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Osthole Improves Aspects of Spatial Performance in Ovariectomized Rats

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Abstract: The present study was designed to investigate the ameliorating effects of *Cnidiuim monnieri* L. Cusson (CM) and osthole, a constituent of CM, on the spatial performance deficit in scopolamine (SCOP)-treated or ovariectomized (OVA) rats. CM improved the deficit of spatial performance, and reversed the lower plasma estradiol levels caused by SCOP in female rats. In addition, osthole (3 and 10 mg/kg, sc) improved the performance deficit in OVA rats. It (10 and 30 μg/brain, icv) also improved the performance deficit caused by SCOP in intact female rats, and at 30 μg/brain improved the deficit in OVA rats. However, osthole did not alter the latency swum to reach the visible target in SCOP-treated and OVA rats. Accordingly, we suggested that osthole is an active constituent of CM, and possesses ameliorating effects on the spatial performance deficits in SCOP-treated female rats or OVA rats. The action mechanism of the effects of osthole on performance deficits was related to the estrogen-like properties and activating the central cholinergic neuronal system.

Keywords: *Cnidiuim monnieri*; Umbelliferae; Osthole; Spatial Performance; Scopolamine; Ovariectomized Rats.

Introduction

Cnidiuim monnieri L. Cusson (Umbelliferae), or CM, was used to cure volvovaginitis described in ancient Chinese herbal books. Modern pharmacological studies have proven that CM possesses anti-pruritic and anti-allergic effects (Chen *et al*., 1988; Matsuda *et al*., 2002). Qin *et al*. (1997) pointed out that total coumarins of CM improved learning impairment induced by continuous administration of hydrocortisone acetate in rats. However, learning

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is associated with the central neuronal systems, especially the cholinergic neuronal system and the central cholinergic dysfunction, usually caused by amnestic syndrome (Fibiger, 1991). The muscarinic receptor antagonist scopolamine (SCOP) impairs learning acquisition and short-term memory in rodents, and is used as a valuable model in the screening of anti-amnesia drugs (Quartermain and Leo, 1988). Therefore, the first purpose of this present study was to investigate the ameliorating effect of petroleum ether layer of CM and osthole, a constituent of CM, on the deficit of spatial performance caused by SCOP on Morris water maze in female rats. Furthermore, some researchers indicated that CM and osthole possessed estrogen-like effects and prevented post-menopausal osteoporosis in ovariectomized (OVA) rats (Li *et al*., 1994; Li *et al*., 1996; Jiang and Li, 2001; Li *et al*., 2002). Estradiol improved the deficit of spatial memory caused by OVA or SCOP in female animals (Packard and Teather, 1997a and b; Fader *et al*., 1999; Lacreuse *et al*., 2002). The second purpose of this study was to investigate the ameliorating effects of petroleum ether layer of CM and osthole on the deficit of spatial performance on Morris wafer maze in OVA rats.

Materials and Methods

Animals

Female Sprague-Dawley rats, three months old and weighing 300–380 g, were obtained from National Laboratory Animal Breeding and Research Center. All rats were used in the following experiments according to the Guiding Principles for the Care and Use of Laboratory Animals. They were randomly housed six per wire-mesh cage ($39 \times 26 \times 21$ cm) for at least 1 week prior to experiments. Temperature (23 ± 1°C) and humidity (60%) were regulated with free access to standard food in pellets (Fwusow Industry Co. Ltd., Taiwan) and tap water, on a 12 hour–12 hour light/dark cycle (light phase: 08:00 to 20:00 hours). Two or three cages were randomly assigned into the same group.

Plant Preparation and Drug Administration

CM was purchased from the Taiwan market and authenticated by Dr. Hsieh Ming-Tsuen. Seeds of CM (10 kg) were chopped and extracted with methanol (3×5 l) by macerating for 2 weeks, and the extract reduced to desiccate with a vacuum rotary evaporator. A yield of 1428 g (14.28%) was obtained. The extract was dissolved in water, and the solution was partitioned with petroleum ether in order to obtain the petroleum ether extract in yields of 89.4 g (6.26%).

