

# THE EFFECT OF CURCUMIN ON CONSOLIDATION AND RETRIEVAL MEMORY FOLLOWING UNILATERAL LABYRINTHECTOMY

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## ABSTRACT

**Objectives.** Nowadays, vestibular system disorders are common. The aim of this study was to evaluate the effect of curcumin on Vestibular compensation (VC) and behavioral changes following unilateral labyrinthectomy in male rats.

**Methods.** Forty adult male Wistar rats were randomly divided into 5 groups: control; without labyrinthectomy and injection of curcumin with passive avoidance learning and memory retrieval testing in 3, 6 and 10 days after learning, group 2; labyrinthectomy with passive avoidance learning and memory retrieval testing, group 3; labyrinthectomy with passive avoidance learning and injection of curcumin then memory retrieval testing, group 4; passive avoidance learning, labyrinthectomy and memory retrieval testing and group 5; passive avoidance learning, labyrinthectomy with injection of curcumin and memory retrieval testing.

The rats were anesthetized at the end of behavior experiment. The brain tissue was removed and brain stem was taken for morphological assessment.

**Outcomes.** The results of this study showed that labyrinthectomy significantly reduced the latency of entering the dark section of the shuttle box compared to the other groups on the 6th and 10th day learning ( $P < 0.05$ ). In the curcumin groups with labyrinthectomy (groups 3 and 5), learning which was performed before and after labyrinthectomy, the latency of the entrance to the dark section was decreased but not significant in comparison to the control group ( $P > 0.05$ ). In group 4, after learning and then labyrinthectomy, the latency of the entering the dark section was decreased but not significant in comparison to the control group ( $P > 0.05$ ).

In the morphological assessment, in the labyrinthectomy group, the increase of supportive cells in the brain stem and hippocampus was observed in comparison with the control group and also deformation and decrease of neurons in the brain stem was observed, but it was not significant change in curcumin group compared to the control group.

**Conclusion.** Unilateral labyrinthectomy caused behavioral disorders and also tissue changes of brain stem on labyrinthectomy side. curcumin had a protective effect on the nervous system maybe by neurotropic, anti-inflammatory and anti-apoptotic properties.

**Keywords:** vestibular compensation, unilateral labyrinthectomy, vestibular system, learning and memory, curcumin

## INTRODUCTION

Vestibular disorders are common and often disabling. Unilateral labyrinthectomy (UL) causes a syndrome of oculomotor, postural and sensory disorders, which diminish over time in a process of behavioural recovery known as vestibular compensation (VC) (1,2). The vestibular system is the sensory system that contributes to the sense of balance and spatial orientation as well as stabilizes gaze for head velocities and frequencies in the range of natural head movements for the purpose of coordinat-

ing movement with balance (3). The results of some studies have shown that Collateral sprouting occurs following UL in amphibians (4), although it does not occur in vertebrates brainstem at any post-lesional time (5). The findings from one of our previous study showed that the dieters neuron degeneration in ipsilateral occurs after UL (6). Herbal medicine has been commonly used over the years for treatment and prevention of diseases. One of them is curcumin, the main active ingredient in turmeric (7). Due to the phenolic group in curcumin molecular structure, it has antioxidant effects (8,9).

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Oral administration of curcumin significantly reduces lipid peroxidation and increases glutathione in the brain which is considered as a central nervous system protector (10). Curcumin improves cognitive impairment and increases the level of acetylcholine in the brain cortex of diabetic rats (11). Destruction of the vestibular system causes a person to experience visual and motor disorders. curcumin plays a role in processes such as cell proliferation, differentiation and cell migration (12) and is highly effective in preventing the degeneration of neuroglial support cells due to its anti-apoptotic effects (13). Therefore, this study examines the protective effects of curcumin in VC and the study of behavioral changes following UL.

## MATERIALS AND METHODS

### Study design

Experiments were carried out on 40 adult male Wistar rats ( $230\pm 30$  g body weight). The animals were purchased from the animal house of Urmia University of Medical sciences and kept in standard condition (12h light/dark cycle and  $22\pm 2$  temperature) and ad libitum feeding. The animals were randomly divided into 5 groups; control or 1 (without unilateral labyrinthectomy and curcumin, only injection of 0.2 ml ethyl oleate, IP, every day for 15 days and passive avoidance learning with shuttle box then memory retrieval testing on 3, 6 and 10 days after learning), 2 (the next day after unilateral labyrinthectomy, 0.2 ml ethyl oleate, IP, every day for 15 days and Passive avoidance learning with shuttle box then Memory retrieval testing on 3, 6 and 10 days after learning), 3 (the next day after unilateral labyrinthectomy, 20  $\mu$ mol/kg, IP every day for 15 days and passive avoidance learning with shuttle box then memory retrieval testing on 3, 6 and 10 days after learning), 4 (the next day after Passive avoidance learning unilateral labyrinthectomy, 0.2 ml ethyl oleate, IP, every day for 15 days and memory retrieval testing on 3, 6 and 10 days after learning), 5 (the next day after Passive avoidance learning unilateral labyrinthectomy, 20  $\mu$ mol/kg, IP every day for 15 days and memory retrieval testing on 3, 6 and 10 days after learning). All animal experiments were performed according to the instructions of the Urmia University Medical Sciences Ethics Committee.

