EFFECT OF INHALATION OF CHAMOMILE OIL VAPOUR ON PLASMA ACTH LEVEL IN OVARECTOMIZED-RAT UNDER RESTRICTION STRESS

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We found that inhaling chamomile oil vapour decreased restriction stress-induced increases of plasma ACTH level in ovariectomized rat. The plasma ACTH level decreased further when diazepam was administered along with inhaling chamomile oil vapour. Flumazenil blocked the decrease in plasma ACTH level induced by inhaled chamomile oil vapour.

KEY WORDS Chamomile Oil Vapour; Inhalation; ACTH; Diazepam; Flumazenil

Chamomile is an herb known to cause sedative, hypnotic, analgesic and anti-inflammatory actions in humans.^{1,2)} It is also reported to strengthen the immune system.³⁾ Inhalation of herb oil vapours is used in aromatherapy. Research on the action of some herbs in humans has been reported, but there are few reports of animal experiments examining the fundamental pharmacology.⁴⁾

We found that inhalation of chamomile oil vapour reduced restriction stress-induced increases in plasma ACTH level in ovariectomized rats. Therefore, we examined the application of chamomile oil as a medication for anxiety or depression in menopausal women. Ovariectomized rats were used as an experimental menopausal model.^{5,6)}

Female Wistar rats (Saitama Experimental Company Ltd., Saitama, Japan) weighing 290--530 g were used in this investigation. Chamomile oil was obtained from *Matricaria chamomilla* L. and purchased from Maggie Tisserand Company (Paston Place, Brighton, England).

Ovariectomized rats were used 20 weeks after surgery (performed at 7 weeks of age). The inhalation apparatus was a lidded plastic cage (L32xW21xH13 cm). Cotton was soaked in 0.7 ml of chamomile oil and placed in the cage. The inhalation was carried out twice a day for 3 days in the morning and afternoon. After rats had inhaled the chamomile oil vapour for one hour, restriction stress was induced for 30 min in the same cage while vapour inhalation continued. Diazepam (Horizon, Yamanouchi Pharmaceutical Company Ltd., 1.0 mg/kg) was administered i.p. 30 mins before the restriction stress. Flumazenil (Anexate, Yamanouchi Pharmaceutical Company Ltd., 0.1 mg/kg) administered i.p. injection 30 mins before restriction stress. As a control, a rat was restricted in a cage without chamomile oil or drugs for the same period of time. Temperature in the experimental room was $23 \pm 1^\circ\text{C}$. After the final restriction stress, rats were decapitated and plasma was obtained from heparinized trunk blood by centrifugation (3000 rpm x 15 min). The plasma ACTH level was determined by radioimmunoassay (SRL, Tokyo).

Restriction stress-induced plasma ACTH level in the ovariectomized rats was significantly greater than that of normal rats (Table 1, 2). Restriction stress-induced increases in plasma ACTH level in normal rats and ovariectomized rats were decreased by administration of diazepam and inhaling chamomile oil vapour. Inhaling chamomile oil vapour induced greater decreases in plasma ACTH levels in ovariectomized rat than treatment with diazepam. This difference was not observed in normal female rats.

Furthermore, the inhalation of chamomile oil vapour induced a decrease in

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plasma ACTH level that was blocked by pretreatment with flumazenile (Table 3). Flumazenile is a potent and specific benzodiazepine receptor antagonist.⁷⁾

Table 1. Effect of Inhalation of Chamomile Oil Vapour and Diazepam on Restriction Stress-induced Plasma ACTH Level in Normal Rats

Treatment	ACTH (pg/ml)
Non stress	13.2 $\pm$ 6.0
Stress	123.3 $\pm$ 4.2 ^{a)}
+Diazepam	62.8 $\pm$ 18.4 ^{b)}
+Chamomile Oil	73.6 $\pm$ 9.4 ^{b)}
+Diazepam+ Chamomile Oil	85.8 $\pm$ 14.9 ^{c)}

Degree of stress and chemical treatment: twice a day for 3 days

Diazepam: 1.0 mg/kg, i.p., Chamomile oil: 0.7 ml/one trial

Restriction stress time: 30 min. Normal female rats were used at 10 weeks after birth. Data are means $\pm$ S.E. n=5 a) $p < 0.01$ vs. Non stress b) $p < 0.01$ vs. Stress c) $p < 0.05$ vs. Stress (Student's t-test)

Table 2. Effect of Inhalation of Chamomile Oil Vapour and Diazepam on Restriction Stress-induced Plasma ACTH Level in Ovariectomized Rats

Treatment	ACTH (pg/ml)
OVX	22.7 $\pm$ 17.7
Stress	155.0 $\pm$ 8.7 ^{a, b)}
+Diazepam	92.2 $\pm$ 20.8 ^{c)}
+Chamomile Oil	63.0 $\pm$ 21.6 ^{c)}
+Diazepam+ Chamomile Oil	18.3 $\pm$ 4.8 ^{d)}

OVX: Ovariectomy, OVX-rat used at 20 weeks after operation (at 7 week). Data are means $\pm$ S.E. n=5 a) $p < 0.01$ vs. OVX, b) $p < 0.05$ vs. Stress of Normal (Table 1), c) $p < 0.01$ vs. Stress, d) $p < 0.01$ vs. Diazepam and Chamomile (Student's t-test)

Many researchers have reported that a variety of stressful stimuli cause a marked increase in catecholamine, serotonin and GABA metabolism in the rat brain as well as ACTH release in plasma.^{8,9)}

Diazepam, one of the benzodiazepine derivatives, is extensively used in the treatment of anxiety. It has been reported that diazepam attenuated restriction stress-induced increase in catecholamine turnover in the rat brain; it also enhanced the activity of GABA neurons. Therefore, the stress-induced increase in plasma ACTH level is attenuated via the decrease in corticotropin-releasing hormone

release in the rat hypothalamus. ^{10,11,12)}

Table 3. Effect of Flumazenile on Inhalation of Chamomile Oil Vapour and Treatment of Diazepam-induced Plasma ACTH Level

Treatment	ACTH (pg/ml)
OVX	18.6 $\pm$ 3.5
Stress	164.5 $\pm$ 6.1 ^{a)}
+ Flumazenile	159.6 $\pm$ 11.5
+ Diazepam + Flumazenile	172.3 $\pm$ 8.2
+ Chamomile + Flumazenile	168.1 $\pm$ 10.1

Flumazenile: 0.1 mg/kg i.p. x 2/dayx3, Diazepam: 1.0 mg/kg i.p. x2/dayx3

Chamomile oil: 0.7 ml/one trial

Degree of stress and chemical treatment: twice a day for 3 days

Data are means $\pm$ S.E. n=5 a)p < 0.01 vs. OVX (Student's t-test)

These findings suggest that chamomile oil may have an effect on GABAergic systems in the rat brain and may have an activity similar to benzodiazepine agonists.

Further investigation is being undertaken to clarify the underlying mechanism.

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