Effects of Acute Administration of *Urtica dioica* on the Novel Object-Recognition Task in Mice

Nasrin Hashemi-Firouzi, Marjan Akhavan, Alireza Komaki, and Siamak Shahidi

1Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, IR Iran

*Corresponding author: Siamak Shahidi, Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, IR Iran. Tel: +98-8138380462, Fax: +98-8138380208, E-mail: siamakshahidi@yahoo.com*

Received 2015 June 1; Accepted 2015 August 15.

**Abstract**

**Background:** *Urtica dioica* (nettle) has a variety of uses in traditional medicine for the treatment of certain urogenital problems, gastrointestinal disorders, and diabetes.

**Objectives:** Recent studies have implicated the effect of *U. dioica* on brain functions such as pain and memory. However, there is no direct evidence of the acute effects of this plant on cognition. The aim of the present study was to evaluate the effect of *U. dioica* aqueous extract on the novel object-recognition task (NOR) in mice.

**Materials and Methods:** First, *U. dioica* aqueous extract was prepared, then adult male mice were randomly divided into four experimental groups. During the training session, the mice were placed in a box and given 5 minutes to explore two identical objects. The next day, they were again placed in the box and allowed to explore one familiar and one novel object. They received intraperitoneal injections of saline or *U. dioica* aqueous extract (100 mg/kg) before or immediately after the training session or before the test session of the NOR task.

**Results:** The results showed that there was a preference for the novel object compared to the familiar one in each of the experimental groups. The object-recognition discrimination index in the group of mice that received *U. dioica* before training was significantly less than in the other experimental groups. There was no significant difference in the discrimination index between the other groups. *U. dioica* did not decrease the time spent exploring familiar and unfamiliar objects, or the total time spent exploring both objects.

**Conclusions:** Acute administration of *U. dioica* impairs the object-recognition task if it is used only before the training session. This may be due to its modulation on the acquisition processing of object-recognition. *U. dioica* has no significant effects on the consolidation or retrieval processing stages of the NOR task. These results emphasize the unfavorable effect on cognitive function of pre-training with acute supplementation of *U. dioica*.

**Keywords:** Mice, Novel Object Recognition, *Urtica dioica*

---

1. **Background**

Memory is the ability of an individual to record events and information, and to retain them over short and long periods of time (1). The effects of numerous herbal medicines used to treat cognitive disorders, including in memory and learning, have been studied. *Urtica dioica* (UD) is widespread throughout Europe, North America, North Africa, and parts of Asia (2). It belongs to the Urticaceae family, and is commonly known as English common nettle (3). The nettle is an herbaceous perennial flowering plant used in traditional medicine for the treatment of many diseases (2).

The plant’s parts have different chemical constituents (4). UD has been reported to have a protective effect against neuronal dysfunction (4-8), hyperglycemia (9, 10), hypercholesterolemia (11), arthritic pain (12), depressive behavior (13), and diabetic neuropathy (14). It also improved cognition and reduced memory dysfunction with chronic treatment in diabetic animals (13, 14).

2. **Objectives**

The *U. dioica* plant is rich in many active pharmaceutical ingredients, such as carotenoids, flavonoids, glycosides, quercetin, kaempferol, caracole, 5-hydroxytryptamine (5-HT), acetylcholine steroids, and sterols (5-6, 8) which could have valuable effects on learning and memory. Therefore, the aim of this study was to evaluate the effect of acute administration of UD aqueous extract using the object-recognition test in male mice.

3. **Materials and Methods**

3.1. **Animals and Experimental Groups**

Adult male mice weighing 20 - 40 g were obtained from the Pasteur Institute of Tehran, Iran. All animals were acclimatized to the departmental animal house, and housed under standard laboratory conditions with a temperature of 22 ± 1 °C and a 12-h light/dark photoperiod.
cycle. All experiments were performed between 9:00 a.m. and 4:00 p.m. Tap water and chow pellets were available ad libitum. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1985).

