

# *ANOECTOCHILUS FORMOSANUS* ATTENUATES AMNESIA INDUCED BY SCOPOLAMINE IN RATS

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The effect of the water extract of *Anoectochilus formosanus* (AFW) on SCOP-induced amnesia was investigated in a step-through passive avoidance task in rats. It was observed that AFW at the doses of 1.0 and 2.0 g/kg attenuated SCOP-induced amnesia. The anti-amnesic effect of AFW on the SCOP-induced amnesia was significantly reversed by a peripheral cholinergic muscarinic receptor antagonist, SCOP N-methylbromide and augmented by neostigmine and physostigmine. These results suggest that the anti-amnesic effect of AFW on SCOP-induced amnesia may be related to the increase in the systematic cholinergic neuronal system activity.

**Key words:** *Anoectochilus formosanus*, Scopolamine, Passive avoidance.

## INTRODUCTION

*Anoectochilus formosanus* (AF) Hayata (Orchaceae), one of the nourishing and health drugs, is a popular folk medicine that possesses antioxidant, anti-inflammatory and liver-protective effects and has been used to cure hepatitis, hypertension and cancer in Taiwan.<sup>1-3</sup> It contains polysaccharides, trace elements (eg: Fe, Co, Cu, Mn, Zn, Mo, Cr)<sup>4</sup> and taurine.<sup>5</sup> The polysaccharide possesses elevating effect of organism immunology.<sup>4</sup> The trace elements play an important role in the anti-aging.<sup>6</sup> However, there is no any other report about the effect of AFW on the amnesia induced by scopolamine (SCOP) in rats.

Alzheimer disease is characterized by memory loss that is accompanied by degeneration of basal forebrain cortical

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cholinergic neurons. It is well known that the cholinergic neuronal system plays an important role in learning and memory in humans and animals.<sup>7-10</sup> SCOP, a muscarinic antagonist, impaired learning and memory in rodents and humans, especially learning acquisition and short-term memory.<sup>11-13</sup> Therefore, SCOP is used as a useful model in the screening of anti-amnesic drugs.<sup>14</sup> In the recent studies, several cognitive enhancers can improve the SCOP-induced amnesia, but their effects can be inhibited by peripherally cholinergic muscarinic receptor antagonist, example for SCOP N-methylbromide (M-SCOP) which was substituted by methyl group and did not pass through the brain-blood-barrier.<sup>15</sup> On the other hand, adrenalectomy can inhibit the cognitive enhancers improvement on SCOP-induced amnesia.<sup>16</sup> The theory from these results suggest that the memory-improving effects of several cognitive enhancers could be partially due to peripherally automatic system.<sup>17</sup>

In the present study, we attempted to investigate 1) whether AFW attenuated SCOP-induced amnesia, 2) whether its anti-amnesic effect will be antagonized by M-SCOP (peripheral muscarinic antagonist), or be potentiated by physostigmine (PHY, central and peripheral acetylcholine transferase (AChE) inhibitor) and neostigmine (NEO, peripheral AChE inhibitor).

## MATERIALS AND METHODS

### Animals

Male Sprague-Dawley rats, weighing 200-250 g, were housed in groups of six with free access to food and water and kept in a regulated environment ( $22 \pm 1$  °C), wherein a 12 hr. light-dark cycle (8:00 to 20:00, light) was maintained. Each experimental group included 12 to 18 rats.

### Preparation of AF

AF was purchased from Pu-Li where they were cultivated and was authenticated by Dr. Chung-Chuen Chen, Institute of Chinese Pharmaceutical Sciences where a plant specimen was deposited, China Medical College, Taichung, Taiwan.

Eight hundred liters of water were added to 90-kilogram fresh whole plants of AF, and were decocted for 4 hr. after filtration, the aqueous extracts were concentrated into 336.6 mg/mL under reduced pressure at 50 °C. We obtained the water extract of AF (AFW) and stored at cooling room until use. In vivo study, the extract was diluted to 50 or 100 mg /mL in water was administrated orally to rats at volume of 1 mL per 100 g body weight. To the control rats, water was administered in a similar way.

### Passive Avoidance Task

Rats were trained in a step-through passive avoidance task. The apparatus consisted of two compartments having a steel-rod grid floor (36 parallel steel rods, 0.3 cm in diameter set 1.5 cm apart). One of the compartments (48 × 20 × 30 cm) was equipped with a 20W lamp located centrally at a height of 30 cm, and the other was dark compartment of the same size, connected through a guillotine door (5 × 5 cm). The dark room was used during the experimental sessions that were conducted between 09:00 and 17:00 hs.

