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Original Research Article

Effect of methanol-aqueous extract of *Satureja montana* and two of its bioactive components on memory in rats

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Abstract

Purpose: *To investigate the effect of Satureja montana Lamiaceae extract on cognition in rats.*
Methods: *A total of 128 male, 8-week-old Wistar rats were used in this study. The rats were randomly and equally assigned to 16 groups (n = 8), which were orally given either saline and olive oil (two negative and two positive controls), S. montana Lamiaceae (250 and 500 mg/kg), RA (15 mg/kg), or carvacrol (500 mg/kg). For each experimental model, acute cold stress and chronic unpredictable mild stress models were used to induce memory impairment in rats. A new object recognition test was used to investigate cognition.*
Results: *Acute stress did not produce a significant impact on cognition (p > 0.05). However, S. montana at a dose of 500 mg/kg significantly increased the discrimination index, relative to positive control rats (p = 0.001), while the lower dose of dry extract and RA had significant impacts only on time spent on new object investigation (p = 0.025 and p = 0.014, respectively). Chronic stress significantly reduced the duration of new object investigation but not the discrimination index (p = 0.037 and p = 0.009). Both doses of Satureja montana and RA increased levels of all studied parameters when compared to positive saline group, as well as novel object recognition when compared to RA and carvacrol (p < 0.05).*
Conclusion: *Satureja montana and RA (but not carvacrol) have moderate effects on cognition abilities in rat models of acute and chronic stress. More research is required to establish the specific biochemical processes through which S. montana and RA affect memory.*

Keywords: *Satureja montana, RA, Carvacrol, Stress, Cognition, Recognition*

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INTRODUCTION

Exposure to increased levels of acute or chronic stress affects cognitive function [1]. From available scientific literature, it is known that acute stress activates areas regulating responses of a predominantly adaptive nature that are important for survival [2]. However, some researchers associate acute stress exposure with increased inflammation in the central nervous system (CNS), which is thought to be involved in the pathogenesis of diseases, including Alzheimer's disease [3,4]. Chronic stress may lead to the depletion of adaptive

mechanisms, thereby resulting in changes in the effectiveness of feedback responses which are associated with the development of abnormal behavior [2].

One way of decreasing the negative effects of stress is the use of adaptogens which are substances of natural origin such as St. John's Worth, turmeric, and red sage, which increase the resistance of an organism against stressors [5].

Satureja montana is a medicinal plant, which is employed traditionally as a remedy for diseases of the digestive system, as well as bronchial, lung and kidney ailments [6]. In the available scientific literature, different extracts of *Satureja montana* have been reported to exert antioxidant, antibacterial and anti-proliferative effects [6].

The present study was aimed at investigating the impact of acute and chronic stress on memory impairment, and the influence of dried extract of *S. montana* Lamiaceae, RA and carvacrol on memory impairment induced by both acute and chronic stress. The justification for carrying out this study was based on the increased levels of stress associated with today's lifestyle, and its negative impact on memory, as well as the lack of literature data about the effect of *Satureja montana* Lamiaceae on cognition.

EXPERIMENTAL

Animals

A total of RA128 male, 8-week-old Wistar rats were used in this study. The rats were randomly assigned to 16 groups (n = 8) comprising 2 negative controls, 2 positive controls, 2 *S. montana* dose groups (250 and 500 mg/kg), carvacrol group (500 mg/kg) and RA (rosmarinate, RA) group (15 mg/kg) in each experiment. The 2 negative control groups received either saline or olive oil (1 ml/100 g), but without stress, while the 2 positive controls were given the same dose of saline or olive oil, and subjected to stress. All experiments were approved by the Ethical Authority of Medical University of Plovdiv (protocol no. 01-2/10.04.2020) and were conducted in accordance with standard protocols on the care and use of laboratory animals [7,9].

Preparation of extract

The methodology outlined by "Veselino EOOD", Kazanlak was employed in the preparation of CH_3OH: aqua (70:30 v/v) extract from the dry leaves of *S. montana* supplied by a local herbal shop. The extract was spray-dried at 40 ^oC until complete volatilization of both solvents.

Drugs and chemicals

Sigma-Aldrich was the source of rosmarinic acid and carvacrol. Prior to administration via the oral route, the extract and rosmarinate were dissolved in distilled H_2O, while carvacrol was solubilized in olive oil.

Inducing memory impairment

Memory impairment was induced via acute and chronic stress models.

Acute stress

Acute cold stress was used for the establishment of rat model of acute stress, with some modifications. In this process, positive control and experimental rats were subjected once to freezing temperature (-4 ^oC) for 60 min in the refrigerator in plastic boxes in which they moved freely [7]. The exposure to this stress factor occurred immediately after administration of the various substances.

