EVALUATION EFFECTS OF "AN TRI KHANG" HARD CAPSULE ON SCOPOLAMINE-INDUCED MEMORY IMPAIRMENT IN EXPERIMENTAL ANIMALS

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This study was performed to investigate the effects of An Tri Khang (ATK) hard capsule on scopolamine-induced memory impairment in experimental animals. The effects on memory of oral administration ATK were evaluated on scopolamine-induced memory impairment in experimental animals. The memory improvement effects were conducted on 2 experimental models: the Morris water maze (MWM) and the Multi-T maze (MTM). In the MWM model, ATK significantly reduced the time spent and pathlength to platform, increased the percentage of time swimming in platform's quadrant. Swiss mice performed the MTM with shorter in both the time spent and the pathlength to the goal box ($p < 0.01$). Both doses of ATK attenuated scopolamine-mediated impairment of memory. ATK has potential effects on memory improvement. It is suggested that further clinical trials should be undertaken to have thorough assessment on human.

Keywords: An Tri Khang, Morris water maze, Multi T maze, scopolamine, experimental animals.

I. INTRODUCTION

Dementia is a syndrome – usually of a chronic or progressive nature – in which there is deterioration in cognitive function beyond what might be expected from normal ageing. It affects memory, thinking, orientation, comprehension, calculation, learning capacity, language, and judgement. Dementia is one of the major causes of disability and dependency among older people worldwide.[1] This condition has a tremendous burden on patients, healthcare system and society. There is no cure for dementia. Therefore, the development of novel treatment which helps to prevent progress of the disease and enhance patient's life quality.

Alzheimer's disease (AD) is the most common type of dementia, accounting for at least two-thirds of cases of dementia in people age 65 and older.[2] One pharmacological strategy for AD is the use of acetyl cholinesterase inhibitors (AChEIs), which inhibit the degradation of acetylcholine (ACh), and therefore increase its concentration in neuronal synapses to compensate for the loss of cholinergic neurons, which is characteristic of AD.[3] Despite the reported benefits of AChEIs for AD, their use is limited since they have severe side-effects.[4]

For many Vietnamese generations, products from herbs have been used as complementary medicine because of supply availability and reasonable cost for most people. There has been a great increased interest in treating diseases with herbal medicine, so our study is conducted to determine the safety and efficacy of An Tri Khang (ATK) hard capsule in dementia treatment.

An Tri Khang (ATK) hard capsule is the combination of herbal ingredients: *Valeriana officinalis, Gingko biloba, Plumula Nelumbinis,*

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Cratoxylum pruniflorum and *Nattokinase*. Some of the components are reported to have bioactivities in the prevention and treatment of dementia in experimental animals.[5–8] Therefore, we conducted the study: Evaluation effects of An Tri Khang hard capsule on scopolamine-induced memory impairment in experimental animals to investigate the effect of ATK capsule on memory improvement in experimental animals.

II. METHODS

1. Materials

Each capsule *An Tri Khang* (*ATK)* contains: 175 mg *Valeriana officinalis*; 120 mg *Ginkgo biloba extract*; 100 mg *Plumula Nelumbinis*, 40 mg *Magnesium Oxide*, 30 mg *Cratoxylum formosum extract*, 300FU *Nattokinase (*equivalent 15 mg *Nattokinase)*, 5 mg *Sodium Citicolin*, 2 mg, *Vitamin B6*. ATK product belongs to An Chau Pharmaceutical Joint Stock Company. This product is manufactured by Phuong Dong Pharmaceutical and Trading Company. This capsule is dissolved in distilled water before oral administration by gastric tube.

2. Chemicals and Equipment

Scopolamine hydrobromide (Sigma Aldrich, USA).

Donepezil hydrochloride (Alpezil, Egis Pharmaceuticals Private Limited Company).

Saline 0.9% 500ml (B.Braun, Vietnam).

Morris water maze: a circular pool (150cm diameter, 60cm height) filled with water (20 ± 2°C). Small black pieces of plastic were placed on the surface of the water to obscure the platform (10cm diameter, 25cm height).

Multi T maze: The MTM is constructed of wood and consists of a wooden platform with seven choice points; the dimensions are 150cm x 130cm x 15cm and a path width of 8cm.

EZVIZ camera.

ANYMAZE tracking software (US Biotech, USA).

3. Experimental animals

The Morris water maze and the Multi-T maze experiments: Swiss mice (18 - 22g) of either sex were purchased from the National Institute of Hygiene and Epidemiology (NIHE). Mice were reared in a laboratory of the Department of Pharmacology, Hanoi Medical University for 5 - 7 days before the experiments with a standard food for mice (provided by the NIHE), water available *ad libitum*.

