

THE INFLUENCE OF *BIDENS TRIPARTITA* EXTRACTS ON PSYCHOMOTOR ABILITIES AND COGNITIVE FUNCTIONS IN RATS

CĂTĂLINA ELENA LUPUȘORU¹, ELIZA GRAȚIELA POPA², RAUL BOGDAN SANDU¹,
BEATRICE ROZALINA BUCA¹, LILIANA MITITELU-TARȚĂU^{1*}, RAOUL VASILE LUPUȘORU³

“Grigore T. Popa” University of Medicine and Pharmacy, 16 Universității Street, 700115, Iași, Romania

¹Faculty of Medicine, Department of Pharmacology-Algesiology

²Faculty of Pharmacy, Department of Pharmaceutical Technology

³Faculty of Medicine, Department of Patho-Physiology

*corresponding author: lylytartau@yahoo.com

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Abstract

The aim of the present study was the investigation of the effects of two *Bidens tripartita* (*B. tripartita*) extracts on the spatial working memory in rat. The experiments were carried out on white Wistar rats (4 groups of 6 animals each), treated intraperitoneally, as follows: Group I (Control): saline solution 0.1 mL/10 g b.w.; Group II (BT-alcoholic): 200 mg/kg b.w. *B. tripartita* alcoholic extract; Group III (BT-aqueous): 250 mg/kg b.w. *B. tripartita* aqueous extract; Group IV (Mg): 1 mmol/kg b.w. magnesium chloride. The effects on the psycho-motor abilities and cognitive functions were evaluated using the T-maze test, in order to investigate the influence of these two vegetal extracts on the short-term memory of laboratory animals. *B. tripartita* aqueous extract determined a significant increase of the spontaneous alternation percentage, but less accentuated than magnesium in the T-maze test in rats. The administration of *B. tripartita* alcoholic extract was associated with a significant augmentation of the spontaneous alternation rate, but less intense than those produced by the aqueous extract in this behavioural model. The treatment with both *B. tripartita* aqueous and alcoholic extracts resulted in a facilitation of spatial learning, an improvement of the short-term memory and a weak anxiolytic effect in rat.

Rezumat

Scopul prezentului studiu a fost investigarea efectelor a două extracte de *Bidens tripartita* (*B. tripartita*) asupra memoriei spațiale de lucru la șobolan. Experimentele s-au efectuat pe șobolani albi Wistar (4 grupe a câte 6 animale fiecare), tratate intraperitoneal, astfel: Lot I (Control): ser fiziologic 0,1 mL/10 g; Lot II (BT-alcoolic): 200 mg/kg extract alcoolic de *B. tripartita*; Lot III (BT-apos): 250 mg/kg extract apos de *B. tripartita*; Lot IV (Mg): 1 mmol/kg clorură de magneziu. Efectele asupra abilităților psihomotorii și a funcțiilor cognitive au fost evaluate utilizând testul *T-maze*, pentru a investiga influența acestor două extracte vegetale asupra memoriei de scurtă durată la animalele de laborator. Extractul apos de *B. tripartita* a determinat o creștere semnificativă a procentului de alternare spontană, dar mai puțin accentuată decât magneziul la testul *T-maze* la șobolan. Administrarea extractului alcoolic de *B. tripartita* s-a asociat cu mărirea semnificativă a procentului de alternare spontană, dar mai puțin intens decât efectul produs de extractul apos în acest model de comportament. Tratatamentul cu ambele extracte, apos și alcoolic de *B. tripartita*, a determinat facilitarea învățării spațiale, îmbunătățirea memoriei de scurtă durată și un slab efect anxiolitic la șobolan.

Keywords: *Bidens tripartita*, behaviour, T-maze, rat, cognitive functions

Introduction

The active principles existing in various plants possess the ability to equilibrate the endocrine and immune systems activity. Moreover, they help the body to maintain the optimal homeostasis and to control or stabilize organ and system functions within the organism [12]. Also, these active compounds mediate the body response to stress (physical, environmental, psychological) and help control the interrelations between endocrine, immune, and nervous systems [1].