### **SCOP-treated rats in Passive Avoidance Performance**

During the trial training, the guillotine door connecting the light and dark compartment was kept closed. After each rat was placed in the light compartment with its back to the guillotine door, the door was opened and simultaneously the time (step-through latency, STL) taken by the rat to enter the dark compartment was measured with a stopwatch. Once the rat entered the dark compartment, the door was closed. An inescapable scrambled footshock (1.0 mA for 2 sec.) was then delivered through the grid floor. The rat was removed from the dark compartment 5 sec. after administering the shock. The rat was then put back into its home cage until the retention trial, which was carried out twenty-four hours later. The rat was once again placed in the light compartment and as in the case of training trial, the guillotine door was opened and the step-through latency was recorded and used as a measure of retention.<sup>18</sup> An upper cut-off time of 300 sec. was set.

In the first series of experiments, SCOP (1.0 mg/kg, i.p.) was administered 30 minutes before the training trial.<sup>18</sup> AFW (1.0 and 2.0 g/kg) was administered to SCOP-treated rats orally. The training trial was carried out 1 hr after the treatment.

In the second series of experiments, AFW (1.0 g/kg, p.o.) was administered to SCOP-treated rats. The training trial was carried out 1 hr. after the treatment. Physostigmine (0.02 mg/kg, i.p.) was administered 20 min. before the training trial. Neostigmine (0.02 mg/kg, i.p.) was administered 20 min. before the training trial. SCOP N-methylbromide (0.5 mg/kg, i.p.) was administered 30 min. before the training trial.

### **Motor Activity Measurements**

Immediately following AFW administrations, each rat was placed in an Omnitech Digiscan Animal Activity Monitor (Model Opto-Varimex, Columbus Co., USA) for 20 min. The acrylic cage within the monitor measured approximately 42 cm wide by 42 cm long and 31 cm high. The monitor was equipped with 16 beams 2.54 cm apart from front to back and from side to side, as well as 16 beams 2.54 cm apart from side to side on the upper level. Every 100 msec., the computer sampled the status of all the beams. The Digiscan analyzer converted the patterns of beams broken into different measures of locomotor activity. In this study, the measure automatically analyzed by the computer is the moving time, resting time and traveling distance.<sup>19</sup>

AFW (1.0 and 2.0 g/kg, p.o.) was administered orally, and the record was carried out 1 hr after the treatment. SCOP (1.0 mg/kg, i.p.) was administered 90 minutes before the record. Physostigmine (0.02 mg/kg, i.p.) was administered 20 min. before the training trial. Neostigmine (0.02 mg/kg, i.p.) was administered 20 min. before the training trial. SCOP N-methylbromide (0.5 mg/kg, i.p.) was administered 30 min. before the training trial.

## Drugs

SCOP hydrobromide, PHY (Eserine), NEO, SCOP N-methylbromide were purchased from Sigma. All drugs were dissolved in 0.9 % saline.

## Statistics

All data obtained during the passive avoidance task was expressed in terms of medians and interquartile ranges and further analyzed by using a Kruskal-Wallis non-parametric one-way analysis of variance, followed by Mann-Whitney's U-test. In addition, the data collected during motor activity was analyzed using a one-way analysis of variance, followed by Scheffé multiple range test. The criterion for statistical significance was  $p < 0.05$  in all the above statistical evaluations.

# RESULTS

## Passive Avoidance

As shown in Fig. 1, SCOP (1.0 mg/kg, i.p.) remarkably reduced the step-through latencies in the retention test. AFW orally administered to SCOP-treated rats at 1.0 and 2.0 g/kg doses remarkably increased the retention latencies which were shortened by SCOP.