Petroleum ether extract of CM (0.3 and 0.6 g/kg) and osthole were dissolved with 95% ethanol (about 2% of total volume). One to two drops of Tween80 were added, and diluted with distilled water to fixed volume (2 ml). Osthole of the 99% purity was purchased from Yoneyama Chemical Industries Ltd., Japan. In systemic treatment, CM at 0.3 and 0.6 g/kg (p.o.) and osthole at 3 and 10 mg/kg (s.c.) were administered 60 minutes before the spatial performance per day for 3 consecutive days. The vehicle-treated rats received the same

volume (0.2 ml/100 g body weight). In intracisternal treatment, rats were anesthesized with pentobarbital (45 mg/kg, i.p.) and internal cannula was implanted into lateral ventricle (Bregma AP: −0.08 mm, L: −0.15 mm, D: −0.40 mm from dura). Then, rats were left to recover for 3 days from surgery and were administered osthole at 10 and 30 μg/20 μl/brain intracisternally 15 minutes before the spatial performance per day for 3 consecutive days. SCOP was purchased from Sigma-Aldrich Co., USA and dissolved in saline.

SCOP and OVA

SCOP (0.5 mg/kg, i.p.), administered to female rats 30 minutes before spatial performance per day for 3 consecutive days, which induced the deficit of spatial performance (Quartermain and Leo, 1988). Female rats were operated under anesthesia with pentobarbital (45 mg/kg, i.p.), and then divided into two groups: the sham-operated and OVA groups. The sham-operated group was anesthetized, laparatomized and sutured without removing the ovaries. On the 14th day after surgery, behavioral studies were performed in sham-operated and OVA rats (Packard and Teather, 1997a).

Apparatus of Morris Water Maze

Water maze testing was performed in a stainless circular pool (160 cm in diameter; 60 cm in height) with an inner white surface. The pool was filled with water to a depth of 35 cm (maintained at 23 ± l°C) that covered an invisible 10 cm circular white platform. The platform was submerged 1.0 cm below the surface of the water and placed in the center of the northeast quadrant. Swimming activity of each rat was monitored via a ccTV camera mounted overhead, which relayed informations including the latency to find the platform, total distance of traveling, time and distance spent in each quadrant, to a video tracking system (VIDEOMEX-V water maze program, Columbus Instruments, Columbus, USA).

Spatial Learning

Each rat was given four trials per day for 3 consecutive days to find the hidden platform. A trial was initiated by placing the rat into the water facing the pool wall in one of the four quadrants. The daily entry into individual quadrants was randomized so that all four quadrants were applied once per day. For each trial, the rat was allowed to swim a maximum of 120 seconds to find the platform. When reaching to the platform, the rat was allowed a 30-second rest on the platform. If it was unsuccessful within the aborted period, the rat would be given a score of 120 seconds, then physically placed on the platform and also allowed a 30-second rest period. In either case, the rat was immediately given the next trial after the rest. The swimming latency of each trial was recorded and the data of four trials per day were averaged (Conway, 1998).

Non-Spatial Performance with Visible Platform

The next day after spatial performance, the platform was re-introduced into the pool in the same position but raised 1 cm above the surface of the water. Each rat was also given four trials to locate the platform visually. The latency to find the platform was recorded, and the data of four trials per day was averaged as a comparison of visual acuity (Conway, 1998).

Measurement of Plasma Estradiol and Progesterone Levels

Blood was collected by tail vein and drawn into EDTA-coated Eppendorf tubes. Plasma obtained by centrifuge was stored at −80°C. Then, concentrations of plasma estradiol and progesterone were measured with an automated direct competitive chemiluminoimmunoassay (CIA) (Chiron Diagnostics Corporation, Emeryville, CA, USA) (Rojanasakul *et al.*, 1994).

