seconds and then was opened to facilitate entrance of animals into the bright compartment. If they did not enter, they were directed by hand. This step was repeated each 30 minutes. If the rats did not go into the bright compartment after 100 seconds in each session, the animal was excluded from the experiment.

The training session was undertaken 30 minutes after the habit session. To establish an electric shock, the animal's feet were dipped into saline solution. The guillotine was opened after 30 seconds to facilitate the entrance of the rats and was slowly closed as soon as the animals arrived the dark compartment. A shock of 1 mA and 100 Hz was applied for 23 seconds to their feet until the animal left the dark compartment and then they were transferred into their cage. The animal was placed in the bright compartment after a few minutes and the guillotine was opened again after 30 seconds. If the animal did not go into the dark compartment after 2 minutes, the training session was terminated or the shock process was repeated.

In memory retention session, 24 hours after the training session, the animals were placed in the bright compartment of the machine and the guillotine was slowly opened after 30 seconds. The time period until the animals could completely enter the dark compartment was recorded as step-through latency (STL). Maximum time to enter the dark compartment was considered to be 600 seconds. The mean and standard error (SE) were

determined for each group. ANOVA, Tukey and Scheffe tests were used to compare the groups, The significance level for all statistical tests was set at $p<0.05$.

RESULTS

Comparing the control and the sham group, there was no significant difference between the two groups. Comparing the 3rd, 4th and 5th groups receiving different doses of *G. biloba* (40, 65 and 90 mg/kg) and the control group regarding the delay time to enter the dark compartment, no significant difference was noticed before training sessions whereas a significant difference was visible between the fifth group receiving the dose of 65 mg/kg of the herbal in comparison to the control group (Figures 1 and 2) resulted into production of the highest levels of cortisol regarding the delay time of the animal to enter the dark compartment of shuttle box.

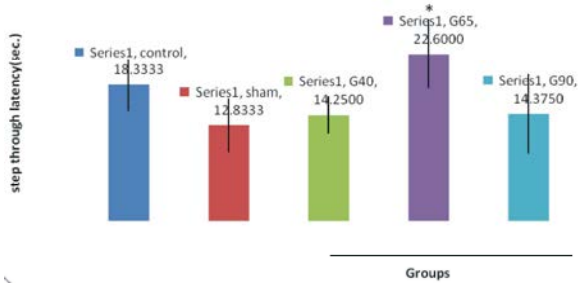

Fig. 1: Comparing of control, sham and experimental groups receiving 40, 65 and 90 mg/kg of *G. biloba* extract over STL (step-through latency) time. Every column shows the mean±SE. The group receiving dose of 65 mg/kg is significantly different from the control and other groups (*P<0.05).

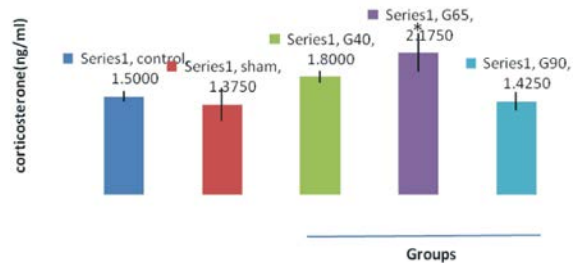

Fig. 2: Comparing the control, sham and experimental groups receiving 40, 65 and 90 mg/kg of *G. biloba* extract regarding corticosterone plasma level. Every column shows the mean±SE. The group receiving dose of 65 mg/kg is significantly different from the control and other groups (*P<0.05).

DISCUSSION

The use of herbal medicines in treatment of diseases such as amnesia and also to improve the memory is still common in traditional medicine even their adverse effects and mode of actions are not completely clarified. *G. biloba* extract was introduced for enhancement of the memory and preventing age-related amnesia due to Alzheimer's disease [12].

In our study, the most effective dose of *G. biloba* was 65 mg/kg which resulted into production of the highest levels of cortisol regarding the delay time of the animal to enter the dark compartment of shuttle box. Indeed, the animal had learned the habit and training sessions that if they entered the dark compartment, their feet would receive shock. Therefore, they did not go into the dark compartment in the memory retention session and took more time to enter the dark compartment. The reason why the dose 65 mg/kg was recognized as the effective dose may be because of *G. biloba* property for vasodilatation and an effective blood supply to the brain and promotion of the memory.

Huang *et al*. [24] showed that *G. biloba* can be a competitive antagonist of GABA receptors Amri *et al.* [25] showed that *G. biloba* inhibited the ligand binding to central and peripheral benzodiazepine receptors in adult male mice. The influence of *G. biloba* on reinforcement of memory was previously shown [26]. Stackman *et al.* [27] showed that in animals suffering from Alzheimer's disease and treated with *G. biloba,* their memory improved.

G. biloba was demonstrated to have antioxidant properties as it improve the function of platelets, nervous system and blood supply to nervous system and brain. It can also reduce blood viscosity and cause dilatation of the blood vessels [12]. Indeed *G. biloba* extract could restore large parts of damages due to old age, especially damages in the circulatory and nervous systems. The extract could also affect blood vessels leading to vasodilation [12].

Our findings suggest that *G. biloba* could improve the induced memory impairment and may be clinically beneficial in treatment of cognitive impairments. These findings may support its use as a food supplement or an adjunct treatment for memory impairment.

ACKNOWLEDGEMENT

The authors would like to thank Islamic Azad University, Arsanjan Branch for financial support. Also we appreciate the helps of laboratory animal center of the university.

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