Statistical data show that almost 80% of the world's population depends basically on traditional herb medicine. Most of these natural remedies consist of

medicinal herbs, about 35000 plant species being in use at present [12]. Plants have developed many protective mechanisms against pests and infection in the form of phytonutrients. These defences are better developed in edible plants, due to selective adaptive pressures necessary to resist pests, infections, and variable climate conditions [25].

Bidens tripartita is an herb of the genus *Bidens*, *Compositae*, *Asteroideae* subfamily. It is an annual flowering plant, known under various popular names such as: Bur Marigold, Three-lobe Beggarticks, Threelobe Beggarticks, Three-part Beggarticks, Leafy-bracted Beggarticks. It grows in wet places, such as the edges of ponds, streams and ditches or in any running water, in humid to wet soil with lots of

sun. The plant grows from July to September, blooming in numerous solitary bright yellow flowers [26].

The phytochemical analysis of different parts of this plant has revealed the presence of flavonoids, coumarines, xanthophylls, volatile oil, acetylenes and polyacetylenes, sterols, auronones, chalcones, caffeine and tannins [7, 20]. From these active principles, two main present groups were proved to possess curative properties: polyacetylenes, which inhibit numerous pathogenic organisms and flavonoids, which decrease inflammatory reactions [11, 13]. The antimicrobial activity of *Bidens tripartita* is supposed to be also connected with the presence of phenylheptatriene, linolic acid and linolenic acid. The existence of another active compound (friedelin) was associated with the anti-inflammatory activity of this plant [22].

Bidens tripartita is a popular herb in ancient Chinese, African, Caribbean and Peruvian medicine, appreciated for its antiseptic, astringent, diuretic, emmenagogue, febrifuge, narcotic, sedative, anti-oxidant and sudorific properties [5, 24]. Literature data show that in traditional medicine, infusions of *B. tripartita* are commonly used in the treatment of catarrhal rhinitis, angina, acute respiratory infection, and as an anti-inflammatory remedies for colitis and gout [21].

Materials and Methods

Plant material

The plants were cut into smaller pieces and ground to coarse powder using a blender. The powder was dissolved in absolute chloroform (96%) and re-extracted seven times. The extracted liquid was filtered through filter paper, dried using a Rota-vapour device, followed by removal of residual solvent smell from the dry extract by jet air. For obtaining the aqueous extract, the dried and powdered vegetal product was extracted in boiling water, left for cooling at the laboratory temperature for 30 minutes, and filtered off thereafter, to obtain the aqueous extract of plant. For the alcoholic extract, after complete drying, the product was consequently extracted seven times with ethanol, following the same method of evaporation. In the final step the extract was weighed, packed in glass containers and stored in the laboratory refrigerator for further research [27].

Animals

The present experiment was carried out on white male Wistar rats (200 - 250 g) distributed into 4 groups of 6 animals each, treated intraperitoneally as follows: Group I (Control): saline solution 0.1 mL/10 g b.w.; Group II (BT-alcoholic): 200 mg/kg b.w. *Bidens tripartita* alcoholic extract; Group III (BT-aqueous): 250 mg/kg b.w. *Bidens tripartita* aqueous extract; Group IV (Mg): 1 mmol/kg b.w. magnesium

chloride. The retained doses of *B. tripartita* extracts administered were 1/20 of the LD50. Magnesium was used as a positive control drug, with known effects of improving spatial memory performances and learning capacities in laboratory animals [23]. The present study and also the previous studies we have performed, involve investigations in order to reveal the main characteristics exhibited by *Bidens tripartita* extracts, which are not yet validated.

Standard laboratory food and tap water were freely available, except during the time of the experiments. Before the experiment, the rats were placed on a raised wire mesh under a clear plastic box and allowed for 2 hours to acclimate to the testing room.

The T-maze assay

To assess the possible involvement of *B. tripartita* extracts in the maintenance of spatial cognition, the present study investigated their effects on rat memory performance on a T-maze assay (Panlab Harvard, USA) [2, 3]. The T-shaped maze consists of three plastic arms (30 cm long, respectively 10 cm wide), whose walls show specific design all over the inner area, allowing the animals to distinguish one from another. This experimental model, together with Y-maze and radial arm maze, belongs to a group of behavioural tests used to evaluate the capacity of the animal to remember the arm it had just explored and would therefore enter one of the other arms of the device [14, 15].