4. Methods

Assess the memory improvement effect of ATK on 2 experimental models: Morris water maze and Muti-T maze.[9,10] *Swiss* mice were randomly divided into 5 groups each group ten animals. (as the table below)

Table 1. Doses and administration of Swiss mice groups

Group	Oral administration	Intraperitoneal Injection
1	Distilled water 0.2ml/10g	0.9% saline 0.1ml/10g
2	Distilled water 0.2ml/10g	Scopolamine 0.1ml/10g
3	Donepezil 2.4mg/kg, 0.2ml/10g	
4	ATK 0.47g/kg, 0.2ml/10g	
5	ATK 1.41g/kg/day (three times dose as group 4), 0.2ml/10g	

The Morris water maze

This study followed the modified Morris water maze according to Lee et al.[10]

- *Learning phase: Familiarization session*: At the first day, the platform was 1cm above the water surface. A mouse was trained twice per day, 20 min apart. A trial was started when the rat was released from one of three randomly chosen start positions. After the mouse found and climbed onto the platform, the trial was stopped and the escape latency was recorded. The maximum trial length was last for 2 mins. If the mouse had not climbed onto the platform in 2 min, the researcher guided the mouse by hand to the platform. The trial ended as soon as the animal climbed on the platform and remained on it for ≥ 15sec. *Acquisition sessions*: from 2nd to 5th day, the platform was 1 cm below the water surface, and the protocol was the same as the first day.

- *Probe trial:* 24h after the last acquisition session. During this trial, the platform was removed from the maze, and the mouse was placed into the water at the opposite quadrant to the escape one. Each mouse was allowed to search the pool for 60s before being removed. *Evaluation indexes:* time spent in escape platform's quadrant *(s)* and the pathlength to reach the platform (*m).*

The Multi-T maze

The experiment is followed the protocol of Falsafi et al.[9] The mice were trained in this maze for 8 consecutive days. Prior to testing, mice were deprived of food for 16h to motivate food searching. Mice were placed in a start box in a black cylindrical start chamber. Each trial started with them leaving the start box and was completed when mice had reached the goal box or, if failed, after 8 min. Upon arriving in the goal box, mice were allowed to consume a small piece of a food pellet as provided reward and transferred to their home cage. Immediately after each trial, the entire maze was cleaned with 70% alcohol solution.

*Learning phase: t*he training is conducted from the 1st to the 5th day. *Probe trial:* at the 8th day (after 2-day-rest), the mouse is placed into the MTM to find the way to the goal box. *Evaluation indexes:* time *(s)* and the pathlength *(m)* to reach the goal box.

5. Statistical Analysis

Data were analyzed using Microsoft Excel software version 2010. The data were expressed as the mean ± standard deviation (SD) and statistical analysis was carried out employing student's T-test. The p-value < 0.05 was considered to be statistically significant.

III. RESULTS

1. Effects of ATK on memory improvement in the Morris water maze experimental model.

In the MWM task, the time spent in the escape platform's quadrant was significantly decreased in scopolamine-treated mice compared with that of the control group ($p < 0.001$), and treatment with ATK (0.96 and 1.41g/kg) was found to markedly reverse this effect ($p < 0.01$). ***(Table 2)***

Table 2. Effect of ATK on the percentage of time in platform's quadrant

Group	% time
Control	26.52 ± 8.86***
Scopolamine-treated	12.80 ± 3.18
Donepezil 2.4 mg/kg	21.65 ± 6.15***
ATK 0.47 g/kg	19.50 ± 3.30***
ATK 1.41 g/kg	18.97 ± 3.53***

****p < 0.001 vs scopolamine-treated group*

In groups treated with donepezil and ATK 1.41 g/kg, the percentage of the pathlength in platform's quadrant increased compared with that of scopolamine-treated group, and it was not statistical significance. ($p > 0.05$) ***(Table 3)***

Table 3. Effect of ATK on the percentage of time in platform's quadrant

Group (n=10)	% pathlength
Control	32.79 ± 10.88***
Scopolamine-treated	17.90 ± 4.92
Donepezil 2.4 mg/kg	23.12 ± 4.68*
ATK 0.47 g /kg	20.88 ± 6.77
ATK 1.41 g/kg	22.05 ± 3.75*

****p < 0.001 vs scopolamine-treated group*

Effects of ATK on memory improvement in the Multi -T maze experimental model.

