NEUROPROTECTIVE POTENTIAL OF *AJUGA GENEVESIS* AND *AJUGA REPTANS* EXTRACTS IN PROTECTING AGAINST MEMORY DYSFUNCTIONS AND COGNITIVE DECLINE IN ANIMALS

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Abstract

Cognitive dysfunction, also known as dementia, is a disease that mainly affects dogs and cats and is is directly related to the aging of the animal, being marked by a decline in brain functions and occurs in 28% of cats and 50% of dogs over 11 years of age. The signs and symptoms of dementia are easily visible by simply observing the animal's behavior: it will learn very difficult new behaviors or they will not learn at all, and the dispositions and commands will be executed with difficulty compared to how they were done before the disease set in. In the present study, we investigated the potential of some extracts from the plant species Ajuga genevensis and Ajuga reptans to counteract cognitive dysfunctions, respectively of short-term memory (STM) and long-term memory (LTM). The behavior of seeking food (reward) and of immobility operationalize the concept of motivation for survival and are directly associated with the functionality of the central dopaminergic pathways and inversely associated with cognitive decline. Motor dementia is associated with cognitive dementia, and from the realization of a motor behavior to the activation of the neurogenesis process, studies have shown a direct relationship. A number of 30 white, male Wistar rats, aged 4 months, with a weight of 320±10g at the beginning of the experiment, were used for the research. According to the data obtained in the in vivo tests, in the three-arm maze test (Y-maze) all groups treated with extracts of Ajuga sp. prove an improvement in STM. The group treated with Ajuga reptans at a high dose (75 mg/kg body weight) showed the most intense STM-improving activity, comparable to that of healthy control animals. Regarding LTM, all groups treated with extracts of Ajuga sp. have had improvements. The batch treated with Ajuga genevensis extract in a low dose (of 25 mg/kg body) showed the most intense action of improving both LTM and STM, the intensity of the effect remaining relatively constant during the 7 days of administration. The obtained results open new directions of intervention in the case of cognitive decline and memory disorders in animals based on natural plant extracts.

Key words: *animal model, dementia, neuroprotection, memory, Ajuga, Alzheimer*

INTRODUCTION

In the present study, we investigated the potential of some extracts from the plant species *Ajuga genevensis* and *Ajuga reptans* to counteract cognitive dysfunctions, respectively of short and long-term memory on an animal model, Wistar rats.

Ajuga reptans L [syn. *Ajuga repens Host*.] is known in our country as "veneriță" or "vinețică" and *Ajuga genevensis L* is called as "suliman" [1, 2]. Both species are common in our country, the suliman developing also on soils very poor in nutrients. Regarding the chemical

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composition of the two species, it is known today that they contain: flavonoids, polyphenolic acids, non-volatile monoterpenes, diterpenes, triterpenes. [3, 4]. Active components are known to have antioxidant action, especially polyphenolic compounds (flavonoids and polyphenolic acids); but also anti-inflammatory and anabolic action. [5]

Ajuga species are used in the ethnomedicine of many European, African or Asian peoples. Starting from the uses in ethnomedicine [6, 7] of the species *Ajuga remota* from Kenya [8] and prescribed by indigenous herbalists in the treatment of more than 70% of malaria patients. Pharmacological studies undertaken for these have shown that some of them develop (concentration-dependent) inhibition of chloroquine-resistant *Plasmodium falciparum* parasites and *Mycobacterium tuberculosis*. Inhibition of the development of parasitemia was demonstrated in the mouse model using *Plasmodium berghei* which causes lethal malaria.

In our country, the fresh leaves are applied raw in the treatment of erysipelas, and the decoction was used in vaginal washes for women suffering from leukorrhea (for antimicrobial action). Suliman decoction (*Ajuga genevensis*) is recommended to be administered internally in indigestion [1]. Based on the data evaluated so far, we assume that the neuroprotective effect of the extracts could be due on the one hand to the anti-inflammatory action and on the other hand to the antioxidant qualities, but at the same time it is known that the phytocomplex itself could have a specific action.

MATERIAL AND METHOD

For the experimental part, a number of 30 animals were used, white, male Wistar rats, 4 months old, weighing 320±10g at the beginning of the experiment. The animals were procured from the Laboratory Animal Breeding Unit of the Cantacuzino Institute in Bucharest. The animals were housed in their maintenance room (room B-108) within the Faculty of Biology in Iași under conditions of: controlled temperature (22°C), artificially ventilated; 12 hour light/dark cycle (starting at 8 am) and 50% humidity. Animals were housed in 1500U (480x 325x 210 mm) polysulfone boxes with free access to food and water.