Each animal was placed at the end of a start arm facing one back wall, and allowed to move unreservedly through the maze during an 8 minutes session. The first 2 minutes were set for habituation and the last 6 minutes for the alternation between arms, recorded with photo beam breaks positioned at the midpoint of each arm. Repeated activation of the same detector was not considered as an arm-entry until the detector in another arm was activated. Spontaneous alternation was defined as the entry in an arm that was least occupied recently. Alternation was defined as a consecutive entry in three different arms. The alternation percentage was computed according to the following formula [4]:

$$\text{Number of alternations} \times 100 / (\text{total number of arm visits} - 2)$$

Latency to leave the start arm, latency of first arm visit, number of arms visited, alternate arm returns, and same arm returns were also parameters that were counted [8]. A video camera set over the device and connected to a computer in another room was used to allow us to evaluate the behaviour without distressing the animals. All trials were videotaped and analysed by an observer unfamiliar with the treatment condition. The tasks were performed between 8:00 a.m. and 13:00 p.m..

Statistical analyses

The obtained data were presented as \pm standard deviation (SD) and significance was tested using

the ANOVA test, implemented in SPSS Statistics 17.0 for Windows, followed by Neumann Keuls *post hoc* test. *p*-values less than 0.05 were considered statistically significant compared to those of the Control group. The experimental protocol was implemented following the recommendations of the “Grigore T. Popa” University Committee for Research and Ethical Issues guidelines for the handling and use of experimental animals, according to the ethical standards of the European Community [28].

Results and Discussion

Using the T-maze test, some information regarding the animal psychomotor abilities and cognitive processes were obtained: the number of arm visits, the percent of time spent inside the arms, the number of the returns inside the same arm, the number of alternations, the alternation percentage [6, 19]. The spontaneous alternation or free-running in rats refers to their natural tendency to instinctively choose alternate arms [8, 9]. In this behavioural experimental model, it is estimated that the increase of spontaneous alternation percentage corresponds to an improvement in the short-term spatial memory. In addition, the decrease in the number of arm entries signifies the effect of improving the animal's working memory performances. On the other hand, the increase in the percentage of the time spent in the new arm could be assimilated with a diminution of the animal's ability to explore the environment [19].

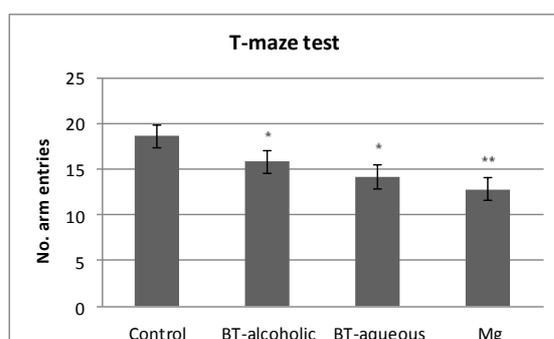


Figure 1.

T-maze test - the effects of *Bidens tripartita* alcoholic and aqueous extracts on the number of arm entries. Values are expressed as mean ± SD of the number of arm entries for six rats. **p* < 0.05, ***p* < 0.01 vs. control

The *B. tripartita* extracts induced a significant diminution of working memory errors number. Intra-peritoneal administration of BT-alcoholic resulted in a decrease of arm entries number (15.83 ± 0.75), statistically significant (*p* < 0.05), compared to the animals treated with saline solution (18.67 ± 5.24) (Figure 1). BT-alcoholic group manifested a tendency

of diminution of the exploration capacity compared to the Control group. The treatment with BT-aqueous was associated with a decrease of arm entries number (14.17 ± 1.72), statistically significant (*p* < 0.05) compared to the Control group (18.67 ± 5.24), but less accentuated than magnesium chloride (12.83 ± 1.47) (*p* < 0.01). Its administration resulted in a decrease of the explorative behaviour in T-maze test (Figure 1).

No significant changes in the latency of the first arm visit between the BT-alcoholic, the BT-aqueous and the saline groups in this behavioural model in rats were observed.