Statistical Analysis

In the hidden platform trials, the latency for each rat was averaged prior to analysis. These parameters were then analyzed by using one-way repeated measures analysis of variance (ANOVA) with treatments as the "between subjects" variable and day as the "within subjects" variable. If the treatment effect was significant, post-hoc comparison would be made by using Dunnett's test (compared to control, family error rate $p < 0.05$ considered statistically significant). The latency swum (or the swimming duration) to reach the platform for non-spatial performance, plasma estradiol and progesterone levels were analyzed by using one-way measures analysis of variance (ANOVA) following by Scheff's test. The criterion for statistical significance was $p < 0.05$ in all statistical evaluations.

Results

Effects of CM on Plasma Estradiol and Progesterone Levels in Female Rats

As shown in Fig. 1, SCOP-treated rats had lower plasma estradiol levels than vehicle-treated rats ($p < 0.01$). However, the plasma progesterone levels of rats between the treatment with vehicle and SCOP showed no difference ($p > 0.05$). CM at 0.3 and 0.6 g/kg reversed the lower plasma estradiol levels caused by SCOP ($p < 0.01$).

Effects of CM on SCOP-Induced Performance Deficit in Female Rats

In vehicle-treated female rats, the latency swum to reach the hidden platform gradually decreased during the consecutive 3 days of spatial performance (Fig. 2). SCOP prolonged the latency swum to reach the hidden platform in comparison with the vehicle-treated female rats ($p < 0.01$). CM (0.3 and 0.6 g/kg) significantly shortened the latency swum to reach the hidden platform caused by SCOP ($p < 0.01$).

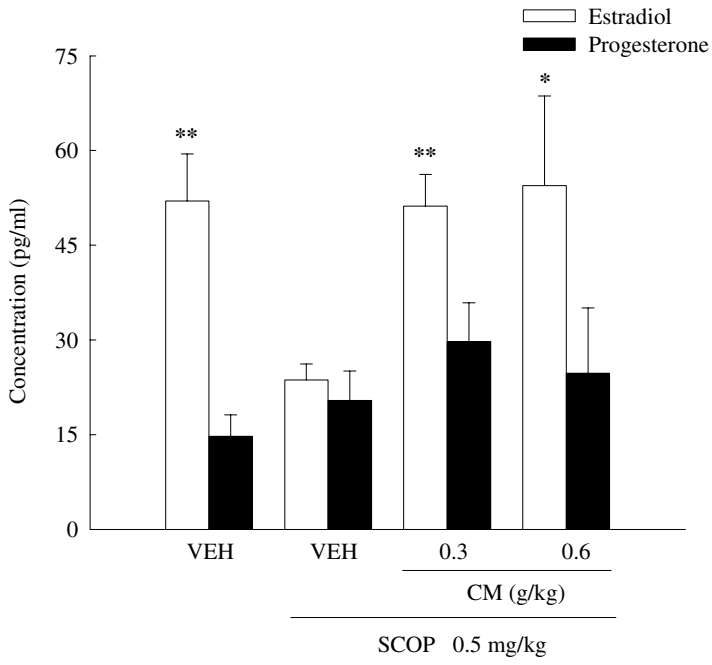

Figure 1. Effects of petroleum ether layer of CM (0.3 and 0.6 g/kg) and SCOP on plasma estradiol and progesterone levels in female rats. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with VEH/SCOP group (n = 12).