In the initial phase of the study, the animals were subjected to a microsurgical procedure called the stereotaxic technique, which approaches with maximum precision a target located intra-cranially using a well-established coordinate system. For the realization of the experimental model, the targeted nervous area was the dopaminergic mesotelencephalic system. The stereotaxic coordinates used were established according to the Stereotaxic Atlas. [9] 6-Hydroxy-dopamine (6-OHDA) is a neurotoxin specific to dopaminergic neurons, by injection the damage of the mesotelencephalic dopaminergic system in the right side of the rat brain is observed. According to specialized literature, the mechanism of intraneuronal action of 6-OHDA consists in the activation of neuronal apoptosis by inhibiting complex I of the electron transport chain at the mitochondrial level. In addition to inducing neuronal apoptosis, 6-OHDA causes neuroinflammation doubled by increased oxidative stress. [10].

In order to administer *Ajuga reptans* and *Ajuga genevensis* extracts to do behavioral tests, rats were divided into six groups two weeks after surgery as follows:

Group 1: " falsely operated " animals, which were administered intraperitoneally serphysiological (also called control group);

Group 2: animals operated and treated with neurotoxin 6-OHDA, which were administered intraperitoneally with physiological serum (also called 6-OHDA group);

Group 3: operated animals, treated with 6-OHDA, and injected after two weeks with 25 mg/mL *Ajuga reptans* extract administered intraperitoneally;

Group 4: operated rats, treated with 6-OHDA, and after 2 weeks with *Ajuga reptans* extract 75 mg/ mL intraperitoneally;

Group 5: operated animals, treated with 6-OHDA, and injected after two weeks, with 25 mg/mL *Ajuga genevensis* extract administered intraperitoneally;

Group 6: operated rats, treated with 6-OHDA, and after two weeks. with extract of *Ajuga genevensis* 75 mg/mL intraperitoneally.

The two studied variables were the administration of *Ajuga* extracts in different doses (the independent variable) and the improvement of cognitive deficits and memory deficiencies in animals treated with the neurotoxin (the dependent variable).

From the 15th day after the operation of the experimental animals, they were injected intraperitoneally, depending on the group to which they belonged, either extract of *Ajuga reptans* (groups 3 and 4) or of *Ajuga genevensis* group 5 and 6, while groups 1 and 2 were administered saline by the same method. All groups were composed of 5 animals each, and the amount of extract injected intraperitoneally was calculated according to body weight.

RESULTS AND DISCUSSIONS

Starting from the idea that the surgical intervention and the injection administration of 6-OHDA produce, in addition to the induction of neuronal apoptosis, and an inflammation doubled by an increased oxidative stress, we considered that the administration of the two *Ajuga* extracts could ameliorate experimentally induced cognitive deficits. We performed two behavioral tests: the three-arm maze test and the radial arm maze test.

Three-arm maze test: this test determines the influence of short-term memory [11[. To carry out this test, a device in the shape of a Y-shaped maze was used. The arms of the device are marked with one of the letters: A, B, C, each having a length of 35 cm, height of 25 cm and width of 10 cm (fig. 1). [11].

The test is based on the tendency of rodents to explore the arms of the Y-maze, thus providing information on exploratory and reward-seeking behavior and short-term memory. The spontaneous alternation behavior is defined as the successive input in the three arms (denoted in triplet sets: ABC, BCA, CAB, CBA, ACB, BAC), and the formula for calculating and expressing the result is rendered by the spontaneous alternation percentage.

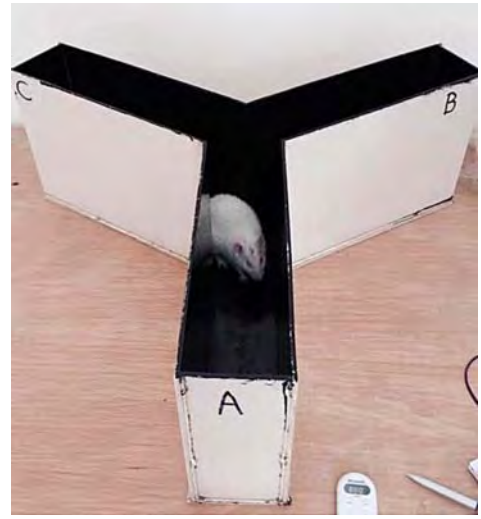

Fig. 1 The three-arm maze

The number of entries in the 3 arms assesses the locomotor activity which implies the presence of reward-seeking-exploratory behavior in which the central dopaminergic pathways are involved. Spatial memory as a component of short-term memory is assessed by: recognition of the new arm, time spent in the new arm, and sequence of entry into the three arms. The percentage of spontaneous alternation is defined as the ratio of the number of alternations performed to those possible

(calculated as the total number of arm entries minus two, all multiplied by 100) [12].

