

## Original Research Article

# Achillea millefolium Aqueous Extract does not Impair Recognition Memory in Mice

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### Abstract

**Purpose:** To investigate the effect of the aqueous extract of *Achillea millefolium* on recognition memory in mice.

**Methods:** Male mice (35) were used. The aqueous extract of *A. millefolium* was prepared using a Soxhlet apparatus and injected intraperitoneally in a dose of 50, 250, 500 or 1000 mg/kg daily for 20 days. The control group was treated with saline 1 ml/mouse/day. Tactile learning was assessed using the novel object recognition test (NORT) in a dark room which entailed measurement of the distance travelled during trial and test phases.

**Results:** Treatment with different doses of *A. millefolium* did not affect activity levels (based on the distance travelled during trial and test phases). The total time and frequency of visits to the sample objects in trial and test phases were not statistically significant between control and *A. millefolium* treated groups ( $p > 0.05$ ). A comparison of the discrimination ratio between the experimental groups revealed no difference. Administration of *A. millefolium* extract for 20 days did not decrease body weight or cause death in the treated animals.

**Conclusion:** The result of this study demonstrates that chronic treatment with different doses of the aqueous extract of *A. millefolium* did not impair recognition memory in mice.

**Keywords:** Recognition, Memory, Learning, Tactile, *Achillea millefolium*

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## INTRODUCTION

*Achillea millefolium* (Asteraceae), known as yarrow ("mil folhas"), is one of the most widespread and frequently used medicinal plants worldwide [1].

*A. millefolium* has been used as a treatment for wound healing, infectious diseases, pain and gastrointestinal complaints as well as many other conditions [2]. Although *A. millefolium* has been suggested as a folk remedy for the traditional treatment of central nervous system diseases,

few data have been published supporting this claimed ethnomedical action. Elmann *et al* reported that *A. millefolium* extract has anti-inflammatory effects on lipopolysaccharide (LPS)-activated primary cultures of brain microglial cells. Therefore, they concluded that *A. millefolium* could be beneficial in preventing/treating neurodegenerative diseases like Alzheimer and Parkinson [3]. In another study, Molina- Hernandez *et al* reported anticonflict-like actions of aqueous extract of the flowers of *A. millefolium* in rats [4]. Also recently, Baretta *et al* reported that acute and chronic oral

administration of the hydroalcoholic extract of *A. millefolium* exerted anxiolytic-like effects in mice [5].

The objective of the present study was to investigate the effects of chronic treatment with the aqueous extract of *A. millefolium* on learning and memory. To address this question, we investigated the effect of different doses of aqueous extract of *A. millefolium* on tactile learning (measured by novel object recognition test) in mice.

## EXPERIMENTAL

### Animals

We used 35 NMARI male mice (weighing 24 - 34 g). The animal ages were 8-10 week. The animals were purchased from Rafsanjan University of Medical Sciences and maintained on a 12 h light/dark cycle (light on: 07:00 to 19:00 h) with free access to food and water. The animal house temperature was maintained at  $23 \pm 2.0$  °C. Procedures involving animals and their care were conducted in accordance with the Guide to the Care and Use of Experimental Animals [6]. Approval from the institutional animal ethics committee was also obtained (ref no; 1057). During the experiments, all the animals were weighed every day.

### Plant material

The plant material was collected in March 2011 from Isfahan Botany Herbarium. and was identified by Dr. Valiollah Mozaffarian at Botany Research Division, Research Institute of Forests and Rangelands, Tehran-Iran. A voucher specimen has been kept in Isfahan Botany Herbarium voucher specimen no. 9757. The dried aerial parts of the plant (200 g) were rinsed with distilled water, dried, ground into powder and extracted in a Soxhlet apparatus with distilled water. The solvent was evaporated under reduced pressure at 40 °C. The extract was reconstituted by dissolving it in distilled water before use.

### Animal groups

The mice were randomly allocated into 5 groups with seven mice in each group as follows: Control, and four treatment groups in which the mice were treated with 50, 250, 500 or 1000 mg/kg *A. millefolium* aqueous extract, respectively, daily for 20 days. The drug was administered intraperitoneally.

### Object recognition task

The object recognition task assesses recognition memory and is based on a natural tendency of animals to preferentially explore novel objects, as opposed to familiar objects [7]. The apparatus was a Plexiglas box (35 × 35 × 35 cm) with a black plastic floor placed in a dimly illuminated room [8]. The objects to be discriminated were square and triangular iron blocks. The behavior of mice was recorded by a camera positioned directly above the box and the data subsequently analysed using Ethovision software (Noldus, Wageningen, Netherlands).