Chronic stress

Chronic stress was induced by exposure to various stress factors for 60 days. The experimental animals were treated daily immediately before exposure to various stressors: food or water deprivation for 24 hours, watering the sawdust bed, exposure to predator sounds, inclining of the cages to 45^o and leaving the light on for 24 hours. The stressors were changed daily to avoid habituation to them [8].

New object recognition test

This was done on three successive days after administering the respective substances. On the first day, rodents were placed in an open field apparatus for 5 minutes for habituation. On the second day, two objects identical in size, form, and color were positioned at opposite ends of the apparatus. The rats were permitted to examine the objects for 10 minutes. The third day was the test day in which one of the already-known objects was positioned along with a new one similar in shape and color. The rats were allowed to examine the objects for 3 minutes.

The time spent surveying each of the objects and the total study time was recorded. An indicator of cognitive memory is the discriminant index (DI). This is the proportion of the time spent by the animal in studying the novel object to total

duration used in examining the 2 objects in a test session. An increase in DI is an index of better recognition memory [9].

Statistical processing

Statistical analysis was processed with IBM SPSS 19.0 software and One-Way ANOVA test, along with LSD and Games-Howell post hoc tests. A Kolmogorov-Smirnov test was performed to determine the distribution of data. Results at a significance level of $p < 0.05$ were considered statistically significant.

RESULTS

Acute stress

Acute stress did not have any significant impact on the recognition memory of the animals in the 2 positive control groups, relative to the analogous negative controls ($p > 0.05$). However, the dry extract of *S. montana* at doses of 250 and 500 mg/kg significantly increased the time spent on investigating the new objects ($p = 0.025$ and $p = 0.042$, respectively) as well as the total time spent on exploration of the object ($p = 0.047$ and $p = 0.003$, respectively), when compared to the positive control (saline) group. However, a significant increase in the discrimination index occurred only with the 500 mg/kg dose, relative to the same control ($p = 0.001$).

Similarly, rosmarinate had a significant effect on discrimination index, relative to positive control group treated with saline ($p = 0.014$). Carvacrol had no statistically significant effect on recognition memory when compared with the group treated with olive oil and exposed to the stressor. These results are presented in Figure 1.

The RA significantly increased the discrimination index, when compared to the dry extract at the lower dose ($p = 0.035$). Compared to carvacrol, the same dose of *Satureja montana* had a significant impact on the time spent investigating new objects ($p = 0.008$) as well as the total time on object exploration ($p = 0.038$).

At a level of 500 mg/kg, the extract significantly increased levels of all studied indicators, when compared to the carvacrol-treated group (time investigating new object: $p = 0.028$, DI: $p = 0.004$, and total exploration time: $p = 0.003$), no significant differences with RA were demonstrated ($p > 0.05$). These data are presented in Figure 2.

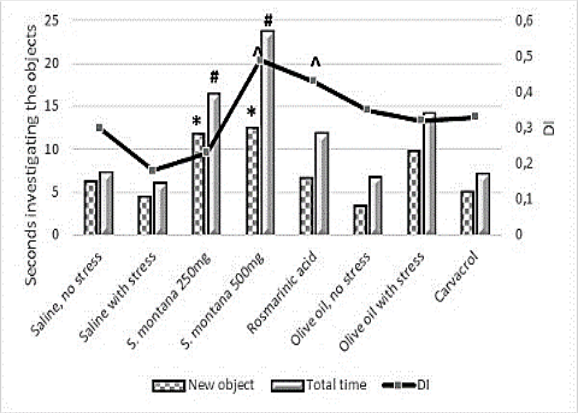

Figure 1: Impact of *S. montana,* rosmarinate, and carvacrol on recognition memory in rat model of severe stress. *Significant increases in time spent in investigation of new objects in groups treated with *Satureja montana* (250 mg/kg; $p = 0.025$) and *Satureja montana* (500 mg/kg; $p = 0.042$), relative to positive control (saline). #Significant increases in total time in groups treated with *Satureja montana* (250 mg/kg; $p = 0.047$) and *Satureja montana* (500 mg/kg; $p = 0.003$), relative to saline control. ^Significant increase in DI in groups treated with *Satureja montana* ($p = 0.001$) and RA ($p = 0.014$), relative to positive control (saline)