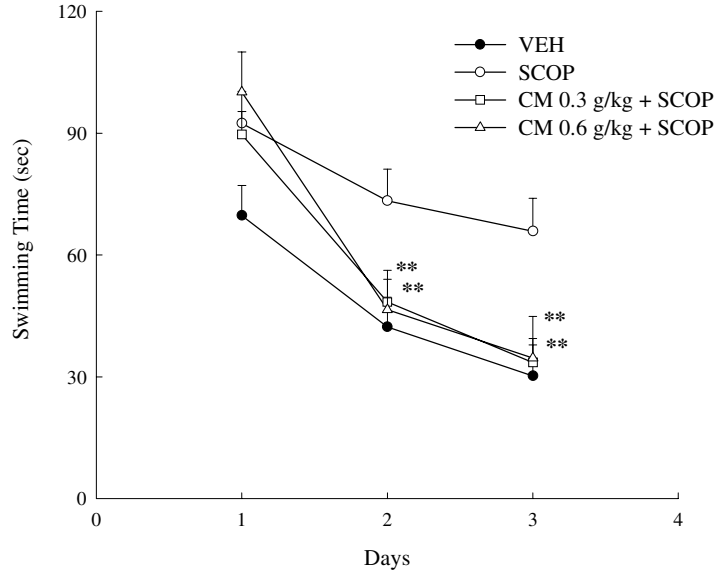

Figure 2. Effects of petroleum ether layer of CM (0.3 and 0.6 g/kg) on the deficit of spatial performance caused by SCOP in female rats. $^{**}p < 0.01$, compared with VEH/SCOP group (n = 12).

Effects of Osthole Administered Subcutaneously or Intracisternally on Spatial Performance Deficit Induced by SCOP or OVA in Female Rats

In sham-operated female rats, the latency swum to reach the hidden platform gradually decreased during the consecutive 3 days of spatial performance (Fig. 3). The latency swum to reach the hidden platform in OVA rats was prolonged ($p < 0.01$). Osthole (3 and 10 mg/kg, s.c.) significantly shortened the latency swum to reach the hidden platform in OVA rats ($p < 0.01$). Furthermore, osthole at 10 and 30 μg/brain administered intracisternally reversed the latency swum to reach the hidden platform by SCOP (Fig. 4) ($p < 0.01$). However, osthole at 30 μg/brain administered intracisternally in OVA rats reversed the latency swum to reach the hidden platform caused by OVA (Fig. 5).

Effects of Osthole Administered Intracisternally on Non-Spatial Performance in Female Rats Treated with SCOP or OVA Rats

The latency swum to reach the visible platform between vehicle- and SCOP-treated rats was not different (Fig. 6). Osthole at 10 and 30 μg/brain administered intracisternally to SCOP-treated rats did not alter the latency swum to reach the visible platform. On the other hand, the latency swum to reach the visible platform between sham-operated and OVA rats was not different (Fig. 6). Osthole at 10 and 30 μg/brain administered intracisternally to OVA rats did not alter the latency swum to reach the visible platform.

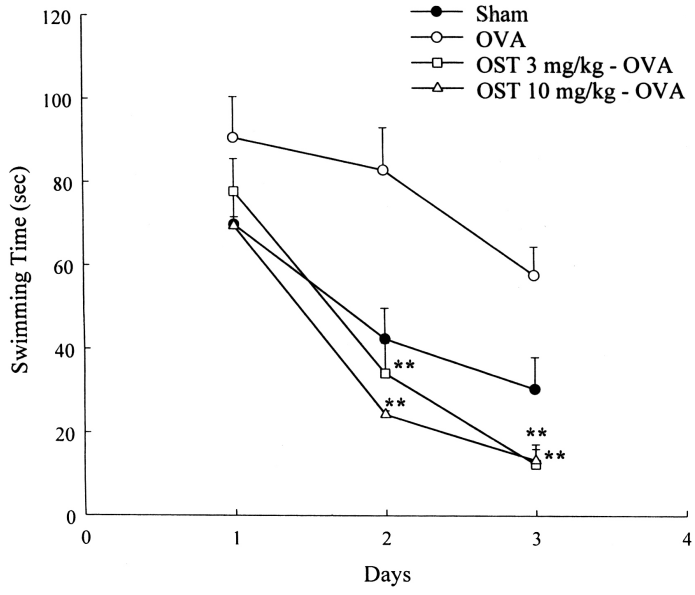

Figure 3. Effects of OST (3 and 10 mg/kg, sc) on the deficit of spatial performance in OVA rats. $^{**}p < 0.01$, compared with VEH in OVA group (n = 12).