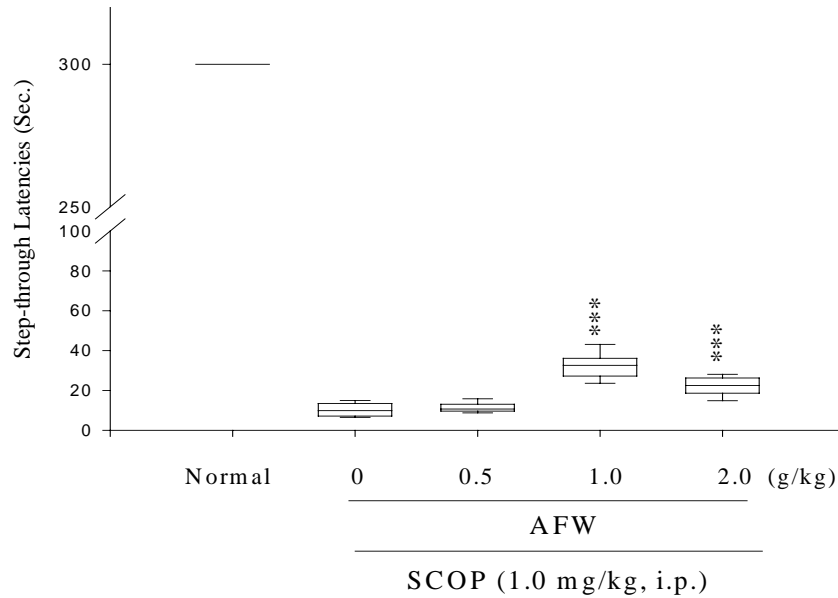
M-SCOP (0.5 mg/kg), a peripherally muscarinic receptor blocker, did not affect the step-through latencies but could attenuate the anti-amnesic effect of AFW (1.0 g/kg) on the SCOP-induced amnesia (Fig. 2).

PHY (0.02 mg/kg), a systematic acetylcholinesterase inhibitor, did not affect the step-through latencies which was shortened by SCOP but could augment the anti-amnesic effect of AFW (1.0 g/kg) on the SCOP-induced amnesia (Fig. 3).

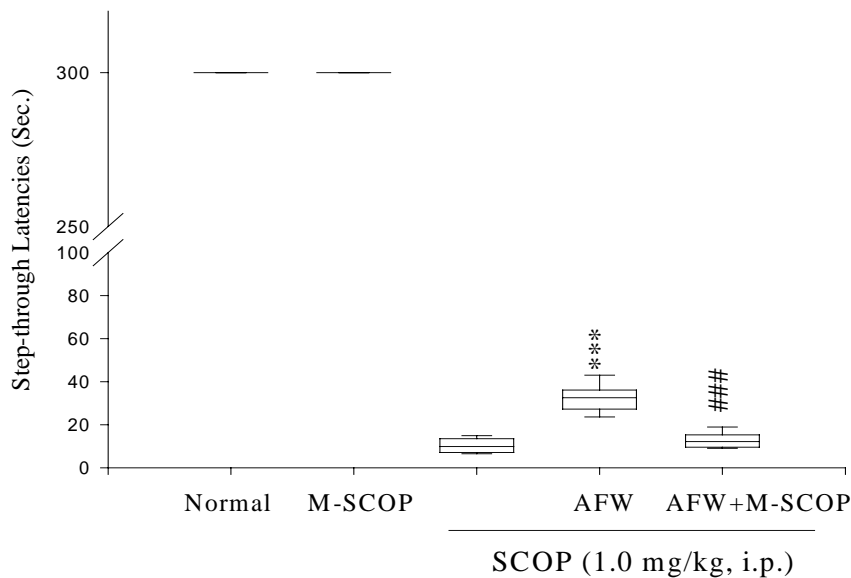
NEO (0.02 mg/kg), a peripherally acetylcholinesterase inhibitor, did not affect the step-through latencies which was shortened by SCOP but could augment the anti-amnesic effect of AFW (1.0 g/kg) on the SCOP-induced amnesia (Fig. 4).

