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Sedative, anticonvulsant and behaviour modifying effects of *Centranthus longiflorus ssp. longiflorus*: a study of comparison to diazepam

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The aqueous extract of *Centranthus longiflorus* ssp. *longiflorus* (CLE) was investigated for sedative, anticonvulsant and behaviour modifying activity using thiopental sleeping, caffeine induced convulsion and forced swimming depression tests. When the effects of the aqueous extract of CLE (100 mg/kg) was compared to diazepam, it showed similar sedative and anticonvulsant effects to those produced by diazepam (5 mg/kg).

1. Introduction

The genus *Centranthus* (Valerianaceae) is represented by three species in the flora of Turkey [1]. 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 [2]. 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 [3–5]. *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 other compounds possessing flavonoid and triterpene structures [7].

The aim of the present study was to investigate the nervous depressant activity of *Centranthus longiflorus* ssp. *longiflorus* prepared under defined conditions and to compare its activity with reference drugs.

2. Investigations, results and discussion

In the present experiments, the sedative, anticonvulsant and behaviour-modifying activities of the aqueous extract of *Centranthus longiflorus* ssp. *longiflorus* (CLE) were investigated and the effects were compared with classical sedative and anticonvulsant drugs.

γ-Aminobutyric acid (GABA) is generally considered to be the major inhibitory neurotransmitter within the mammalian CNS, playing a crucial role in the pharmacology of stress and anxiety [8]. *Valeriana officinalis* has been reported to induce the release of [^3H] GABA in rat brain synaptosomes, yet its mechanism of action *in vivo* is still unclear [9]. Sedative, anticonvulsant and antidepressant activity of *Valeriana* spec. has been attributed to GABA-ergic effects [8]. The sedative activity of *Centranthus longiflorus* ssp. *longiflorus,* which belongs to the same family and has similar chemical composition to *Valeriana* sp. [7], was investigated using a short-effect CNS depressant thiopental sodium sleeping test [10]. The activity of thiopental is increased with benzodiazepines (diazepam etc.). CLE significantly increased the sleeping time induced by thiopental sodium in mice in a dose-dependent manner, producing an effect 1.24, 1.74, 2.48 and 2.07 times greater than the control group at the doses of 25, 50, 100 and 200 mg/kg respectively, while with diazepam the sleeping time was increased 1.93 times. With 25 mg/kg CLE treated animals no significant activity was observed, but at higher doses of CLE (50, 100, 200 mg/kg) the activity was similar to that of diazepam and was found to be significant when compared to that of the control ($p < 0.001$). No significant difference in activity was found between diazepam (5 mg/kg) and CLE at 50, 100 and 200 mg/kg doses ($p > 0.05$). Our results show that the thiopental sodium induced sleeping time was extended by CLE at doses up to 100 mg/kg whereas at 200 mg/kg it caused no further increase (Table).

100 mg/kg doses of CLE extended sleeping time 2.5 times when compared to the control group. In a similar study Rosercans et al. [11] showed that 50, and 100 mg/kg doses of valerenic acid isolated from *Valeriana officinalis* increased pentobarbital (60 mg/kg) induced sleeping time in mice 1.6 and 2 times more than the control group. In another study where the effect of 100 and 200 mg/kg doses of alcoholic extracts of *V. officinalis* on 35 mg/kg pentobarbital-induced sleeping time in rats, results similar to our study were obtained. A sleeping effect was not observed with the increased dose [12]. When compared to the results of this study, our results show that the aqueous extract of *Centranthus longiflorus* ssp. *longiflorus* possesses stronger activity than *Valeriana*.

Adenosine inhibits the release of various neurotransmitters from the nerve endings and as a result of this, the sensitivity to the extatory neuromediator of the post-synaptic membrane decreases. It has been suggested, that inhibition of adenosine uptake in the brain increases neuronal activity. In this case adenosine acts partly as a mediator for the sedative and anticonvulsant activities of benzodiazepines and barbiturates [10]. It has also been claimed that the

Table: Effect of CLE and diazepam on thiopental sleeping time in mice, latent period time for caffeine-induced seizure in mice and immobility time in the forced swimming test in rats

Drug	Dose (mg/kg)	Sleeping time (min) Mean ± S.E.M.	Latent period time (sec) mean ± S.E.M.	Immobility time (sec) mean ± S.E.M.
Vehicle	–	36.3 ± 5.7	110.0 ± 2.2	215.8 ± 5.7
CLE	25	45.0 ± 2.4	–	–
CLE	50	63.3 ± 4.5*	116.2 ± 6.6	179.2 ± 4.9*
CLE	100	90.0 ± 12.1*	169.8 ± 5.3*	149.2 ± 3.5*
CLE	200	75.3 ± 9.7*	124.0 ± 3.6*	184.7 ± 3.8*
Diazepam	5	70.0 ± 8.8*	152.3 ± 9.2*	91.0 ± 3.6*

N (number of rats per group) = 6 for all groups
*$p < 0.01$ vs. control (by ANOVA, Tukey test)

convulsant effects of both caffeine and theophylline at higher doses are due to the inhibition of adenosine receptors. Additionally, caffeine antagonises various effects of diazepam by affecting the benzodiazepine receptors [13]. In our study, oral administration of caffeine (300 mg/kg) induced convulsions, whereas 50, 100 and 200 mg/kg doses of CLE did not show any significant activity ($p > 0.05$). However a 100 mg/kg dose of CLE extended the latency time. The 100 mg/kg dose of CLE showed more activity than 5 mg/kg of diazepam but this difference was not significant ($p > 0.05$). CLE antagonised the convulsant effect of caffeine more than diazepam. It can be concluded that the sedative effect of CLE was higher than that of diazepam. With increasing doses of CLE (200 mg/kg) the activity was decreased (Table).