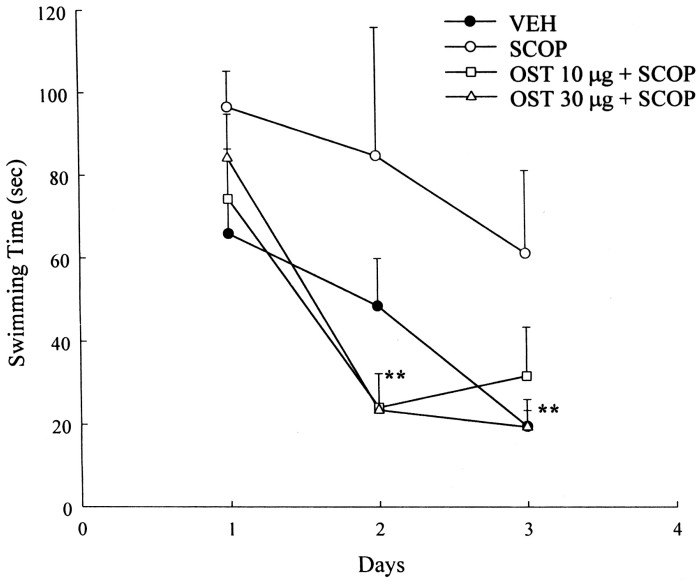

Figure 4. Effects of OST (10 and 30 μg/brain, icv) on the deficit of spatial performance caused by SCOP in female rats. $^{**}p < 0.01$, compared with VEH/SCOP group (n = 12).

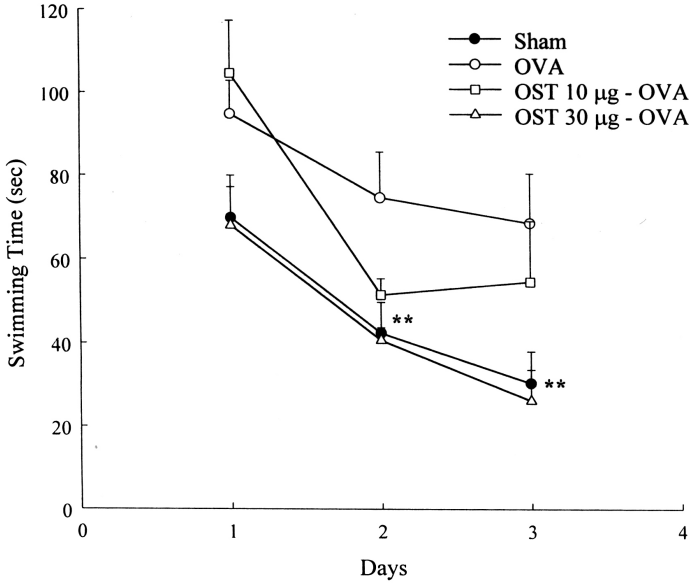

Figure 5. Effects of OST (10 and 30 μg/brain, icv) on the deficit of spatial performance in OVA rats. $^{**}p < 0.01$, compared with VEH in OVA group (n = 12).

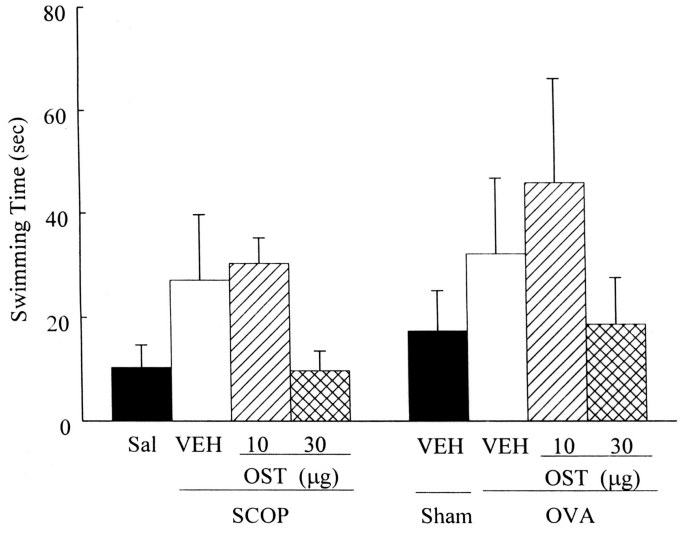

Figure 6. Effects of OST (3 and 10 μg/brain, icv) on the non-spatial performance in SCOP-treated and OVA rats (n = 12).

