УДК 615.322 +635.9+612.015

H. M. GALSTYAN
Yerevan State Medical University after M. Heratsi
Pharmacognosy department

SOILLESS CULTIVATION AND APPLICATION PERSPECTIVES OF GERMANDER IN MEDICINE

The opportunity and efficiency of introducing Germander (Teucrium Polium L. Lamiaceae) into soilless culture has been confirmed by us for the first time. Plants grown in gravel differed with yield and high content of biologically active substances. They were nourished with Davtyan's normal density nutrient solution. Due to low toxic influence and high content of biologically active substances, the water fraction of hydroponics T polium ethanol extract was studied by electrophysiological method on female rats with two-sided ovarian ectomy. Intramuscular injection confirms the anticholinesterase and impulse transfer effect.
Keywords: *hydroponic Teucrium polium, ovariectomy, basal nucleus of Meynert, neuroprotection.*

Introduction

Not all herbs applied in folk medicine are officially recognized and Germander (Teucrium polium Lamiaceae) ranks among them. Traditionally, Teucrium polium has been used for different pathological conditions such as gastrointestinal disorders, inflammations and type 2 diabetes. During the past 40 years, different classes of compounds have been isolated from various parts of T. polium of which the main groups are terpenoids and flavonoids [2]. The plant has several varieties, which chemical compositions definitely differ from each other [16]. Anticholinesterase effects of Teucrium polium in the nervous system are less studied [1]. On the other hand numerous clinical and experimental studies of postmenopausal women and ovariectomized (OVX) animals testify about neurodegenerative alterations in various structures of the brain, associated with mental function (in particular, hippocamp and basal nucleus of Meynert (bnM)) [21, 18]. In diabetic rats Teucrium poliu treatment reversed learning and memory deficits: antioxidant, anticholinesterase and hypoglycemic effects of Teucrium may be involved in the obtained effects [12]. Earlier by us was showed that, hydroponic Teucrium polium contains flavonoid, terpenoid, iridoid and phenilpropanoid glycosides, exhibiting high biological activity [9, 14, 4]. We advanced a hypothesis about neuroprotective efficiency of hydroponic Teucrium polium in rats with chronic OVX.

Materials and methods. The plants of Teucrium polium L were imported from Khosrov reserve of Garni district and planted in the open-air hydroponics and soil culture conditions of the Ararat valley. The trials were carried out in hydroponics vegetative vessels with 2m^2 nutrient surface, 6 plants on account of 1m^2. As a substrate 3-15mm particles' diameter gravel, volcanic slag and their mixture with 1:1 ratio were used. The plants were nourished by Davtyan nutrient solution [7]. Soil culture served as a control, where accepted rules in agrotechnician were preserved. The trials were conducted by 4-6 replications. The data were subject to mathematical working out [8]. Two harvests were made in June (blossoming period) and in August (budding period) substrate selection and one harvest at the beginning of June (budding period) - the test of the nutrient solution's influence. At the same time the dynamics of biological active substances' accumulation during the plant's budding, blossoming and insemination periods on gravel substrate, as well as on the samples brought from Garni were investigated.

The microelectrophysiological study was performed on Albino rats (220±20) in the groups Norm (n=4 rats), OVX (n=5), OVX+Teucrium polium (n=5). Under Nembutal anesthesia (35 mg/kg, i/p) was performed bilateral ovariectomy (OVX). After 6 and 8 weeks of OVX was performed extracellular registration of background and induced spike activity of single neurons nbM under high-frequency stimulation (HFS) of ipsilateral hippocamp. In acute experiment under Urethan anesthesia (1 mg/kg, i/p) the animals were immobilized by 1 % ditiline (25mg/kg i/p) and was given artificial breathing. The stimulatory electrode was input in hippocamp according to stereotaxic coordinates (AP -3.5, L ±2.0, DV +3.5 mm) and glass recording electrode with 1µ diameter of tip, repeatedly have submerged in nbM (AP-1.08, L±3, DV+7.4 mm). HFS (100 Hz during 1 second) had performed by means of charge by 0.16 mA amplitude. On-line registration and mathematical analysis of spike activity carried out on the basis of program (worked up by V.S Kamenetski), which provide the selection of spikes and exclusion of artefacts during HFS by amplitude discrimination, which allows to evaluate not only tetanic, but also posttetanic activity. On the base of analyisis of peristimul spiking was built the timing, frequency and cumulative histograms for single neurons, as well as of those averaged for whole massive of different types of responses in compared experimental groups. For statistic evaluation of differences of peristimulus interspike intervals uses t-criteria of Student's, as well as non-parametric method of checking by using a biselected test of Vilkokson-Mann-Whitney. Data were compared with tabulated values of normal distribution. For analysis was used the spike of neuronal activity by statistic significant level ($P \leq 0.05$).