Statistical analysis of the data revealed that the alternation rate of BT-alcoholic group rats (50.17 ± 2.86) was statistically higher (*p* < 0.05) than the spontaneous alternation percent of the Control group rats (39.67 ± 1.75) in the T-maze assay, thus signifying a facilitation of the extinction learning (Figure 2).

The use of BT-aqueous produced a statistically significant (*p* < 0.05) augmentation of the spontaneous alternation rate (53.33 ± 2.73) compared to the Control group (39.67 ± 1.75), suggesting the favourable effects on the spatial memory, especially on the short-term memory (Figure 2). Its effects were less intense than those of magnesium chloride (64.83 ± 3.19) (*p* < 0.01) in the same behavioural model.

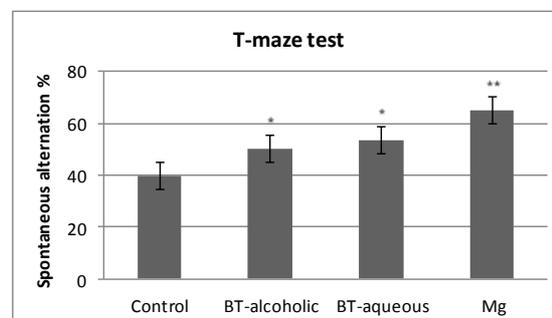


Figure 2.

T-maze test – the effects of *Bidens tripartita* alcoholic and aqueous extracts on spontaneous alternation percentage. Values are expressed as mean ± SEM of spontaneous alternation percentage for six rats.

p* < 0.05, *p* < 0.01 vs. control

No significant modifications of the same arm return number and alternate arm return number between the groups treated with BT-alcoholic, BT-aqueous and saline solution were observed.

Throughout time, traditional medicine experience has proved the existence of many herbs (such as chamomile, *Ginkgo biloba*, lavender, valerian etc.), found useful in overcoming human anxiety and different affective instability states [17]. Literature data has demonstrated positive neurochemical,

endocrinological, and epigenetic effects of various plants administered in patients with depression, anxiety, sleep disturbances and other behavioural disorders. Thus, the clinical trials have provided beneficial evidence of the antidepressant activity of some medicinal plants: *Echium amoenum*, *Crocus sativus*, *Rhodiola rosea*, and anxiolytic effect of other herbs: *Matricaria recutita*, *Ginkgo biloba*, *Passiflora incanata*, *E. amoenum*, *Scutellaria lateriflora* etc. [17, 18].

We previously demonstrated that both *Bidens tripartita* extracts induced a slightly decrease of the escape attempts number, corresponding to a decrease of the exploratory behaviour and locomotor activity of rats in the Actimeter test. The effects of *Bidens tripartita* aqueous extract were more intense than those of *Bidens tripartita* alcoholic extract on spontaneous motor activity in this behavioural experimental model. The observed actions of diminishing both the global motor behaviour and the number of escape attempts could correspond somehow to the general behavioural inhibition or to sedation in humans [16]. Extrapolation of these results is however difficult, due to of the enormous differences in behaviour, cognitive function and motor coordination skills between lab animals and humans. Other researches have proved the antinociceptive effects of *Bidens tripartita* alcoholic extract in tail flick test in rats [10].

The studies performed on the T-maze test in order to assess aspects of cognitive function and learning in rats showed an improvement of the spatial memory in the animals treated with both *Bidens tripartita* extracts. This was proved by an increase of the spontaneous alternation percentage compared to the Control group, demonstrating beneficial effects on the short-term memory. Our results indicate that the intraperitoneal administration of both *Bidens tripartita* extracts was associated with an enhancement of cognitive functions in rats. Statistical analysis of the results shows that the treatment with *Bidens tripartita* aqueous extract is more efficient in facilitating the extinction learning memory than *Bidens tripartita* alcoholic extract.

Conclusions

We can conclude that the administration of both *Bidens tripartita* extracts facilitated the spatial learning and working memory, thus suggesting an improvement of cognitive functions in rats. *Bidens tripartita* herb contains a mixture of various active principles whose characteristics were not previously completely established, and need extensive studies. A comprehensive investigation of the pharmacologic effects of different extracts of this plant is required.

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