The object recognition task was done in 3 phases (habituation, training and test phases) with 24 h interval between habituation and training phases and 4 h interval between training and test phases. During the habituation phase, the mice were allowed to freely explore the box in the absence of objects for 30 min. In the training phase (T1), each mouse was placed in the box with one object and allowed to explore for 10 min. To prevent side preference affecting the results, the position and shape of the object were changed after each animal was tested. All mice were placed in the box at the same point and they were facing the same direction. In the test phase (T2), each mouse was returned to the box where it was presented with a familiar object from the training trial (the position of this object was consistent between both training and test phases) and a novel object. Exploration time in T2 was 10 min similar to T1. Care was taken to avoid olfactory stimuli by cleaning the box and objects with 70% ethanol between tests [9]. The time spent (in seconds) for exploring the objects was recorded. Exploration was defined as pointing the nose to the object at a distance  $\leq 2$  cm. Climbing or sitting on an object was not considered as exploration. In T2 phase, a discrimination ratio was calculated using the following formula: [total time spent in exploring both objects divided by the time spent exploring novel objects only] × 100. Mice showing a total exploration time < 10 s in either training or testing phases were excluded [8].

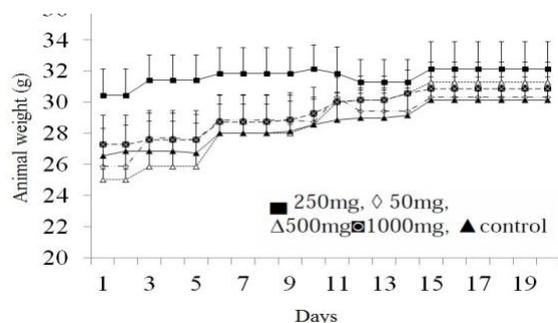
### Statistical analysis

The statistical analysis was performed using Excel and SPSS software. All data are expressed as mean  $\pm$  SEM. Differences between the groups were determined using ANOVA followed by Tukey post hoc test. Paired t-test was also used to compare activity level between trial and test phases and also to compare weight changes in each group. A *p*-values < 0.05 was considered statistically significant.

## RESULTS

### Weight change

The body weight of animals in all the groups (both control and treatment) increased ( $p < 0.05$ ) during the 20-day administration of *A. millefolium* extract (Figure 1). We did not observe any mortality in animals during the treatment period.



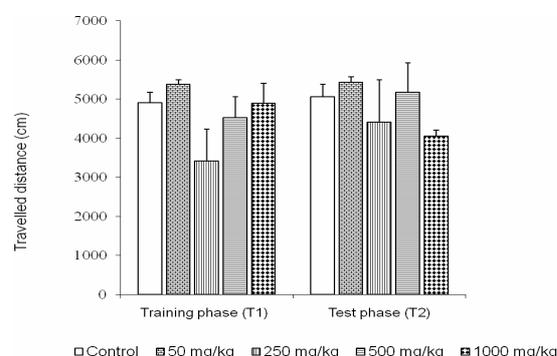
**Figure 1:** Weight changes in control and *A. millefolium* aqueous extract-treated animals over a 20-day period. Data are expressed as mean  $\pm$  SEM ( $n = 7$ )

### Novel object recognition

#### Activity level

Activity level was assessed by measuring the distance travelled during trial (T1) and test (T2) phases (Figure 2). In control group, the distance travelled in T1 and T2 were not significantly different ( $p = 0.6$ ). In animals that received 50, 250, 500 and 1000 mg/kg extract, the distance travelled in T1 and T2 were not significantly different ( $p > 0.05$ ).

In extract-treated groups, the distance travelled in T1 and T2 did not differ significantly compared to the related phases in control group ( $p > 0.05$ , Figure 2).



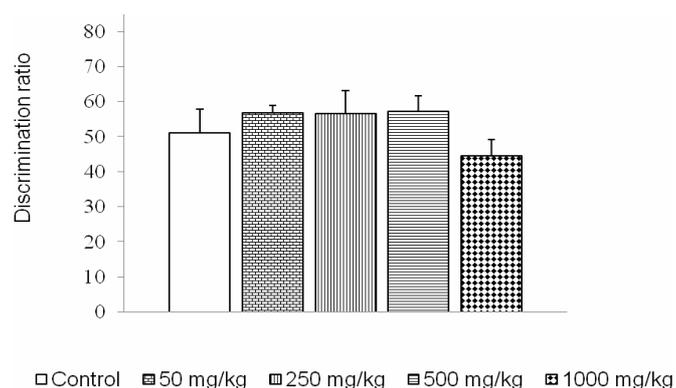
**Figure 2:** Activity level of control and *A. millefolium* aqueous extract-treated animals. Activity levels measured by distance travelled in 10 min in both T1 and T2 phases. Data are expressed as mean  $\pm$  SEM ( $n = 7$ )

#### Object recognition task: Trial phase (T1)

The total time spent exploring one object in T1 (Table 1) was not statistically significant between control and extract-treated groups ( $p = 0.5$ ). Similarly, no reliable differences were found for the frequency of visits to the sample objects between experimental groups (Table 1,  $p = 0.09$ ).

#### Object recognition task: Test phase (T2)

Object exploration times during the test phase (T2) in experimental groups are also shown in Table 1. No significant differences were found for the time to explore novel ( $p = 0.1$ ) and familiar ( $p = 0.9$ ) objects between experimental groups. The mean of total exploration time of both objects (familiar + novel) were not statistically significant between control and extract-treated groups ( $p = 0.75$ ). Moreover, no significant reliable differences were found for the frequency of visits to the novel ( $p = 0.6$ ) and familiar ( $p = 0.6$ ) objects between experimental groups (Table 1). A comparison of the discrimination ratio (Figure 3) between the experimental groups revealed no difference ( $p = 0.3$ ).