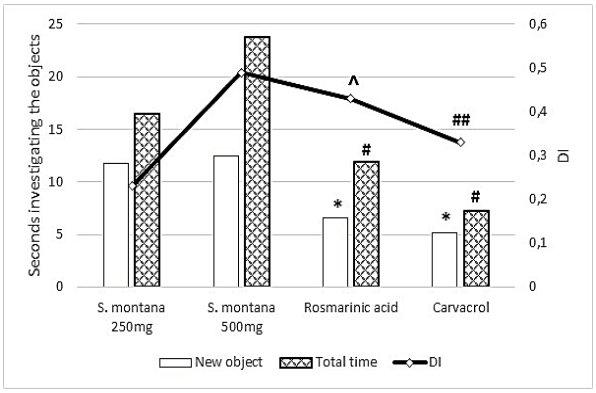

Figure 2: Comparative impact of the 3 substances RA on recognition memory in acute stress model. *Significant decreases in time spent investigating new objects ($p = 0.008$) and in total time ($p = 0.038$) in carvacrol-treated rats, relative to the group treated with *S. montana* (250 mg/kg). #Significant decreases in time spent investigating new objects ($p = 0.028$) and in the total time ($p = 0.003$) in carvacrol-treated rats, relative to the group treated with *S. montana* (500 mg/kg). ##Significant decreases in DI ($p = 0.004$) between carvacrol-treated and *S. montana* (500 mg/kg)-treated groups. ^Significant increase in DI ($p = 0.035$) in RA-treated rats, relative to rats treated with *S. montana* (250 mg/kg)

Chronic stress

Chronic stress significantly reduced the time spent investigating the new object in both the positive and negative control groups (saline controls: $p = 0.037$, and olive oil-treated groups: $p = 0.009$). No significant changes in terms of the

discriminant index were demonstrated. The positive control group treated with olive oil had significantly reduced total time for exploration of the object when compared to the corresponding negative control (p = 0.009). No such differences were established between the 2 saline-treated groups.

Relative to the positive saline group, both doses of *S. montana* caused significant increases in all studied parameters {250 mg/kg increased time spent on investigating the new object (p < 0.001), DI (p = 0.025) and total exploration time (p = 0.002); and 500 mg/kg increased time spent investigating new objects (p < 0.001), DI (p = 0.042), and total exploration time (p = 0.007)}. Compared to the same positive control, RA had a significant impact only on the time spent investigating new objects (p = 0.013) and the total exploration time (p = 0.003), but no effect on DI was found (p > 0.05). The lower dose of the extract had significant impacts on the time spent investigating the objects (p = 0.012) and the total time for exploration of new objects relative to negative control rats (p = 0.022). Such an effect was not seen with *S. montana* at the dose of 500 mg/kg. Carvacrol had no significant impact on recognition memory. These results are presented in Figure 3.

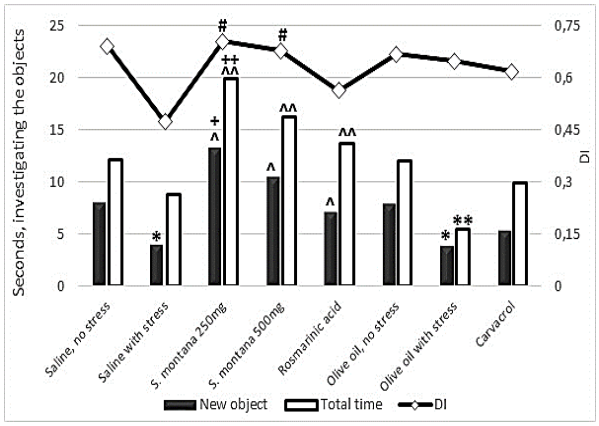

Figure 3: Impact of the 3 substances on recognition memory in rat model of chronic stress. *Significant decreases in time spent in the investigation of new objects in both negative control groups, relative to both positive groups (p = 0.037 and p=0.009, respectively). **Significant decreases in the total exploration time in positive control rats, relative to negative control group treated with olive oil (p = 0.009). ^Significant increases in time spent in investigation of new objects in both groups treated with dry extract (250 and 500 mg/kg) and RA, relative to positive saline control (p < 0.001, p < 0.001, and p = 0.013, respectively). ^^Significant increases in total exploration time for new objects in both groups treated with dry extract (250 and 500 mg/kg) and RA, relative to saline positive control (p = 0.002, p = 0.007 and p = 0.013, respectively). #Significant increases in DI in positive saline control and both groups treated with *S. montana* (p = 0.025 and p = 0.042 respectively). +Significant decrease in time spent in investigation of new objects in negative saline control, relative to *S. montana* (250 mg/kg; p = 0.012). ++Significant difference in total exploration time between negative saline control and *S. montana* group (250 mg/kg; p = 0.022)

Compared to carvacrol, *Satureja montana* at doses of 250 and 500 mg/kg significantly increased the total exploration time of the objects (p = 0.001 and p = 0.049, respectively). Both experimental doses of *Satureja montana* extract significantly increased the time spent in investigation of new objects, relative to RA (p = 0.001 and p = 0.013, respectively) and carvacrol (p < 0.001 and p = 0.003, respectively). No significant differences in terms of the discriminant index were demonstrated. These results are presented in Figure 4.