## Motor Activity

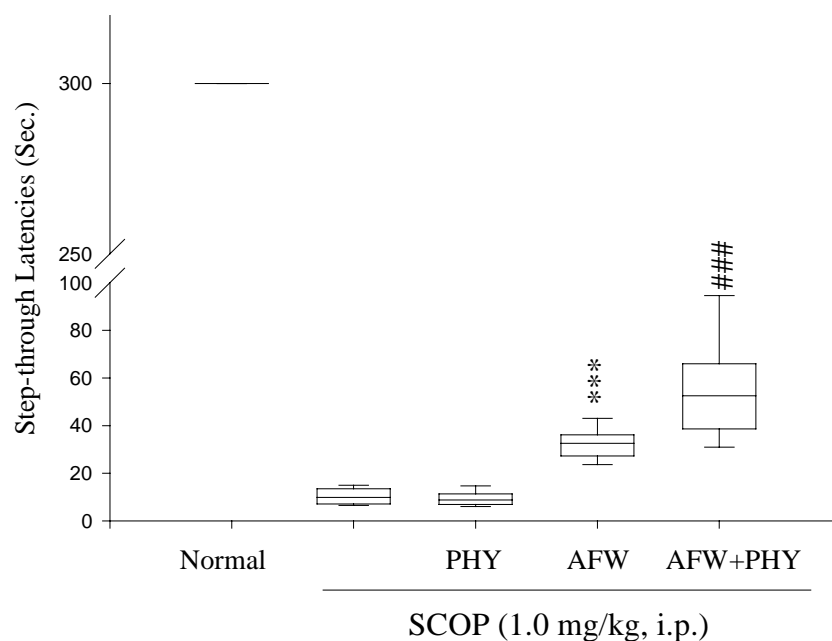
As shown in Fig. 5, the moving time, traveling distance and resting time of rats administered with AFW was not different from those of the vehicle group. The moving time, traveling distance and resting time of rats administered with AFW in combination with SCOP was not different from those the PHY, NEO or M-SCOP group, respectively.



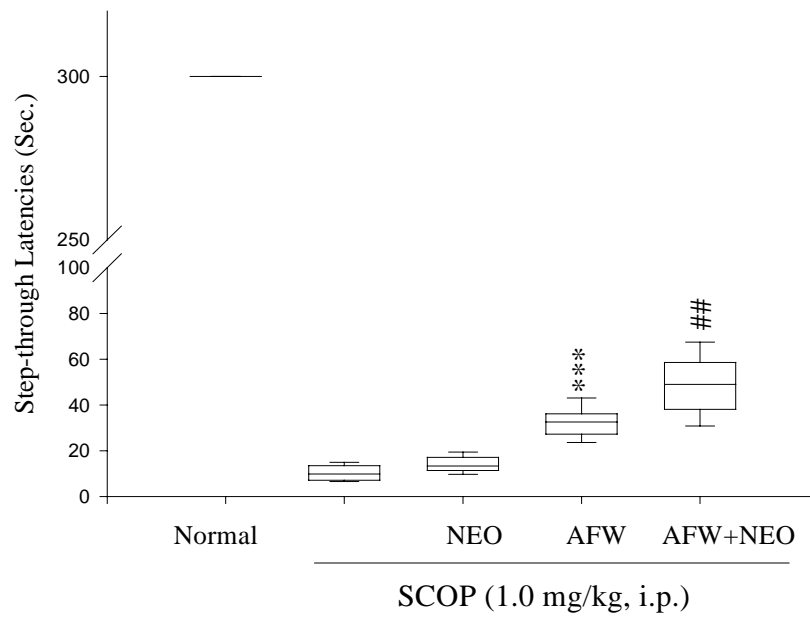
**Fig 1. Effects of water extract of *Anoectochilus formosanus* (AFW, 1.0 g/kg) on SCOP- induced amnesia in rats. Each column, center line in the column and the bars represent the 95 % confidence interval, median and range of 12-18 rats, respectively. \*\*\* $p < 0.001$ , compared with the SCOP group.**