Discussion

Some researchers and our previous report pointed out CM and its constituent, osthole, improved learning dysfunction caused by SCOP (Qin *et al*., 1997; Shen *et al*., 1999; Hsieh *et al.*, 2003). However, CM was usually used to cure women's diseases, especially post-menopausal-associated disorders and possessed estrogen-like effects (Jiang and Li, 2001). Some researchers suggested that estradiol improves deficits of spatial memory caused by SCOP in female animals (Packard and Teather, 1997b; Fader *et al*., 1999). Our present data showed that CM at 0.3 and 0.6 g/kg reversed the deficit of spatial performance caused by SCOP in female rats. Longley *et al*. (1968) pointed out that autonomic drugs modulated ovarian hormone secretion. We further found that CM at 0.3 g/kg reversed lower plasma estradiol level caused by SCOP in female rats. Hence, the reversal caused by CM from SCOP-induced performance deficit in female rats might be related to modulating plasma estradiol levels or its estrogen-like properties.

As a constituent of CM with approximately 1% concentration, osthole also improves the memory deficit induced by SCOP in male rats (Shen *et al*., 1999). Therefore, we further evaluated whether osthole improved spatial performance deficit caused by SCOP or OVA in female rats. OVA rats of the 14th day after surgery have long latency swum to reach the hidden platform in comparison with sham-operated rats. Osthole at 3 and 10 mg/kg, reversed the long latency in OVA rats. Moreover, post-mentern report on post-menopausal women associated dementia indicated largely lost in spine density of hippocampal granule and pyramidal cells (Gibbs, 2000). Estrogens increased spine density in the hippocampus, and then intra-hippocampal estrogen enhanced memory in SCOP-treated or OVA rats (Packard and Teather, 1997a and b). Osthole (10 and 30 μg/brain, icv) also reversed the long latency

induced by SCOP in female rats, but only at 30 μg/brain reversed the long latency in OVA rats. Our present data suggested that osthole might be an active constituent of CM possessing the anti-amnestic activity. Shen *et al*. (1999) pointed out that the enhancing effects of CM and osthole on learning and memory were partially due to activating central cholinergic neuronal system. Therefore, the reversal caused by CM in female rats might be partially related to activating the cholinergic system directly or via its estrogen-like properties indirectly because estrogen enhances central cholinergic activity (Gibbs and Aggarwal, 1998; Gibbs, 2000).

Finally, the latency swum to reach the visible platform between vehicle- and SCOP-treated rats was somewhat divergent but not statistically significant. Osthole at any dose did not alter the latency in SCOP-treated female rats. The latency swum to reach the visible platform between sham-operated and OVA rats was also not statistically significant, osthole at 10 and 30 μg/brain did not alter it. Therefore, the deficits in spatial performance caused by SCOP and OVA were due to alteration of memory processes but not attention or motor activity, and the ameliorating effects of osthole on performance deficits were also due to alteration of memory processes.

In summary, the results of the present studies proved that CM improved the deficit of spatial performance on Morris water maze induced by SCOP in female rats, and osthole possesses the anti-amnestic activity. The ameliorating effects of osthole on performance deficits were similar to those of estrogen and might be related to the cholinergic neuronal systems and ovarian hormones. For the detailed mechanism on the ameliorating effects of osthole on the performance deficits, the central neuronal systems including cholinergic, glutaminergic and monoaminergic neuronal system in hippocampus shall be further demonstrated in the future, because the enhancing effects of estrogen on memory function were due to activating of the neuronal systems (Luine *et al*., 1998; Farr *et al*., 2000; Gibbs, 2000).

Acknowledgments

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