Twenty-eight animals were randomly divided into four groups (n=7 each): one control group and three treatment groups. The three experimental groups received intraperitoneal (i.p.) injections of U. dioica extract, at a dose of 100 mg/kg, either before training, immediately after training, or before the retention test (24 hours after training). The control animals received the same volume of saline.

3.2. Preparation of Extract

The prepared aqueous extract of U. dioica was gifted to the Tehran University of Medical Sciences.

3.3. Novel Object-Recognition Test

The apparatus consisted of an open brown wooden box (38 × 48 × 42 cm) with a white floor. Two identical objects and one different object were used. The objects were symmetrically fixed to the floor of the box, 15 cm apart. The arena and the objects were cleaned with 10% ethanol before each test.

All experiments were carried out in a quiet room under light conditions. Before the test, the mice were habituated to the arena (without any objects) for 5 min. The training test session was then done, followed by retention tests 24 hours later.

During the training trial, two identical objects were symmetrically fixed to the floor of the box, 15 cm apart. The mice were allowed to explore freely for 5 minutes. After training, the animals were returned to their home cages, and the box was carefully cleaned with wet tissue paper (10% ethanol solution) to eliminate any remaining odors. The retention test was performed 24 hours later, in which one of the objects was replaced by a novel one. The animals’ behavior was recorded with a video-camera system, and the time spent investigating each object was measured. The exploration process of an object was defined as smelling the object. The discrimination index was measured. The exploration process of an object was defined as smelling the object. The discrimination index was defined as the difference between the exploration time for the novel object and the familiar object during the retention test (15-17).

3.4. Statistical Analysis

One-way ANOVA was used to determine the statistically significant differences in the discrimination index between the experimental groups, followed by a post hoc Tukey test. All results are presented as mean ± SEM. A P value of < 0.05 was considered significant.

4. Results

All of the experimental groups exhibited a stronger tendency to explore the new object. One-way ANOVA showed significant differences in the discrimination index between the groups 24 hours after training. A significant difference was also found among the treated groups compared to the control group. The Tukey test illustrated that the discrimination ratio of rats receiving UD before training was significantly lower than in the control group (P < 0.05; Figure 1).

One-way ANOVA clarified that there was no significant difference in the discrimination ratios 24 hours after training between the control group and the groups receiving UD.

5. Discussion

In the present study, the amnesic effect of U. dioica consumption on learning and memory was evaluated using the NOR task, which has emerged as a popular method for testing nonspatial memory in rodents. This task exploits the natural tendency of rodents to explore novel items; based on the amount of time that rodents spend exploring the presented objects, inferences about memory can be established (18).

Our results showed that in an object-recognition task 24 hours after a training trial, the mice that had been treated with UD immediately before the training session demonstrated a lower ability to recognize a previously presented object. In contrast, the other groups that received nettle showed some memory activity during the test period. This result probably indicates that memory impairment is a side effect of acute administration of aqueous extract of U. dioica.

Unlike our results, there are some reports on the effect of UD in spatial memory, using a Morris water maze task in diabetic mice; for example, the consumption of hydro-alcoholic extract of UD (50 mg/kg i.p.) improved memory after 60 days (14). The chronic administration of hydro-alcoholic extract of UD (100 mg/kg/day p.o.) enhanced spatial and associative memory at the end of 12 weeks.
One physiological action of the aqueous extract of UD is an antihyperglycemic effect through the reduction of intestinal glucose absorption (10). Evidence indicates that peripheral glucose levels exert an important influence on memory storage (20). Glucose administration attenuates spatial memory deficits (21) and enhances memory in humans and rodents (22, 23). Glucose can also temporarily enhance hippocampal-dependent memories during encoding, which creates delayed memories (24). Hippocampal function is facilitated by glucose administration in learning and memory processes, such as inhibitory avoidance conditioning (20), spontaneous alternation (25), delayed recall (26), and the radial arm maze (27). It seems that the hyperglycemic properties of UD cause memory impairment 24 hours prior to the object-recognition test.