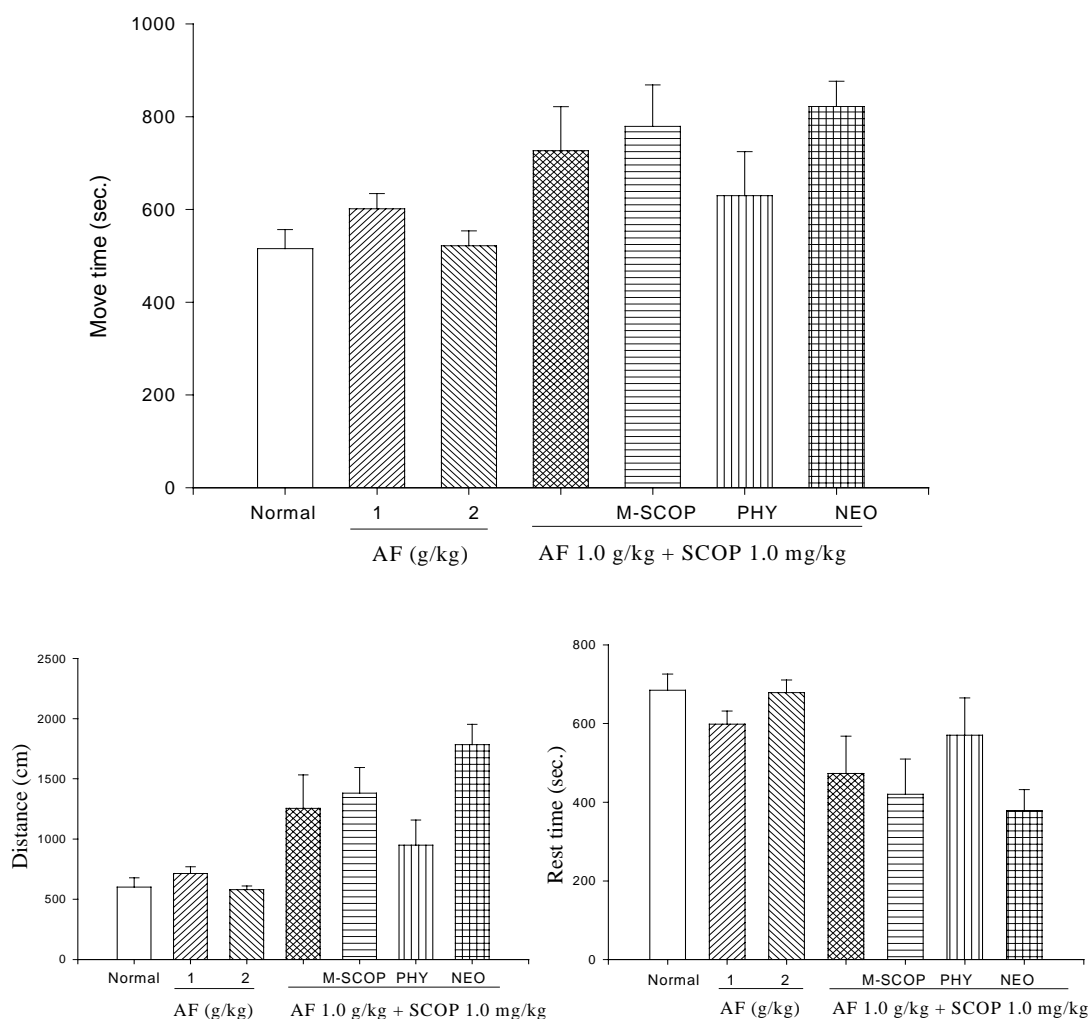
**Fig 2.** Effects of scopolamine methylbromide (M-SCOP, 0.5 mg/kg) on the water extract of *Anoectochilus formosanus* (AFW, 1.0 g/kg)-induced attenuation from SCOP-induced amnesia in rats. Each column, center line in the column and the bars represent the 95 % confidence interval, median and range of 12-18 rats, respectively. \*\*\* $p < 0.001$ , compared with the SCOP group. #### $p < 0.001$ , compared with the group given SCOP in combination with AFW.



**Fig 3.** Effects of physostigmine (PHY, 0.02 fig/kg) on water extract of *Anoectochilus formosanus* (AFW, 1.0 g/kg)-induced attenuation from SCOP-induced amnesia in rats. Each column, center line in the column and the bars represent the 95 % confidence interval, median and range of 12-18 rats, respectively. \*\*\* $p < 0.001$ , compared with the SCOP group. #### $p < 0.001$ , compared with the group given SCOP in combination with AFW.



**Fig 4.** Effects of neostigmine (NEO, 0.02 mg/kg) on water extract of *Anoectochilus formosanus* (AFW, 1.0 g/kg)-induced attenuation from SCOP- induced amnesia in rats. Each column, center line in the column and the bars represent the 95% confidence interval, median and range of 12-18 rats, respectively. \*\*\* $p < 0.001$ , compared with the SCOP group. ## $p < 0.01$ , compared with the group given SCOP in combination with AFW.