The results showed that in the group treated with 6-OHDA (group 2) locomotor activity decreased significantly ($p<0.01$) compared to the control group (group 1). This indicates that the administration of 6-OHDA affects the nigrostriatal dopamine pathway, but without inducing a severe impairment of locomotor activation, so as to prevent the animals from moving through the maze (Fig. 2). In the group pretreated with 6-OHDA and injected with *Ajuga reptans* extract 25mg/kg body weight (group 3), the locomotor activity was reduced compared to rats operated and treated only with 6-OHDA. When the dose of *Ajuga reptans* extract administered increased to 75 mg/kg body, the parameter (locomotor activity) improves, but without being able to reach the level recorded in the group 1 (control). (fig. 2).

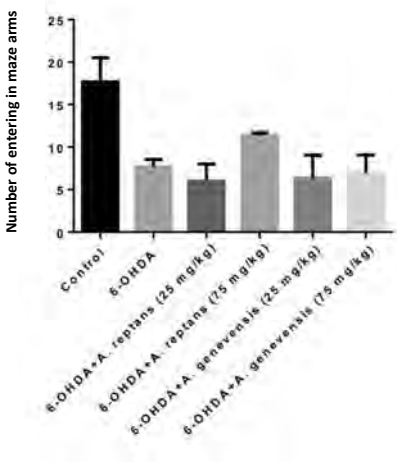

Fig. 2 Locomotor activity evaluated by the number of arm entries

Comparing the locomotor activity of all the animals enrolled in the experiment, we find, on the one hand, that it was reduced due to the injection into the substantia nigra of 6-OHDA, a marked reduction in three of the groups treated with *Ajuga* extracts, except for group 4, in which the animals were treated with 75 mg/kg body *Ajuga reptans* extract. This means that only high-concentration *Ajuga reptans* extract could counteract the degenerative effect induced by 6-OHDA on the dopaminergic pathways.

Regarding the percentage of spontaneous alternation (fig. 3) that evaluates short-term memory, the results show that group 2, treated with 6-OHDA, had significant decreases ($p<0.0001$) in short-term memory compared to the group 1 (control). Animals in group 3 were treated with 6-OHDA, after which 14 days later they started the intraperitoneal administration of *Ajuga reptans* extract at a dose of 25 mg/kg body weight showed decreases in spontaneous alternation compared to group 1 (control), but some better than group 2. For the 75 mg extract dose, spontaneous alternation is almost as good as in the control group (group 1) animals, suggesting that at this dose the active principles in the extract are effective.

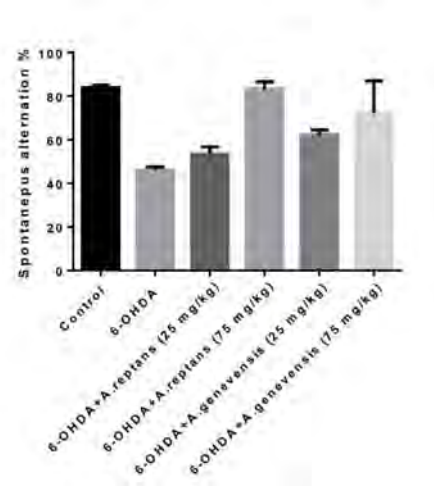

Fig. 3 The percentage of spontaneous alternation that evaluates short-term memory

Regarding the response of the experimental animals to the treatment with the two doses (respectively 25 mg, 75 mg/kg body) of *Ajuga genevensis* extract, we find that in this case the treated animals

show an improved spontaneous alternation compared to group 2 (6-OHDA group). After as can be seen from the image, the best percentage of spontaneous alternation was obtained for group no. 4 (6-OHDA + *Ajuga reptans* 75 mg/kg body) followed by group 6, in which the treatment consisted of the intraperitoneal administration of 75 mg/kg *Ajuga genevensis* extract; however, high-dose *Ajuga reptans* counteract the neuronal degradation effect induced by 6-OHDA, coming closest to the situation identified in the control group (group 1).

