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Preliminary reports

PHARMACOLOGICAL *IN VITRO* SCREENING OF THE CENTRAL MONOAMINERGIC EFFECTS OF *VALERIANA OFFICINALIS* EXTRACTS

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Pharmaceutical preparations of valerian root and rhizome (*Valeriana officinalis* L., Valerianaceae) have widespread use for sedation and treatment of hysteric states, anxiety, stress and insomnia (MORAZZONI and BOMBARDELLI, 1995; HOUGHTON, 1999; WEISS and FINTELMANN, 2000; CROPLEY *et al.*, 2002). Pharmacological investigations of valerian were mostly concentrated on the components of the essential oil - valerenic acids, non-glycosidated iridoids named valepotriates and its degradative products - baldrinals, but also on several lignans. For some of these components, interaction with GABA, benzodiazepine,

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adenosine, melatonine and barbiturate receptors were found (MORAZZONI and BOMBARDELLI, 1995; HOUGHTON, 1999; MULLER *et al.*, 2002; ABOURASHED *et al.*, 2004). Some recent findings indicated that most of the pharmacological effects of valerian, and especially of valerenic acid, are mediated through modulation of GABA A receptor function (YUAN *et al.*, 2004). However, the mechanism of the valerian neuropharmacological action is not completely resolved yet and it remains the object of actual research.

Based on the evidence that monoaminergic systems and primarily central dopamine (DA) and/or serotonin (5-HT) system(s) participate essentially in the control of virtually all mental processes and sleep (Nestler *at al.*, 2001), we screened *in vitro* total dichlormethane (CH_2Cl_2) and methanol (MeOH) extracts of *V. officinalis* on DA-ergic and 5-HT-ergic activity. *In vitro* radioassays on rat brain preparations were used to evaluate possible influence of the extracts on DA receptors (D_1, D_2), 5-HT receptors ($5\text{-}HT_{1A}$, $5\text{-}HT_{2C}$, $5\text{-}HT_3$) and 5-HT synaptosomal uptake. The potential of the valerian extracts to inhibit rat microsomal monoamino oxidase (MAO) were also explored.

Air-dried roots and rhizomes of cultivated valerian (Institute "Josif Pančić" Pančevo, Serbia) was freshly powdered (100 g) and successively macerated with 500 (+ 300) ml of either CH_2Cl_2 or MeOH at room temperature. After evaporation of filtrates under low pressure, 3.87g and 4.04g of dry extracts were obtained, respectively. Valepotriates, valerenic acids and terpenes (essential oil) were identified in CH_2Cl_2 extract, while phenolic acids were detected in MeOH extract, by TLC analysis (WAGNER and BLADT, 1996). Both extracts were dissolved in dimethylsulpoxide (DMSO) by sonication to a final concentration of 20 mg/ml (stock solutions). Serial dilutions (1.0 mg/ml - 0.1 µg/ml) of the stock solutions in adequate buffers were made for all experiments. Commercial hydroethanolic extract of St. John's Wort (*Hypericum perforatum)* is a product of the Institute "Josif Pančić", Belgrade.

Table 1 . - The effect of Valeriana officinalis extracts on in vitro DA and 5-HT receptor binding, 5-HT reuptake and MAO activity. Radioassays were performed on rat brain synaptosomal and hepatic microsomal (MAO) preparations. Commercial hydro-ethanolic extract of Hypericum perforatum was used for comparison. The results, expressed as mg of extract per ml of incubation mixture, are mean IC50 values ± S.E.M. obtained from 3-4 experiments

