—Regular Articles—

Sedative Effect of *Centranthus longiflorus* ssp. *longiflorus* in Rats and the Influence of Adrenalectomy on its Effect

Halis SULEYMAN,[a] Zuhal GUVENALP,*[,b] Mehmet KIZILKAYA,[c] and L. Omur DEMIREZER[d]

[a]*Department of Pharmacology, Faculty of Medicine, Ataturk University, Campus 25240, Erzurum, Turkey,* [b]*Department of Pharmacognosy, Faculty of Pharmacy, Ataturk University, Campus 25240, Erzurum, Turkey,* [c]*Department of Anesthesiology, Faculty of Medicine, Ataturk University, Campus 25240, Erzurum, Turkey, and* [d]*Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Sıhhiye 06100, Ankara, Turkey*

(Received February 13, 2007; Accepted March 20, 2007)

Sedative effect of the aqueous extract of *Centranthus longiflorus* ssp. *longiflorus* (Cle-1) on intact and adrenalectomized rats was investigated using a thiopental sleeping test to clarify the relationship of this effect on adrenal gland hormones, particularly glucocorticoids. Adrenal gland hormones were found to play an important role in inhibiting the sedative effect of the investigated drugs. It is clear, however, that these hormones are not glucocorticoids. Glucocorticoids were not responsible for shortening the sleep period.

Key words——*Centranthus longiflorus* ssp. *longiflorus*; sedative effect; rat; thiopental sodium

INTRODUCTION

The genus *Centranthus* (Valerianaceae) is represented by three species in the flora of Turkey.[1] Pharmacological studies were undertaken as long as ago as 1907 which demonstrated experimentally that *V. officinalis* extracts possessed a sedative effect. Many subsequent studies have confirmed these early findings but the identity of the compounds responsible has been a matter of controversy which has still not been fully resolved.[2] Aqueous preparations of valerians which belongs to the same family as *Centranthus* have been used as a major sedative in phytomedicine. However, the biochemical mechanism of *Valeriana officinalis* extract in the nervous system is still unknown.[3] Iridoids and valepotriats are among the constituents of the aqueous extract of *Valeriana officinalis* and are thought to be responsible for the central nervous depressant activity associated with these extracts.[4,5] *Centranthus longiflorus* ssp. *longiflorus* is a perennial herb and is traditionally used as a sedative.[6] The chemical constituents of this plant have been reported in our previous study. Iridoids and valepotriats were isolated as well as some flavonoid and triterpene structures of other compounds.[7]

The goal of this study has to investigate the sedative effect of *Centranthus longiflorus* ssp. *longiflorus* on intact and adrenalectomized rats based on the relationship of this effect on adrenal gland hormones, particularly glucocorticoids.

*e-mail: zguvenalp@yahoo.com

MATERIALS AND METHODS

Plant Material The aerial parts of *Centranthus longiflorus* ssp. *longiflorus* were collected in July 1999 from Erzurum, eastern Anatolia, in the vicinity of Ispir. A voucher specimen is deposited in the Herbarium of Hacettepe University, Faculty of Pharmacy (HÜEF 99042) Ankara/Turkey.

Preparation of the Extract The powdered herb of *Centranthus longiflorus* ssp. *longiflorus* was extracted with methanol at 40°C under reflux for 4 h. The solvent was filtered and evaporated in a vacuum using a rotary evaporator. The residue was dissolved in water and partitioned with petroleum ether. The water extract was evaporated and the final residue was stored after lyophylization (Cle-1).

Animals 54 adult male Wistar albino rats weighing between 175–185 g from the experimental animal laboratory of Atatürk University were used. Animals were provided with standard laboratory diet, and were assigned to groups, each consisting of 6 animals.