**Fig 5. Effects of AF and combined drugs on the move time, rest time and travel distance in rats. AF: Anoectochilus formosanus, SCOP: scopolamine hydrobromide, M-SCOP: scopolamine methylbromide, PHY: physostigmine, NEO: neostigmine. Each column and vertical bar represent the mean  $\pm$  SEM. (N = 6).**

## DISCUSSION

There is evidence both from animal and human studies indicating that learning and memory can be modified by drugs which affect central cholinergic function.<sup>7-9</sup> For instance, a muscarinic antagonists, such as SCOP, have been shown to impair memory,<sup>11-13</sup> whereas acetylcholinesterase inhibitors, such as physostigmine, facilitate cognitive process in animals and humans.<sup>19-22</sup> SCOP, the muscarinic receptor antagonist, can decrease the activity of the central cholinergic neuronal system, and impair the performance on the passive avoidance task in rodents.<sup>11</sup> Therefore, SCOP has been proposed as a short-term amnesia model representative of dementia and useful for screening the cognitive enhancer drugs in animals. In the present study, it was observed that SCOP also significantly shortened the step-



through latency of the retention trial in passive avoidance task. AFW at a dose of 1.0 and 2.0 g/kg prolonged the step-through latency which was shortened by SCOP in passive avoidance task.

On the other hand, the effect on sensory, motivational or motor systems can in turn affect the acquisition of the avoidance response. Several aspects shown the possibility that the passive avoidance response in drug-treated animals can be related with the sensitivity to nociceptive stimuli or motor alteration during training. It was noted that AFW did not change the motor activity as shown by the animal activity meter. Therefore, the present results were shown that AFW did not alter the motor activity and nociceptive stimuli (data not shown). Furthermore, AFW in combination with SCOP also did not alter the motor activity. The present data demonstrated that the improving effect of AFW on the SCOP-induced impairment of learning acquisition in passive avoidance task could be only related the memory-related process.

In the previous study, several cognitive enhancers can improve the SCOP-induced amnesia by partially peripheral nervous system.<sup>18</sup> SCOP N-methylbromide (M-SCOP), a peripherally muscarinic receptor antagonist, is a quaternary amine compound and is known to reduce the activity of cholinergic neuronal system in peripheral nervous system. M-SCOP significantly attenuates the nootropics-reversed step-through latency of the retention trial shortened by SCOP.<sup>16</sup> The anti-amnesic effect of AFW was attenuated by M-SCOP. Neostigmine (NEO), an acetylcholinesterase inhibitor, is known to increase the activity of peripherally acetylcholine neuronal system and significantly strengthened the step-through latency of the retention trial shortened by SCOP.<sup>23</sup> The anti-amnesic effect of AFW on the SCOP-induced amnesia was augmented by NEO. Physostigmine (PHY), an acetylcholinesterase inhibitor, is known to increase the activity of systemic acetylcholine neuronal system and significantly strengthened the step-through latency of the retention trial shortened by SCOP.<sup>21</sup> The anti-amnesic effect of AFW on the SCOP-induced amnesia was enhanced by PHY. From these results, it is suggested that anti-amnesic effect of AFW on the SCOP-induced amnesia acts by enhancing systematic cholinergic neuronal system.

*Anoectochilus formosanus* Hayata possesses antioxidant, anti-inflammatory and liver-protective effects.<sup>1-3</sup> It contains polysaccharide, taurine and trace elements (eg: Fe, Co, Cu, Mn, Zn, Mo, Cr).<sup>5</sup> The polysaccharides possess elevating effect of organism immunology.<sup>5</sup> The trace elements play an important role in the anti-aging.<sup>6</sup> We will be made further studies to clarify whether the major anti-amnesic components of AFW is the polysaccharides or trace elements in the future.

In conclusion, we suggested that AFW could attenuate the SCOP-induced amnesia. The anti-amnesic effect of AFW on SCOP-induced amnesia may be related to the increase in the activity of peripheral cholinergic neuronal system.

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# 台灣金線連減弱東莨菪鹼誘發健忘作用

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(2003年8月14日受理, 2003年10月13日收校訂稿, 2003年10月20日接受刊載)

本研究以被動迴避學習反應儀探討台灣金線連水抽出物(簡稱 AFW)對東莨菪鹼誘發健忘之作用, 結果顯示 AFW 在 1.0 及 2.0 公克/公斤劑量可改善東莨菪鹼誘發之健忘, AFW 之抗健忘作用可被週邊膽鹼毒菌鹼接受器拮抗劑 scopolamine N-methylbromide 所拮抗, 並會被 neostigmine 及 physostigmine 增強。

綜合以上結果顯示 AFW 抗東莨菪鹼誘發之健忘作用與增強全身膽鹼神經系統活性有關。

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