Results and their discussion. An analysis of the substance accumulation dynamics of planting samples in Figure 1 gives us the opportunity to conclude that their maximum accumulation occurs in the budding phase (at the beginning of June) 1.3-1.5 fold, exceeding flowering and insemination phases. The content of biologically active substances in different eco-conditions show that hydroponic culture has medial position compared to wild and soil (control) [10]. The following samples of Teucrium polium have been studied for toxicity: 1. Wild plants grown in the territory of Garni; 2. Plants cultivated in hydroponic conditions in the Institute of Hydroponics Problems (IHP) (substrate, gravel, 1N Davtyan's nutrient solution); 3. Soil plants grown in the IHP (control). The results of toxicity content that the hydroponic culture of Teucrium polium has medial position compared to wild and soil (control) [5]. Earlier by us are defined therapeutic doses of water fraction of ethanol extract of hydroponic TPL (5 % from maximum endurable dose, equal to 400 mg/kg) [5]. As hydroponic Teucrium polium is less toxic and has active enough substances, therapeutic investigation was carried out on water fraction of ethanolic extract (because phenolic and flavonoid glycosides are in that fraction) (Fig. 1).

In the groups of Norm, OVX, OVX+Teucrium polium in neurons of bnM are recorded the excitatory and inhibitory responses under HFS of hippocamp, as well as are revealed the areactive units. The induced responses are in the form: excitation during HFS -

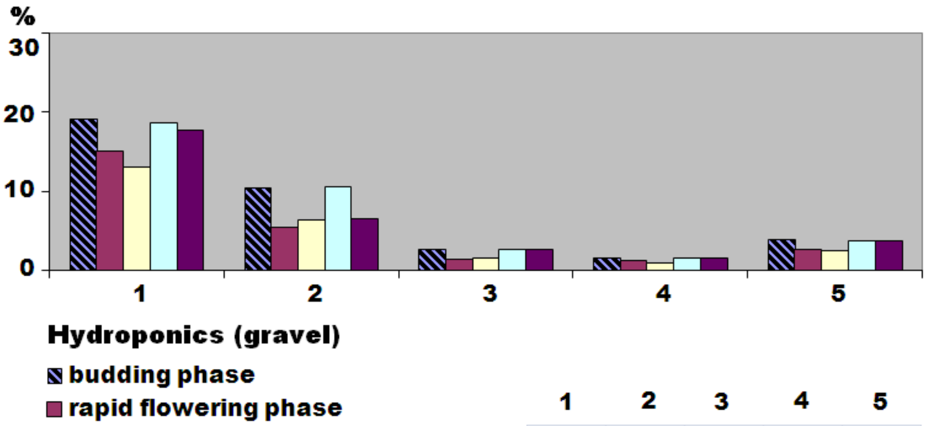
tetanic potentiation (TP), within poststimulus period - posttetanic potentiation (PTP), inhibition during HFS - tetanic depression (TD), within poststimulus period - posttetanic depression (PTD), as well as TD_PTP and without expressed changes of spiking before and after stimulation – areactivity. In the norm there is balance of excitatory (42.85%) and inhibitory (42.35%) responses. TD_PTP responses and areactive units composed 10.70% and 4% accordingly (Fig.2). The balance of excitatory and inhibitory responses registered in norm in single neurons and neural populations is disrupted in the dynamics of the development of OVX- induced neurodegeneration (Fig.2). After 6, 8 weeks of bilateral OVX in placebo control group about deficiency of neurotransmitters and damage of synaptic transmissions testify sharp increase in number of areactive neuronal units (Fig.2). On the 8th week following OVX in the placebo-control the predominant type of responses was the inhibitory (41.17%) and areactive units exceed the same in the Norm fourfold (16.2%: 4% = 4) (Fig.2). I/m injection of Teucrium polium (20 mg/kg within 3 weeks) starting from 3th week following OVX is prevented neurodegenerative alterations noted on the 6th week (according to criteria of expression of excitatory and inhibitory responses, statistical correlation of reactive and areactive units) (Fig.2). In the group of OVX+Teucrium polium in the dynamics of rehabilitation from 6th to 8th week is revealed the tendency of approximation to normal balance of types of responses, i.e, occurs balancing of excitation and inhibition, as well as reduction of the number of areactive units (Fig.2). Teucrium polium restores optimal balance between excitatory and inhibitory responses by means of neurotransmitter's redistribution and prevents progress of neurodegeneration initiated by OVX. These data suggest anticholinesterase activity of Teucrium polium and abilities its bioactive compounds to modulate some neurotransmitter systems. By modulating activity-dependent events, AChRs participate in fundamental aspects of synaptic plasticity.

