

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

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## 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 (4-6, 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  $22 \pm 1$  °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 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.

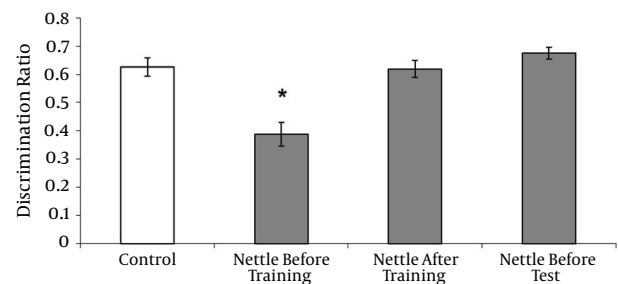
## 4. Results

All of the experimental groups exhibited a stronger ten-

dency 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.

**Figure 1.** Effect of UD on the Discrimination Index on the Object-Recognition Task 24 Hours After the Training Trial



The data were analyzed with one-way ANOVA followed by a Tukey post hoc test. Values are mean ± SEM (n = 7 per group). \*P < 0.05 compared to other groups.

## 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 hydroalcoholic extract of UD (50 mg/kg i.p.) improved memory after 60 days (14). The chronic administration of hydroalcoholic extract of UD (100 mg/kg/day p.o.) enhanced spatial and associative memory at the end of 12 weeks

(13). Nettle supplementation for 8 weeks had a potential effect of decreasing the level of brain injury caused by N-methyl-D-aspartate lesions in rats (19). In previous studies, chronic administration of UD enhanced cognitive function and there were beneficial effects of UD in cognitive disorders (13, 14). UD is a rich source of acetylcholine, 5-hydroxytryptamine, choline acetyltransferase, ferulic acid, carotene, lutein, myricetin, quercetin, kaempferol, and caracole, which are known for their involvement in neuronal function (4-8).

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.

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## 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.

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