In the MTM, scopolamine significantly ($p < 0.01$) increased the time and the pathlength compared with those of the control. However, *Swiss* mice group with donepezil has a greatly decrease in both evaluation index compared with the control group ($p < 0.01$ and $p < 0.001$). The administration of 0.47 and 1.41 g/kg ATK markedly reversed scopolamine-induced memory deficits ($p < 0.01$). No statistical significance of different treatment at two ATK doses. (***Table 4)***

IV. DISCUSSION

Nowadays, it has been highly popular to use ethnomedicine materials in the treatment and symptom improvement of dementia, particularly AD.[11] Many herbs and other plants have been increasingly recognized as effective natural materials for the treatment of diseases and the alleviation of symptoms.[7,10–12] In this study, we investigate the efficacy of ATK in terms of memory improvement in experimental animals.

To assess whether ATK could improve learning and memory capacity or not, the induction of memory deficit is the first task. Scopolamine, an unspecific muscarinic receptor antagonist, has been used as the "gold standard" for memory impairment in both animal and human studies of working memory, was selected in this study.[10] Donepezil hydrochloride is a piperidine derivative and a centrally acting, rapid, reversible inhibitor of acetylcholinesterase. Acetylcholinesterase is an enzyme that degrades acetylcholine after release from the pre-synapse. Donepezil binds reversibly to acetylcholinesterase and inhibits the hydrolysis of acetylcholine, thus increasing the availability of acetylcholine at the synapses, enhancing cholinergic transmission.[13] Therefore, donepezil is used as a positive antagonist control of scopolamine mechanism.

Table 4. Effect of ATK on the time spent and the pathlength to the goal box

Group (n = 10)	Time (s)	Pathlength (m)
Control	144.84 ± 29.15**	19.22 ± 3.36**
Scopolamine-treated	303.04 ± 66.79	29.08 ± 5.80
Donepezil 2.4 mg/kg	202.30 ± 36.60***	20.04 ± 6.8**
ATK 0.47 g/kg	210.98 ± 60.75**	19.91 ± 6.78**
ATK 1.41 g/kg	178.82 ± 41.65**	17.41 ± 2.73***

***p < 0.01; ***p < 0.001 vs scopolamine-treated group*

Two memory and learning capacity evaluation models are selected: MWM and MTM. For the MWM, we assess spatial reference memory which is dependent on stress stimulation and long-term potentiation (LTP) in hippocampus. But primarily, it is essential for the rodent to develop the essential behavioral strategies needed to cope with this stressful, aversive situation, e.g., learning to swim and recognizing that the platform is the only means of escape. These behavioral strategies require the animal to have spatial information about the surrounding cues and the location of the escape platform. The second component is the spatial learning component, meaning that the animal must learn the position of the platform and create swimming strategies to move from one of the randomly chosen starting points toward the platform. The swimming efficiency during the probe trial is the best parameter with which to measure real spatial acuity.[14] For the MTM, the strategy is similar to the MWM. The mice are expected to remember the right path to the goal box (only one right way in entire 7 T maze).

The effects of ATK on memory capacity is primarily due to the recognized effectiveness of main ingredients such as *Valeriana officinalis* and *Gingko biloba.* Many studies have demonstrated that the extracts of *Valeriana officinalis* and its compounds (at least 4 rare tepernoids) have had positive effects on nourishing nerve cells and reducing AchE activity.[7,8,15] Moreover, the extracts of *Ginkgo biloba* are proved to having neuroprotective and antioxidant effects against AD and other neurodegenerative disorders, in which AchE-related mechanisms have been shown.[5,16] In addition, other ingredients in ATK are good choices in dementia supplemental products. Particularly, *Plumula Nelumbinis* and *Cratoxylum formosum extract* play vital roles in cerebral flow increase, radical scavenging activities and brain function.[17,18] Exogenous administration of COP-choline provides both choline and cytidine which access the brain and serve as substrates for the synthesis of phosphatidylcholine, a primary neuronal membrane component; the choline also enhances brain acetylcholine synthesid.[19]

V. CONCLUSION

Based on the results of the study, in memory deficit-induced models, An Tri Khang at two doses (0.47g/kg and 1.41g/kg) on *Swiss* mice in the Morris Water maze: ATK reduced the time spent and pathlength to platform increased the percentage of time swimming in platform's quadrant. In the Multi-T maze, ATK decreased the time and pathlength to the goal box. The effects of ATK at the two doses are similar in both treated groups.

In conclusion, these data suggest that An Tri Khang poses great potential effects on memory improvement. So, further clinical trials should be undertaken to assess the efficacy on human.

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