In conclusion, acute administration of aqueous extract of UD decreases recognition memory. These facts show that further studies on the effects of systemic administration of UD in different subfields of memory and cognition, using pharmacological and physiological approaches, are necessary.

Acknowledgments

This work was supported by a grant from Hamadan University of Medical Sciences.

Footnotes

Authors’ Contribution: Study concept and design, analysis and interpretation of data, and critical revision of the manuscript for important intellectual content: Nasrin Hashemi-Firouzi, Marjan Akhavan, Alireza Komaki, and Siamak Shahidi; acquisition of data, statistical analysis, and drafting of the manuscript: Marjan Akhavan, Nasrin Hashemi-Firouzi, and Siamak Shahidi; administrative, technical, and material support: Siamak Shahidi and Alireza Komaki; study supervision: Siamak Shahidi.

Conflict of interest: The authors declare that there is no conflict of interest.

Funding/Support: This work was supported by a grant from Hamadan University of Medical Sciences.

References

1. Phane M, Korgaonkar D. Pharmacology of learning and memory. The Internet Journal of Pharmacology. 2009;7(1):387.
2. Kuete V. Medicinal plant research in Africa: Pharmacology and chemistry. Newnes: 2013.
3. Bessey CE. The Phylogenetic Taxonomy of Flowering Plants. Annals of the Missouri Botanical Garden. 1915;2(1):99-106. doi: 10.2307/2990030.
4. Wessler L, Rillinginger R, Ritteringer F, Kirkpatrick CJ. The biological role of non-neuronal acetylcholine in plants and humans. Jpn J Pharmacol. 2001;85(1):2-10. [PubMed: 11435686].
5. Otles S, Yalcin B. Phenolic compounds analysis of root, stalk, and leaves of nettle. ScientificWorldJournal. 2012;2012:564367. doi: 10.1155/2012/564367. [PubMed: 22593694].
6. Tahata A, Dixit VK. Ameliorative effects of stinging nettle (Urtica dioica) on testosterone-induced prostatic hyperplasia in rats. Andrologia. 2012;44 Suppl 1:93-940. doi: 10.1111/j.1439-0272.2011.01917.x. [PubMed: 21806658].
7. Holscher C. Diabetes as a risk factor for Alzheimer’s disease: insulin-signalling impairment in the brain as an alternative model of Alzheimer’s disease. Biochem Soc Trans. 2011;39(4):391-7. doi: 10.1042/BST20103989. [PubMed: 21787389].
8. Collier HOJ, Chester GB. Identification of 5-hydroxytryptamine in the sting of the nettle (Urtica dioica). British Journal of Pharmacology and Chemotherapy. 1956;12:186-9. doi: 10.1111/j.1476-5381.1956.tb00151.x. [PubMed: 13329377].
9. Kavalali G, Tuncel H, Gökşen S, Hatemi HH. Hypoglycemic activity of Urtica pilulifera in streptozotocin-diabetic rats. J Ethnopharmacol. 2001;84(2-3):241-5. [PubMed: 12648821].
10. Bnouham M, Merhfour F, Ziyat A, Mekhi H, Aitiz M, Legssyer AZ. Antihyperglycemic activity of the aqueous extract of Urtica dioica. Flora. 2003;197(4):577-81. [PubMed: 14630172].
11. Nassiri-Asl M, Zamanisoltani F, Abassi E, Dameshi MM, Zangivand AA. Effects of Urtica dioica extract on lipid profile in hypercholesterolemic rats. Zhong Yi Yi Xue Ke Xue Bao. 2009;7(5):428-33. doi: 10.3736/cimj20090505. [PubMed: 19435556].
12. Setty AR, Sigal IH. Herbal medications commonly used in the practice of rheumatology: mechanisms of action, efficacy, and side effects. Semin Arthritis Rheum. 2005;34(6):773-94. doi: 10.1016/j.semarthrit.2005.01.011. [PubMed: 15934292].