It was known nicotinic acetylcholine receptors at glutamate synapses facilitate long-term depression or potentiation [11]. The network's circuitry is mainly comprised of glutamatergic neurons and synapses, which are under tight control from intrinsic GABA-ergic inhibitory interneurons and external inputs including cholinergic neurons [3]. Presynaptic nAChRs facilitate the release of a variety of neurotransmitters (both inhibitory and excitatory) throughout the brain [17]. In fact, activation of nAChRs modulates the transmission of information by altering both interneuron and pyramidal cell activation, affecting neuronal circuits at multiple levels. Hence, the net effect of cholinergic inputs on neural activity in the hippocampus can be quite complex [6].

Thus, in electrophysiological studies, is established the neuroprotective efficiency of water fraction of ethanol extract of hydroponic Teucrium polium.

Flavonoids repeates properties of some neurotransmitters and hormones [13]. The effect of luteolin on LTP and memory occurred by activation of CREB (cAMP response element-binding), which supports the therapeutic potential of luteolin for synaptic function [20]. Flavonoids with estrogenic activity abound; among the most commonly studied and discussed are rutin, catechin, apigenin, kaempferol, luteolin, chrysin and puerarin [19]. The isoflavone group of phytoestrogens weakly bind estrogen receptors and one clinical trial reported soy supplementation to improve women's performance of a memory exercise [15]. Thus, hydroponic Teucrium polium is advisable to recommend for approval in clinical practice to postmenopausal women for prevention of mental disturbances.

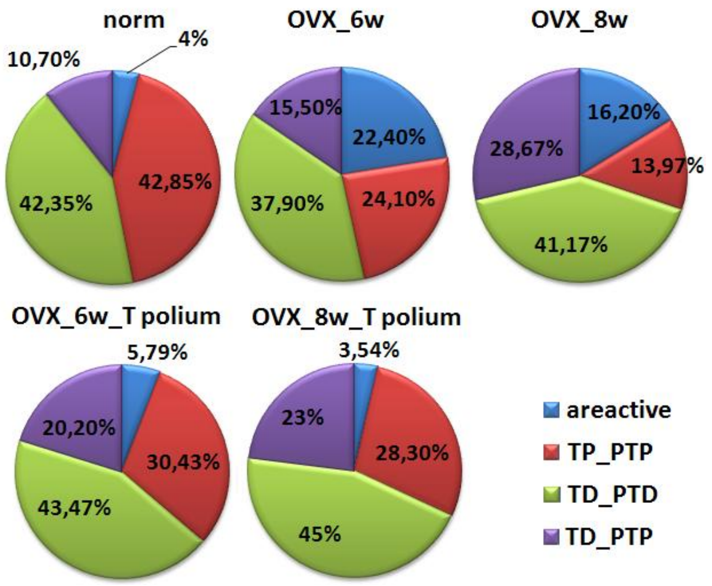
Figure 1. accumulation of polium’s active different conditions. 1. soluble substances; 2. soluble substances; 3. flavonoids; 4. 5. Teupolyozid.



	1	2	3	4	5
Hydroponics (gravel), budding phase	19,1	10,4	2,6	1,5	3,75
Hydroponics (gravel), rapid flowering phase	15	5,4	1,4	1,2	2,67
Hydroponics (gravel), insemination phase	13	6,4	1,6	0,9	2,5
Soil (Garni), budding phase	18,6	10,5	2,7	1,52	3,8
Soil (Garni), rapid flowering phase	17,7	6,5	2,6	1,5	3,79

Dynamics of Teucrium biological substances in growing Water- extractive Alcohol- extractive Sum Polyumozid;

Figure 2. Percental the excitatory (TP_PTP), TD_PTP responses and neurons of bnM in norm, following OVX in the groups OVX+Teucrium polium.



expressed balance of inhibitory (TD_PTD), areactive units of and after 6, 8 week of placebo-control and

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А. М. ГАЛСТЯН

БЕСПОЧВЕННОЕ ВЫРАЩИВАНИЕ ДУБРОВНИКА БЕЛОВОЙЛОЧНОГО И ПЕРСПЕКТИВЫ ПРИМЕНЕНИЯ В МЕДИЦИНЕ

Резюме: Впервые нами установлена возможность и целесообразность выращивания дубровника беловойлочного (Teucrium polium) в условиях гидропоники. Водная фракция этанольного экстракта гидропонического Teucrium polium благодаря малой токсичности и высокому содержанию биоактивных веществ электрофизиологически изучена на овариэктомированных животных. Внутримышечная иньекция Teucrium polium свидетельствует о антихолинэстеразной активности растения и способность биоактивных соединений модулировать некоторые нейротрансмиттерные системы.
Ключевые слова: гидропонический Teucrium polium, овариэктомия, нейропротекция.