**Figure 3:** Discrimination ratio for control and *A. millefolium* aqueous extract-treated animals. Data are expressed as mean  $\pm$  SEM ( $n = 7$ )

## DISCUSSION

The growing number of studies on the possible beneficial effect of *A. millefolium* extract for prevention or treatment of neurodegenerative diseases may be attributed to the plant's potentials. However, this raises important questions about possible side effects of the plant on the nervous system function, especially on learning and memory. Therefore, in this study, we sought to investigate the effect of *A. millefolium* aqueous extract on tactile learning using the novel object recognition task. Our results demonstrated that long-term treatment with different doses (50, 250, 500 and 1000

**Table 1:** Frequencies and times of visits novel or familiar objects in T1 and T2 among extract and control groups

Phase	Parameter	Treatment				
		Control	50mg/kg extract	250mg/kg extract	500mg/kg extract	1000mg/kg extract
Trial phase (T1)	Total exploration time (sec)	83.7±17.4	75.17±15.3	134.7±81.7	48.2±16.6	114.8±23.8
	Frequency of visits to both objects	82.4±15.1	77.8±15	50.5±22.8	49.1±12.3	122.5±25.8
Test phase (T2)	Time to visit familiar object	65.3±28.9	68.9±4.7	52.9±18.4	43.2±10.8	59.8±9.8
	Time to visit novel object	46.9±8.8	93.2±12.8	49.4±15.4	62.7±15	48.5±10.3
	Total exploration time (sec)	112.2±29.3	162.1±17	102.3±32.6	105.9±23.8	108.3±16.9
	Frequency of visits to familiar object	119.0±52.3	74.6±5.9	59.8±22.3	55.5±9.1	154.5±79.4
	Frequency of visits to novel object	147.7±56.3	101.4±11.6	63.5±18.1	92.4±21.6	66.2±11.6

Data are expressed as mean ± SEM; T2 results were obtained 24 h after obtaining T1 results

mg/kg) of the aqueous extract of *A. millefolium* did not impair this ability in mice.

Most neurodegenerative diseases such as Alzheimer, Parkinson, lateral sclerosis and multiple sclerosis have deleterious effect on learning and memory [10] and these diseases mainly destroy integrative and cognitive abilities [10].

*A. millefolium* is one of the oldest known botanicals used by humans. Chandler *et al* compiled a list of 30 medicinal uses *A. millefolium*. They divided its uses into four categories including wounds and skin damage, bleeding conditions, digestive problems and general tonic use [12]. Human use of *A. millefolium* is mainly in the form of the aqueous extract. In several studies, various doses of *A. millefolium* extracts ranging from 8 to 3000 mg/kg were used. However, the most frequent doses for *A. millefolium* extracts were between 50 to 1000 mg/kg [2].

Most of previous studies reported no sign of toxicity for *A. millefolium*. Acute treatment of rats with *A. millefolium* aqueous extract up to 10 g/kg orally and up to 3 g/kg intraperitoneally caused no death [13]. In addition, long-term studies demonstrated little side effects or toxicities in animals treated with *A. millefolium* extract [13]. In a study on the pregnant female rats, animals treated with the ethanol extracts of *A. millefolium*

at a dose of 2.8 g/kg/day for one week, no signs of maternal toxicity were reported [14].

However, there are some reports about toxicity and side effects of *A. millefolium*. One side effect of *A. millefolium* is allergic contact dermatitis. It has been reported that the concentration of sensitizing compounds (guaianolides) of *A. millefolium* diminish in dried or processed material due to degradation [15]. Graf *et al* reported that yarrow tea might have weak genotoxic effect in drosophila [16]. In male rodents, it is reported that high doses of both ethanol [17] and aqueous extracts [18] of *A. millefolium* have some deleterious effects on spermatogenesis. However, our results demonstrated that the aqueous extract of *A. millefolium* has no deleterious effect on recognition memory.

In addition, the anxiolytic-like properties of *A. millefolium* was recently reported by Baretta *et al* [5]. On the other hand, it has been reported that benzodiazepines as standard anxiolytics induce anterograde amnesia [19]. Therefore, it may be suggested that aqueous extract of *A. millefolium* may reduce anxiety with little side effects on memory.

On the other hand, there are reports about biological effects of other forms of *Achillea* extracts. For example, it is reported that a methanol extract of *Achillea* is active against

helicobacter pylori [20]. A lipophilic fraction from *A. millefolium* reduced pain responses in an acetic acid-induced writhing test. Therefore, we suggest that in future studies other forms of *A. millefolium* extracts be tested for their possible effects on learning and memory.

## CONCLUSION

As there might be some new implications for *A. millefolium* in preventing or treating mental disorders [2], the result of present study demonstrated that long term treatment with aqueous extract of *A. millefolium* has no impairing effect of recognition memory in mice.

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