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# Anxiolytic and mnemotropic effects of herbal extracts in an experiment on laboratory animals

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**Abstract.** We research memory, exploratory behavior and anxiety in adult rats in an experiment. These parameters were assessed through the use of behavioral assays, namely, the Suok test and passive avoidance learning. These parameters were studied after treatment with extracts of Ginkgo Biloba and salvia. The aqueous extract of salvia and Ginkgo-Biloba contributed to the activation of patterns of exploratory behavior and a decrease of anxiety in animals in the Suok-test. The Ginkgo-Biloba extract stimulates cognitive functions in rats, which is reflected in an improvement in the elaboration of the conditioned reflex and the preservation of the memorable trace after training in the passive avoidance learning.

**Keywords:** memory, exploratory behavior, anxiety, Suok test, passive avoidance learning.

## 1. Introduction

The reproduction of a memorable track after the elaboration of the passive avoidance learning is a convenient and applicable method for studying the influence of various substances on cognitive processes in experiments with rodents. When the animal is placed in the lighted half, because of the mink reflex, it quickly turns into a dark compartment. The animal passes into the dark compartment of the test not only under the influence of exploratory behavior, but also the innate preferences of dark areas of space (photophobia). In the dark compartment, the rat gets painful irritation (electric shock), which leads to a passive avoidance of the dark compartment. The quantitative measure of remembering the situation in the dark compartment (painful irritation) is the latency period of the stay of the rat in a light compartment during testing a day [1]. The presence of a sharp gradient between light and darkness makes it possible to verify the specificity of the acquired experience. An animal that has produced a memorable trace remains in the illuminated chamber, because active behavior is blocked by a conditioned emotional reaction [2]. This model allows to evaluate the selective action of various compounds at the stages of fixation, consolidation, storage and retrieval of the memory trace. The mnemotropic effect of various compounds can be evaluated with this model as well.

The Suok test is a "hybrid" of several traditional behavioral models; it allows to register a wide range of behavioral responses, from locomotion and exploratory activity to vegetative behavior markers. As a new method, behavioral model is recommended in neurogenetics, in psychopharmacology for screening psychotropic effects of drugs, in neurophysiology for analyzing the physiological activity of the brain.



The aim of the research was to study the effect of plant extracts on neurocognitive functions and behavioral responses in an animal experiment.

## 2. Materials and methods of research

Experiments were carried out on 80 adult male rats. Behavioral testing was always conducted between 10.00 and 12.00 o'clock. Animal care procedures were conducted in accordance with the guidelines set by the European Community Council Directives. The procedures used in this study were in strict accordance with the European legislation and the guidelines of the National Institutes of Health on the use and care of laboratory animals.

The animals were divided into 4 groups. 1 group was a control group; 2-4 groups consisted of experimental animals that received daily herbal extracts (divasa solution, Ginkgo-Biloba extract and water extract of salvia, respectively) instead of water for 21 days. The rats received saline (control group), Ginkgo Biloba extract (experimental group 1; 200 mg/kg), salvia extract (experimental group 2; 200 mg/kg) and divasa (experimental group 3).

In our research we used the method of passive avoidance learning to assess the effect of herbal extracts on learning and memory processes. The main advantage of this method is the rapidity of the elaboration of the reflex (training from one sample) and the ability to differentially affect different phases of memory [3].

When the animal passed from the light compartment to the dark one, the door closed and the animal received a discharge of 0.5 mA during a 3-s period. The time it took the animal to pass from one compartment to another was recorded (crossing latency). The animal was allowed a maximum of 5 min to cross from the light to the dark compartment after which the trial terminated. In the test phase, which began 24 h later, each animal was placed once again in the illuminated compartment for an adaptation period of 60 s. Afterwards, the door opened and the time it took the animal to enter the chamber in which it had previously received the shock was recorded. No shock was administered on this occasion. The animal was allowed a maximum of 5 min to enter the dark chamber, after which the test terminated. If it failed to cross the chamber it was placed by the experimenter in the black compartment for a 5-s period without any shock being administered.

The effect of herbal extracts on anxiety and exploratory behavior was assessed by the behavior of laboratory animals in the Suok-test (light-dark modification) [4, 5]. The anxiety was assessed according to the following indicators: latency to leave the central zone; time spent in the dark and light sections of the Suok-test; autogrooming behaviors.

Additional indicators characterizing the conditioned reflex, emotional and biased activity were a number of segments which travelled; orientation (side-directed exploration); head dips (number of exploratory looks down); stopping activity (number of stops).