Immobility period with 100 mg/kg of CLE was 31% shorter than the control group while in the diazepam administered group it was 58% shorter. A decrease of 17% and 14.5% in immobile response was obtained with 50 and 200 mg/kg, respectively, compared to that of control. The diazepam administered group swum immediately whereas the group given CLE were immobile for 30–60 min and then began to move. This can be attributed to the behaviour modifying effect of CLE as well as to its significant hypnotic-sedative effect, which results in the vigilance and psychomotor reactions of animals falling below the normal level and a decreased orientation to environment. Although antidepressant doses of benzodiazepines did not produce sedation, a sedative effect was produced by higher doses. This observation can be used to explain the mobility of diazepam-administered animals (Table).

100 mg/kg doses of CLE produced the maximum effect in the sleeping, convulsion and immobility tests while the effect was decreased with increased doses. This may be due to the autoinhibitor feed-back mechanism, which depends on excessive stimulation of GABA-ergic receptors of the autonomic nervous system [8]. It appears that the role of valerians include not only a GABA ergic effect, but also cholinergic, dopaminergic, serotonergic and adenosinergic effects. CLE shows more significant sedative, anticonvulsant and behaviour modifying effects than the valerian extract. As CLE has a similar chemical content to the valerian extract, *Centranthus longiflorus* ssp. *longiflorus* can therefore be used in diseases in which valerians are used and may have a commercial value.

3. Experimental

3.1. 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.

3.2. 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 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 lyophylisation.

3.3. Animals

Male and female albino mice weighing between 30–35 g and Sprague Dawley rats weighing between 180–210 g were used.

Animals were provided with standard laboratory diet. Food was withdrawn 16 h before administration. Animals were assigned to 6 groups, each consisting of 6 animals.

3.4. Thiopental sleeping test

The extract of *Centranthus longiflorus* ssp. *longiflorus* (CLE) was given at doses of 25, 50, 100 and 200 mg/kg by oral gavage. Another group of animals was treated with diazepam for comparison. Diazepam was given in a volume of 0.5 ml (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 mice received 25 mg/kg body weight thiopental sodium by intraperitoneal injection.

After thiopental 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 on sleeping time was compared to that of the control and diazepam groups. All the experiments were performed between 2–4 p.m.

3.5. Effect on caffeine-induced convulsions

The first group served as a control which received the vehicle by oral gavage. The remainder of the extract of *Centranthus longiflorus* ssp. *longiflorus* was given at doses of 50, 100 and 200 mg/kg p.o. by gavage. A further group of animals was treated with 5 mg/kg in 0.5 ml diazepam for comparison. One hour after administration, caffeine was injected intraperitoneally at a dose of 300 mg/kg, and then the animals were placed in a Plexiglas cage ($30 \times 30 \times 40$ cm). Animals were watched for seizures and the time between caffeine administration and the first tonic convulsion which persisted for at least 5 s was measured (latency of convulsion) The increase in latency of convulsion was taken as an indication of protection. The effect of CLE on convulsions induced with caffeine was compared with the control and diazepam groups. All tests were conducted between 9–11 a.m.

3.6. Forced swimming test (FST) [14]

The studies were carried out on rats according to the method of Porsolt et al., 1977 [14]. Male Sprague-Dawley rats (180–210 g) were divided into 5 groups. Each group consisted of 6 animals. Briefly, the animals were placed individually in a swimming apparatus (Plexiglas cylinders, 45 cm in height; 28 cm in diameter), containing 30 cm^3 of water maintained at 21–24 °C. After 15 min, the animals were removed to a drying room. Twenty-four hours after their first exposure, the animals were replaced in the swimming apparatus for 5 min and their behaviours were monitored by stopwatch for subsequent analysis. Loss of fore extremity movement was recorded as immobility. 50, 100 and 200 mg/kg of CLE and 5 mg/kg diazepam were administered P.O. by gavage at an application volume of 1 ml one hour before re-test.

The antidepressant activity of CLE was compared to the control and diazepam groups. All testing was conducted between 9–11 a.m.

3.7. Acute toxicity test

Animals were divided into 4 groups which consisted of 6 animals, and then placed in a cage. 500, 1000, 1500 and 2000 mg/kg doses of CLE were given to mice by gavage. The animals were observed for 24 h. At the end of this time the number of animals still alive was determined.

3.8. Chemicals

Caffeine (Sigma-Germany), diazepam (Deva-Turkey), and thiopental sodium (Abbot-Turkey) were used in this study.

3.9. 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 (± SEM).

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