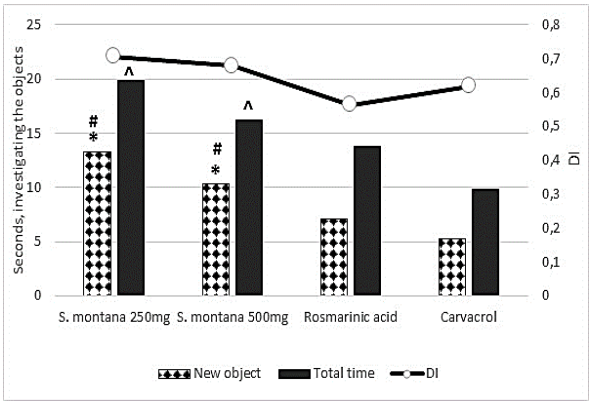

Figure 4: Comparative impacts of 3 substances on recognition memory in chronic stress model. *Significant increases in time spent investigating new objects, relative to RA treatment (p = 0.001 and 0.013, respectively). #Significant increases in time spent investigating new objects, relative to carvacrol treatment (p < 0.001 and 0.003, respectively). ^Significant increase in total exploration time (p = 0.001 and 0.049), relative to carvacrol treatment

DISCUSSION

Stress may have contradictory effects on cognition [1]. Acute stress is associated with no impact on memory or even with the enhancement of cognitive functions, while exposure to chronic stress causes memory impairment [10,11]. This is due to the CNS's response to stress. Acute stress stimulates neurogenesis in the hippocampal area, which has an important role in the memory process [10]. The suppressive effect of chronic stress on cognitive functions is associated with the presence of anxious and depressive behavior [11]. In accordance with scientific data on the frequency of use of different stressors, acute cold stress and chronic unpredictable mild stress

models were chosen in this research to impair cognition in the rats [8]. A novel object recognition test (NORT) examines non-spatial memory in which distinguishing a new object engages many zones in the CNS [1,10,11]. For this reason, NORT was used in this study as a screening model to investigate the effect of the tested substances on cognitive function.

The results obtained in the present study by comparing both positive and negative control groups in both experimental models of stress are in accordance with the available literature on cognition alterations as a consequence of the impact of stress. The results presented for RA and carvacrol are in accordance with previous findings [12,13]. The cognitive improvement in animals treated with RA was likely due to its neuroprotective properties [12]. When discussing the effect of this phenolic acid on stress-induced memory impairment, its anti-inflammatory and antioxidant effects should be considered, as well as the fact that it reduces glucocorticoid levels [14]. Stress impairs endogenic regulation of the release of glucocorticoids, which results in increased plasma concentration of glucocorticoids, with a negative impact on memory [15,16].

Similar to psychostimulants, carvacrol is known to increase norepinephrine, and it exerts anti-inflammatory and antioxidant effects [13,17]. These properties are probably not effective enough to overcome the cognition-impairing effect of corticosteroids. Confirmation or rejection of this hypothesis will be the subject of further studies. No data on the effect of carvacrol on glucocorticoid levels were found in the available literature.

No studies on the effect of *S. montana* on cognition were found in available literature with which to compare the results obtained in the present study. The dry extract of *S. montana* increased the time spent in investigation of the new object. The dose of 500 mg/kg produced significant impacts on discrimination index (DI) in both experimental models, while the lower dose had a significant effect only on the DI in the chronic unpredictable stress model. The effect of the *Satureja montana* extract on cognition was likely due to the presence of RA in amounts corresponding to the standard dose used. The improved impact of a higher dose of *Satureja montana* (500 mg/kg) was most likely due to the higher quantity of RA, relative to the lower dose of extract.

Rodents treated with both experimental doses of *Satureja montana* dry extract had enhanced cognitive potential in both stress experimental models when compared to the rats administered RA and carvacrol. For that reason, it can be assumed a potentiation of the effects of RA in the composition of the dry extract. Further studies are needed to establish the mechanism of action involved in the memory-enhancement effect of the dry extract, and also to investigate its influence on the other types of memory.

CONCLUSION

Satureja montana and RA have a moderate effect on cognitive abilities in acute and chronic stress models, as tested with a novel object recognition test. Carvacrol does not produce any significant attenuating effect on the negative impact of stress on recognition memory.

More research is required to establish the specific biochemical processes through which *S. montana* and RA affect memory, as well as clarify the interactions between the bioactive components of the extract.

DECLARATIONS

Acknowledgements

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Funding

None provided.

Ethical approval

This study was approved by the Ethical Authority of Medical University of Plovdiv (protocol no. 01-2/10.04.2020).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All experiments were designed and performed under

the guidance of Kostadinova I, Delev D, and Kostadinov I. Vilmosh N, Kotetarova M, Zlatanva H, and Kandilarov I, performed the experiments. Each author contributed to writing the article.

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