Long-term memory can be assessed with the radial arm-maze test [12]. The maze with radial arms (fig. 4) consists of a platform with a circular surface located in the center and 8 arms numbered from 1 to 8 arranged radially. The test investigates both short-term and long-term memory. The number of working memory errors was considered as a measure of short-term memory. A working memory error is defined as the animal's re-entry into a previously explored arm in which there was bait, because during each training session the animal is required to memorize which arm contains the bait and which does not. The number of reference memory errors informs us about long-term memory. A reference memory error is the rat's entry into an arm that never contained bait, information that also remained constant throughout each training session, with the animal having to memorize the position of each reward arm [11].

Fig. 4 The labyrinth with radial arms

Following the number of working memory errors (fig. 5) for the groups injected with *Ajuga reptans*, we find the following: while group 2 (treated with 6-OHDA) starts with a large number of memory errors, which during the training days improve, group 1 (control), group 3 (6-OHDA + 25 mg/kg body *Ajuga reptans*) and group 4 (6-OHDA + 75 mg/kg body *Ajuga reptans*), starts in the experiment with a small number of errors which remains reduced until the end, better response having the rats treated with high dose of *Ajuga reptans* extract, better even than that of the animals in the control group (group 1).

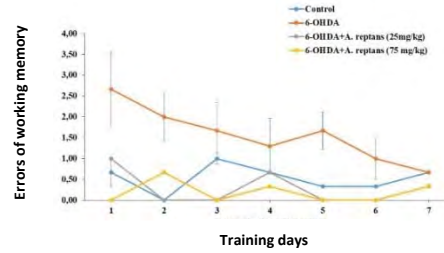

Fig. 5 Number of working memory errors for batches treated with *Ajuga reptans*

Compared to the situation mentioned above, as shown in fig. 6 *Ajuga genevensis* extract proved to be more effective both at the dosage of 75 mg/kg body, and especially at 25 mg/kg body.

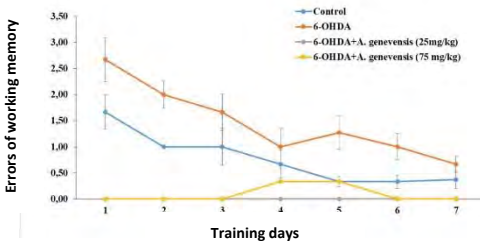

Fig. 6 Number of working memory errors for batches treated with *Ajuga genevensis*

Even if the suliman extract seems to be more effective in small doses (taking into account the reduction in the number of memory errors), we must not forget that there are numerous plant substances that act, depending on the dosage, differently, especially in inflammatory disorders. For example, extracts from *Boswellia serrata* act anti-inflammatory [13, 14, 15] when administered in the usual dose (600-900 mg/day), in adults, while underdosed, they act pro-inflammatory [16, 17].

In fig. 7 shows the results of the reference memory test, in the case of treatment with *Ajuga reptans* extract. Following the number of reference memory errors, we found that in group 2 (treated only with 6-OHDA) their frequency is high compared to the control, which suggests a long-term memory impairment.

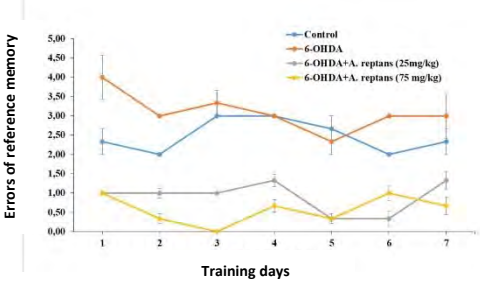

Fig. 7 Number of reference memory errors for batches treated with *Ajuga reptans*

As can be seen from fig. 7 for the group treated with *Ajuga reptans* extract, the reference memory errors fall below those of the control group, which could lead us to the conclusion of an improvement, a fact also found in the case of *Ajuga genevensis* extract treatment (fig. 8).

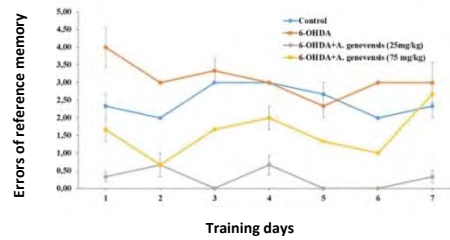

Fig. 8 Number of reference memory errors for batches treated with *Ajuga genevensis*

CONCLUSIONS

In neurodegenerative disorders, the degenerative process itself is accompanied by the appearance of neuroinflammation and increased oxidative stress that accelerates their evolution. The behavior of looking for food (reward) and of immobility operationalize the concept of motivation for survival and are directly associated with the functionality of the central dopamine pathways and inversely associated with cognitive decline.