Receptor/activity	D_1	D_2	$5\text{-}HT_{1A}$	$5\text{-}HT_{2C}$	$5\text{-}HT_3$	5-HT reuptake	MAO
^{3}H-Radioligand	SCH23390	spiperone	8-OH-DPAT	mesulergine	zacopride	5-HT	tyramine
(Ci/mmol)	(91)	(25)	(245)	(86)	(83)	(128)	(^{14}C; 55)
Conc. in assay (nM)	0.4	0.2	1.5	1	1	50	-
V. officinalis extracts:							
CH_2Cl_2	0.081 ± 0.035	0.95 ± 0.15	0.69 ± 0.14	0.64 ± 0.20	> 1	0.090 ± 0.021	0.29 ± 0.09
MeOH	>> 1	>> 1	>>1	>>1	>>1	> 1	> 1
H. perforatum extract:							
EtOH/H_2O	> 1	-	> 1	> 1	-	0.0060 ± 0.0013	0.47 ± 0.11

Brains of adult male Mill-Hill hooded rats (200-240 g b.w.) were dissected and used for synaptosomal preparation. The details of the preparation and a methodology of receptor competitive binding and synaptosomal 5-HT reuptake radioassays are described elsewhere (VOGEL and VOGEL, 1997; TOMIĆ *et al*, 2005), while the concentrations of the specific radioligands in assays, are presented in Table 1. The potency of extracts to inhibit MAO enzymes isolated from rat liver microsomal fraction was estimated by rating the level of *in vitro* ^{14}C-tyramine degradation. (TOMIĆ *et al*, 2005). Competition curves were constructed and analyzed by "GraphPad Prism" (v. 4.0.) software.

The results of the radioligand binding assays (Table 1) show that only the CH_2Cl_2 extract of valerian expressed detectable affinity (IC_{50} < 1 mg/ml) at most of the tested DA and 5-HT receptors. Also, this extract exhibits modest influence on microsomal MAO activity (IC_{50} = 0.29 mg/ml). A notable binding capacity of the CH_2Cl_2 extract at the D_1 receptors (IC_{50} = 81 μg/ml) seems to be rather low to be neuro-pharmacologically significant, when it is compared to the presented nanomolar affinity of the typical dopaminergic drugs (NESTLER *et al.*, 2001). In addition, the CH_2Cl_2 extract showed similar inhibiting potency on *in vitro* synaptosomal 5-HT reuptake (IC_{50} = 90 μg/ml). We collated this level of activity to the analogous inhibitory effect of the hydroethanolic extract of St. John's Wort. The commercial extracts of this herb are well known as over-the-counter antidepressant drugs with the proposed primary active mechanism of the central 5-HT reuptake inhibition (MULLER *et al.*, 1997). In our study, the St. John's Wort preparation expressed significant inhibition of 5-HT uptake with IC_{50} = 6.0 μg/ml, what, in comparison to the determined IC_{50} values for the valerian extracts, excluded this mechanism as a dominant source of valerian psycho-activity. However, it is still possible that in cooperation with already suggested and more evident valerian effects on the other systems for the central neurotransmission (e.g. GABA, adenosine), such degree of valerian interaction with 5-HT reuptake and, possibly, D_1 receptors, may contribute to its complete behavioral effects. The effect of valerian on three subclasses of 5-HT receptor in our study was not so prominent, but some recent studies found more pronounced interaction of valerian extracts on 5-HT_4 , 5-HT_{5A} ,5-HT_6 and 5-HT_7 subclasses of this receptor (ABOURASHED *et al.*, 2004; DIETZ *et al.*, 2005). The pharmacological consequences of these interactions are not yet elucidated.

In conclusion, based on our TLC analysis of the extracts, the majority of approved active components of valerian (e.g. valepotriates, essential oil components) are contained in the CH_2Cl_2 extract. This coincides with our findings regarding specified monoaminergic *in vitro* activity of this extract alone, although the degree of this action seems to be insufficient to explain the proposed valerian psychoactivity. However, these results suggest that particular pure constituent(s) of valerian CH_2Cl_2 extracts could remain stronger inhibitors of *in vitro* synaptosomal 5-HT reuptake, as also of DA D_1, that needs further re-evaluation of the individual liposoluble components of this extract.

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