Thiopental Sodium Sleeping Test on Intact Rats The aqueous extract of *Centranthus longiflorus* ssp. *longiflorus* (Cle-1) was given at doses of 100 and 200 mg/kg by oral gavage. Another group of animals was

treated with diazepam for comparison. Diazepam was given at a dose of 5 mg/kg by gavage. A further group of animals (control group) received the same volume of distilled water. One hour after administration of the test substances and distilled water, the rats received 25 mg/kg body weight thiopental sodium by intraperitoneal injection.

After thiopental sodium administration the beginning of sleeping time was taken to be when the animal assumed a supine position. When the animals turned into a quadruped prone position this was used as the end-point of sleeping time. Sleeping time was measured with a stop-watch in minutes. The effect of Cle-1 on sleeping time was compared to that of the control and diazepam groups.

Thiopental Sodium Sleeping Test on Adrenalectomized Rats In this series of our experiments, the sedative effects of Cle-1 were investigated on adrenalectomized rats. The adrenal glands were removed from rats anesthetized with 25 mg/kg ketamine.[8] After operation, the rats were nourished with 1% NaCl and pellet fodder for a period of 7 days. After the eighth day, 100 mg/kg of Cle-1, 5 mg /kg of prednisolone+100 mg/kg of Cle-1, 5 mg/kg of diazepam, 5 mg/kg of prednisolone+5 mg/kg of diazepam were administered by oral gavage. The control group was administered distilled water. One hour after administration of the test substances and distilled water, the rats received 25 mg/kg body weight thiopental sodium by intraperitoneal injection. The effect of Cle-1 on sleeping time was compared in that of the control and diazepam groups.

Statistical Analysis The Tukey test one-way analysis of variance in conjunction with a Student's *t*-test for independent samples was performed for statistical analysis and a probability level of $p<0.05$ was chosen as the criterion of statistical significance.

Values were reported as mean plus or minus standard error of mean (±SE).

RESULT AND DISCUSSION

The central nervous system has two types of neuromediators. Excitatory neuromediators (*e.g.*, dopamine, noradrenaline, serotonin, acetylcholine) act as stimulators, and inhibitory neuromediators (*e.g.*, GABA, adenosine, glycine) cause sedation. It is well known that the drugs which increase the level of GABA, adenosine and other inhibitory neuromediators produced the sedative effects.[4,9,10] Valeric acid causes inhibition of an enzyme system which degrade GABA. GABA level increases and a sedative effect occurs. *Centranthus* also contains valerianic acid. In our previous study the sedative effect of *Centranthus longiflorus* ssp. *longiflorus* was investigated on mouse.[11]

In this study, the effect of Cle-1 and diazepam were investigated compared for the increase of thiopenthal sodium sleep period in the intact and adrenalectomized rats.

Many pharmacological test method are available to determine the sedative effect of substances. Lengthening of the thiopenthal sodium sleep test is frequently used to evaluate effect. As is shown in Table 1, the thiopenthal sodium sleep period was found to be 84±37 min for Cle-1 (100 mg/kg) and 88.4±40 min for Cle-1 (200 mg/kg) when administered to intact rats, respectively. Sleep period for diazepam is 155±58 min and for control 16.2±14.7 min. One hundred mg /kg of both Cle-1 and diazepam significantly prolonged thiopenthal sodium induced sleeping times in intact rats. One hundred mg/kg of Cle-1 thus increased the sleep period of thiopenthal sodium 5.2 times more than control, but the effect of a double dose of Cle-1 (200 mg/kg) was found statistically meaningless. On the other hand, diazepam showed significant activity and increased the sleep period 9.5 times more than control. In our previous study on Cle-1, the active dose was found to be 100 mg/kg, but in the same study diazepam (5 mg/kg) showed lower activity than 100 and 200 mg/kg of Cle-1. Consequently, it can be said that Cle-1 showed sedative activity similar to diazepam and the latent period increased the sleep period.[11] The case in adrenalectomized rats is different (Table 2). The thiopenthal sodium sleep period was 422±76 min and 212±87 min for 100 mg/kg of Cle-1 and 5 mg/kg of diazepam administered rats, respectively. An interesting result was found from the control group. The thiopenthal