The neurotoxin specific to the central dopaminergic pathways affects the short and long-term memory of the animals (6-OHDA groups) and the administration of the extracts led to the improvement of the memory decline (groups treated with plant extracts).

Groups treated with *Ajuga reptans* 75 ml/kg had an improvement in short-term memory approaching the performance of the control group (not treated with the neurotoxin).

Regarding reference memory (long-term memory), all groups treated with Ajuga extracts recorded fewer memory errors compared to the group treated with

the neurotoxin (control group) and compared to the healthy group (control group).

The animals in the group treated with *Ajuga genevensis* in the dose of 25 mi/kg had the best results in terms of the number of working memory errors, the results remaining constant during the 7 days of training.

REFERENCES

1. Butără V. Encyclopedia of Romanian ethnobotany. *Ed. Scientific and Encyclopedic* Bucharest **1979**; 226;
2. Blaschek W, Ebel S, Hackenthal E, Holzgrabe U, Keller K, Reichling J, Schulz V. Hagers Handbuch der Drogen und Arzneistoffe 7th *Ed. Springer Publishing Berlin Heidelberg NY* **2006**;
3. Hemcinschi Lungu A. Contributions to the study of some indigenous species of Galium and Ajuga, *PhD Thesis*, University of Medicine and Pharmacy Gr. T. Popa Iași **2011**;
4 . Hemcinschi A, Gales R, Stănescu U, Toma C. Comparative histo-anatomy and chemical composition of two Ajuga species from the Romanian flora. Scientific Annals of the University Al. I. Cuza, Iasi, *S. II Plant Biology* **2009**; 55(2):3346;
5. Ciocârlan V. Illustrated Flora of Romania 2nd Ed., *Ed. Ceres Bucharest* **2000**;
6. Cocquyt K, Cos P, Herdewijn P, Maes L, Van den Steen PE, Laekemann G. Ajuga remota Benth.: from ethnopharmacology to phytomedical perspective in the treatment of malaria. *Phytomedicine* **2011**; 18(14):1229-37;
7. Fekete G, Polgár IL, Báthori M, Col J, Darvas B. Per os efficacy of Ajuga extracts against sucking insects. *Pest Management Science* **2004**; 60(11):1099-1104;
8. Muregi FW, Ishih A, Miyase T, Suzuki T, Kino H, Amano T, Mkoji GM, Terada M. In vivo antimalarial activity of methanolic extracts from plants used in Kenyan ethnomedicine and their interactions with chloroquine CQ against CQ tolerant rodent parasite in small. *J Ethnopharmacol* **2006**; 111(1)190-19;.
9. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. *Academic Press,* New York **2005**;
10. Mevissen G. Parkinson in die Praxis translated. *Pharm. Ztg.* **2000;** 145:3935-3938;
11. Hrițcu L, Ioniță R, Motei DE, Babii C, Ștefan M, Mihășan M. Nicotine versus 6-hydroxy-L-nicotine against chlorisondamine induced memory impairment and oxidative stress in the rat hippocampus. *Biomedicine & Pharmacotherapy* **2017**; 86:102-108;
12. Hrițcu L. Experimental animal physiology. *University Ed. Al. I. Cuza Iasi* **2016**;
13. Kimmatkar N, Thawani V, Hingorani L, Khiyani R. Efficacy and tolerability of Boswellia serrata extract in treatment of osteoarthritis of knee - a randomized double blind placebo controlled trial. *Phytomedicine* **2003**; 10(1):3-7;
14. Kohler I. Pharmacokinetische Studien mit einem standardisierten Extract von Boswellia serrata. *Z. Phytother* **2006**; 27:95-97;
15. Sharma S, Thawani V, Hingorani L, Shrivastava M, Bhate VR, Khiyani R. Pharmacokinetic study of 11-Keto beta-Boswellic acid. *Phytomedicine.***2004**; 11(2-3):255-60;
16. Ammon HP T. Boswelliasäuren (Inhaltsstoffe des Weihraunchs) als überwirtschaften Prinzipien zur Behandlung chronisch endzündlicher Erkrankungen. *W.M.E.* **2002**; 152: 373-378;
17. Brenker R. Weihraunch bei rheumatischen Erkrankungen. *Z. Phytother* **2006**; 27:77-79.