## 3. Results and discussion

In the passive avoidance learning it was established that the administration of Ginkgo-Biloba extract and divasa solutions to animals significantly increased the latency of rats entering the dark compartment of the chamber 2 hours after the elaboration of the reflex compared to control group animals and rats receiving salvia extract (Table 1).

We showed that the introduction of solutions of Ginkgo-Biloba and divasa improved memory of rats, in comparison with animals receiving salvia extract and in control group. 24 hours after the training, the latent period of entering the dark compartment increased in control group 3.4 times, in groups of rats that received salvia extract 2.9 times, in groups of rats that received the solution of Ginkgo-Biloba - 11 times, the solution of the divasa - 10.2 times.

It was established that in the control group of rats 24 hours after the training, 55% of the animals remembered the shock and did not enter the dark "dangerous compartment"; among rats that received salvia extract 40% of animals avoided the dark compartment, while rats under the influence of divasa solutions and Ginkgo-Biloba better preserved the memorial track, since only 30 % and 5% of animals accordingly did not enter the dark compartment (Table 1).

**Table 1.** The influence of herbal extracts on the passive avoidance learning

Experimental groups	Control	Ginkgo Biloba	Divasa	Salvia
Latencies, sec				
Before training	34,0±6,94	38,0±12,23	15,6±4,45	13,0±1,54
2 hours after training	146,9±13,38	141,0±15,70	167,4±9,47	165,0±9,80
24 hours after training	116,4±14,82	111,2±19,34	171,6±8,45	132,4±15,77
Attempts to enter a dark chamber after the elaboration of a conditioned reflex, %				
In 2 hours	25	30	10	10
In 24 hours	55	40	5	30

Control group and experimental rats underwent behavioral testing on the Suok test. In the light part of the Suok test rats after treatment with Ginkgo Biloba extract displayed an increased number of head dips and time spent compared to controls. No significant differences in number of segments which travelled, vertical rears, orientation, defecation boli, missteps, stops were detected between control group and rats under treatment of Ginkgo Biloba extract (Table 2).

**Table 2.** The influence of herbal extracts on the parameters of behavior in the light part of the Suok test

Experimental groups	Control	Ginkgo Biloba	Divasa	Salvia
Latency to leave, sec	7,1±2,32	10,1±3,38	16,1±2,43*	15,2±5,64
Vertical activity (number of vertical rears)	0	0	0	1,0±0,45
Horizontal activity (number of sectors visited)	2,4±0,60	2,3±0,65	5,4±0,94 *	5,6±1,12 *
Orientation (side-directed exploration)	1,1±0,29	2,6±0,51*	4,5±0,91*	7,0±2,23*
Head dips (number of exploratory looks down)	47,4±9,44	183,5±53,8*	163,0±25,72*	131,3±31,00*
Time spent, sec	1,0±0,38	3,1±0,81	0,9±0,43	0,9±0,31
Number of defecation boli	0,7±0,24	1,5±0,39	1,4±0,27	0,6±0,20
Number of missteps	2,6±1,87	2,4±0,78	1,5±0,43	2,5±0,94
Stopping activity (number of stops)	36,3±10,19	49,2±9,89	65,1±12,69	65,9±17,51

\* $P < 0.05$  vs. experimental groups

Rats under treatment of salvia extract displayed an increased number of orientation, head dips and spent more time in the light part of Suok test compared to controls. No significant differences in number of segments which travelled, defecation boli, missteps, stops were detected between controls and rats under treatment of salvia extract (Table 2).

The solution of the divasa, which is a pharmaceutical nootropic agent, led to similar but more pronounced changes. In the test rats after treatment with divasa displayed an increased number of segments which travelled, orientation, head dips and time spent compared to controls. No significant differences in number of vertical rears defecation boli, missteps, stops were detected between controls and rats after treatment with divasa (Table 2).

In the dark part of the Suok test no significant differences in studied parameters were detected between control and experimental groups.

Thus, the administration of an aqueous extract of salvia and Ginkgo-Biloba contributed to the activation of patterns of exploratory behavior and a decrease of anxiety in animals in the Suok-test, and these changes were comparable to those of the solution of the divasa. When studying the effect of these substances on the memory, we obtained results that show the presence of an improving effect of these processes only in extract of Ginkgo-Biloba and divasa, whereas the effect of the aqueous extract of salvia did not differ from the values in the control animals. Thus, the administration of Ginkgo-Biloba extract to animals stimulates cognitive functions in rats, which is reflected in an improvement in the elaboration of the conditioned reflex and the preservation of the memorable trace after training in passive avoidance learning.

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