Table 1. The Effect of Cle-1 and Diazepam on Thiopenthal Sleep Period in Intact Rats

Drugs	Animals	Dose mg/kg	Latent period (min)	Sleeping time (min)	*p*
Cle-1	6	100	2.8	84.3±37	<0.05
Cle-1	6	200	2.4	88.4±40	<0.05
Diazepam	6	5	3.1	155±58	<0.05
Control	6	—	3.0	16.2±14.7	—

Table 2. The Effect of Cle-1 and Diazepam on Thiopenthal Sleep Period in Adrenalectomized Rats

Drugs	Animals	Dose mg/kg	Latent period (min)	Sleeping time (min)	p
Cle-1	6	100	2	422±76	>0.05
Cle-1+ Prednisolone	6	100 5	2	399±125	<0.05
Diazepam	6	5	3	212±87	<0.05
Diazepam+ Prednisolone	6	5 5	1.8	204±68	<0.05
Control	6	—	1.7	523±40.7	—

sodium sleep period of the control group was 523± 40.7 min. Comparison of the results of the control group of adrenalectomized rats and intact rats showed that the sleep period of the former was 32 times longer than that of the latter. Similar results are valid for Cle-1. One hundred mg/kg of Cle-1 increased the sleep period in adrenalectomized rats 5 times more than intact rats. Diazepam and Cle-1 potentiated the sedative effect of thiopenthal sodium in intact rats but antagonized it in adrenalectomized rats.

In other words, the sedative effect of Cle-1 and diazepam can change with the presence of adrenal gland hormones. Individual differences affect the results.[12)]

Variability of the sedative effect of these drugs in intact and adrenalectomized rats means that adrenal gland hormones play an important role inhibiting the effect. It is clear, however, that these hormones are not glucocorticoids. Glucocorticoids were not responsible for decreasing the sleep period. There was no statistical significance between the Cle-1 administered groups and the groups given Cle-1 plus prednisolone. A similar effect was observed between diazepam and diazepam plus prednisolone.

The mechanism of the sedative effect of Cle-1 and diazepam in adrenalectomized rats require more experimental studies.

REFERENCES

1) Davis P. H., ''Flora of Turkey and the East Aegean Islands,'' Vol. 4, University Press, Edinburgh, 1972, p. 558.
2) Hölzl J., ''Valerian the Genus Valeriana,'' ed. by Houghton P. J., Harwood Academic Publishers, 1997, pp. 55–75.
3) Santos M. S., Ferreira F., Faro C., Pires E., Carvalho K. P., Cunha A. P., Macedo T., *Planta Med.*, **60**, 475–476 (1994).
4) Houghton P. J., *J. Pharm. Pharmacol.*, **51**, 505–512 (1999).
5) Wagner H., Jurcic K., Schaette R., *Planta Med.*, **39**, 358–365 (1980).
6) Baytop T., ''Theraphy with Medicinal Plants in Turkey (Past and Present),'' Istanbul University Publications, No: 3255, İstanbul, 1984, p. 282.
7) Demirezer L. O., Guvenalp Z., Schiewe H. J., Strietzel I., Harmandar M., Zeeck A., *Phytochemistry*, **51**, 909–912 (1999).
8) Porsolt R. D., Le Pichon M., Jalfre M., *Nature*, **266**, 730–732 (1977).
9) Bloom F. E. ''Goodman Gilman's the Pharmacological Basis of Therapeutics,'' ed. by Brunton L. L., McGraw Hill, New York, 2006, pp. 317–386.
10) Rang H. P., Dale M. M., Ritter J. M., ''Pharmacology,'' Fourth ed., Churchill Livingstone, China, 1999.
11) Buyukokuroğlu M. E., Demirezer L. O, Guvenalp Z., *Pharmazie*, **57**, 559–561 (2002).
12) Kayaalp O. S., *Medical Pharmacology for Rational Therapy*, Hacettepe-Taş Ltd. Sti., Ankara, 2002.