13. Patel SS, Udyanabahu M. Urtica dioica extract attenuates depressive-like behavior and associative memory dysfunction in dexamethasone induced diabetic mice. Metab Brain Dis. 2014;29(1):321-30. doi: 10.1007/s11011-013-9480-0. [PubMed: 24435938].
14. Patel SS, Udyanabahu M. Effect of Urtica dioica on memory dysfunction and hypogalaxia in an experimental model of diabetic neuropathy. Neurosci Lett. 2013;521:84-9. doi: 10.1016/j.neulet.2013.07.029. [PubMed: 23966662].
15. Ennaceur A, Neave N, Aggleton JP. Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. Exp Brain Res. 1997;113(3):509-19. [PubMed: 9108217].
16. Broadbent NJ, Squire LR, Clark RE. Spatial memory, recognition memory, and the hippocampus. Proc Natl Acad Sci U S A. 2004;101(40):14535-20. doi: 10.1073/pnas.0406344101. [PubMed: 15452348].
17. Akirav I, Maroun M. Ventromedial prefrontal cortex is obligatory for consolidation and reconsolidation of object recognition memory. Cereb Cortex. 2006;16(12):2579-93. doi: 10.1093/cercor/bjh074. [PubMed: 16421301].
18. Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats: Behavioral data. Behavioural Brain Research. 1988;31(1):47-59. [PubMed: 3228475].
19. Toldy A, Atabay M, Stadler K, Saviari M, Jakus J, Jung KJ, et al. The beneficial effects of nettle supplementation and exercise on brain lesion and memory in rats. J Nutr Biochem. 2009;20(2):197-81. doi: 10.1016/j.jnutbio.2008.09.001. [PubMed: 19070007].
20. Gold PE. Glucose modulation of memory storage processing. Behavioural and Neural Biology. 1986;45(3):342-9. [PubMed: 3783986].
21. Lu Y, Xu S, Hs M, Chen C, Zhang L, Liu C, et al. Glucose administration attenuates spatial memory deficits induced by chronic low-power-density microwave exposure. Physiol Behav. 2012;106(5):631-7. doi: 10.1016/j.physbeh.2012.04.009. [PubMed: 22564353].
22. Smith MA, Rihy LM, Eekelen JA, Foster JK. Glucose enhance-
ment of human memory: a comprehensive research review of the glucose memory facilitation effect. Neurosci Biobehav Rev. 2011;35(3):770–83. doi: 10.1016/j.neubiorev.2010.09.008. [PubMed: 20883717]

23. Messier C. Glucose improvement of memory: a review. Eur J Pharmacol. 2004;490(1-3):33–57. doi: 10.1016/j.ejphar.2004.02.043. [PubMed: 15094072]

24. Stollery B, Christian L. Glucose, relational memory, and the hippocampus. Psychopharmacology (Berl). 2015;232(12):2113–25. doi: 10.1007/s00213-014-3842-5. [PubMed: 25527036]

25. Ragozzino ME, Pal SN, Unick K, Stefani MR, Gold PE. Modulation of hippocampal acetylcholine release and spontaneous alternation scores by intrahippocampal glucose injections. J Neurosci. 1998;18(4):1595–601. [PubMed: 9454864]

26. Long JM, Davis BJ, Garofalo P, Spangler EL, Ingram DK. Complex maze performance in young and aged rats: Response to glucose treatment and relationship to blood insulin and glucose. Physiology & Behavior. 1992;51(2):411–8. [PubMed: 1313591]

27. Winocur G, Gagnon S. Glucose Treatment Attenuates Spatial Learning and Memory Deficits of Aged Rats on Tests of Hippocampal Function. Neurobiology of Aging. 19(3):233–41. doi: 10.1016/S0197-4580(98)00057-6. [PubMed: 9660998]