### Unilateral labyrinthectomy

Animals were anesthetized with pentobarbital sodium (40 mg/kg, ip). Then unilateral labyrinthectomy was performed by electro coagulation apparatus that composed two electrodes (cathode and anode), the cathode was inserted into inner ear and the anode was connected to skin with 20 m AM voltage for 1 minute. To reduce pain, we can make 10 mA for 2 minutes. After labyrinthectomy to prevent infection, tetracycline was applied to the site. Then the rats were controlled for proper labinterectomy. When we pull the rat from the tail, it bends towards the labyrinthectomy side.

### Shuttle-Box passive avoidance learning

This device has two bright and dark compartments separated by a door. In avoidance training, a light (60 W) was switched on alternately in the two compartments and used as a conditioned stimulus (CS). The CS preceded the onset of the unconditioned stimulus (US) by 5 s and overlapped it for 25 s. In the dark chamber the shock (0.1 mA) as unconditioned stimulus (US) was applied continuously to the grid floor of the device.

At the start of learning test, the rats were placed individually on the right compartment of a shuttle box. Then the lamp in the compartment was turned on and 10 seconds later the door between the two compartments was opened. The rat moves between the two compartments for 10 minutes to get used to the environment. The steps mentioned above were repeated 30 minutes later. As soon as the rat entered the dark chamber, the door was closed and an electric shock was delivered to the floor at 50 Hz, 1 mA for 2 seconds. After using the shock, the door opens and the rat moves from the dark chamber to the light chamber quickly. Then the animal removes it from the compartment and two minutes later, passive avoidance learning was repeated. Learning is done if the rat does not enter dark compartment during 120 seconds.

### Shuttle-Box retrieval and memory consolidation test

To perform a memory retrieval and memory consolidation of passive learning avoidance, the rats were individually placed in a light chamber. Immediately after placement of the rat, the lamp

turned on and the door was opened 10 seconds later. The time until arrival in the dark chamber was measured and recorded by a chronometer. This time was followed up to 600 seconds. The rats that did not enter the dark chamber within 600 seconds had a latency time of 600 seconds and Memory consolidation was occurred.

### Histological study

Hematoxylin and eosin (H&E)-stained brain sections from all studied groups were prepared for histological studies.

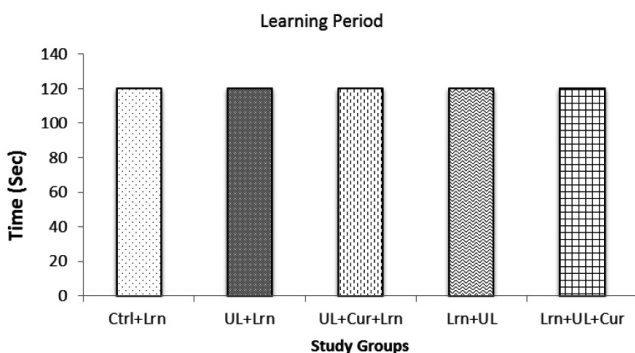
#### Statistical analysis

Data were expressed as the mean  $\pm$  SD. Statistical significance of differences was assessed with one-way ANOVA followed by Tukeys' test.  $P \leq 0.05$  was considered as statistically significant.

## RESULTS

### Learning time at first day

The results showed that the mean of learning time was the same in all groups and was 120 minute. The frequency of shock was also in all groups once. There are no significant differences between study groups (Figure 1).

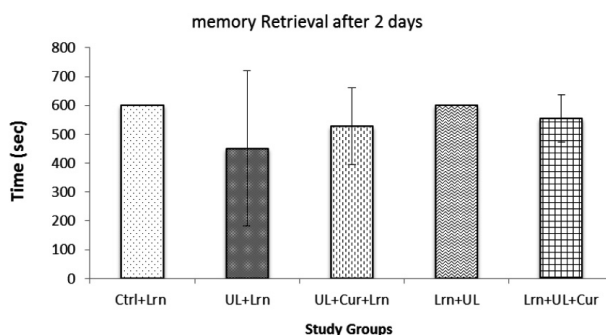


**FIGURE 1.** Comparison of learning time and memory consolidation at the start on study  
Ctrl: Control, UL:unilateral labirentectomy, Cur:Curcumin, Lrn: Learning

### Memory retrieval 48 h after learning

Average latency in entering the dark chamber 48h after was 600, 451 $\pm$ 268, 528 $\pm$ 133, 600 and 555 $\pm$ 83 minute in Control ( Ctrl), Unilateral Labyrinthectomy + learning (UL+Lrn), Unilateral Labyrinthectomy + Curcumin + learning (UL+Cur+Lrn), learning + Unilateral Labyrinthectomy (Lrn+UL) and learning + Unilateral Labyrinthecto-

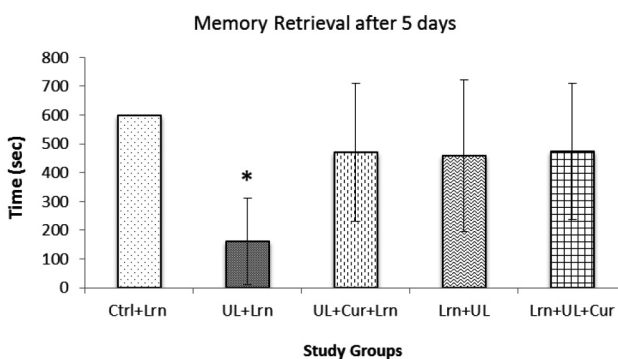
my + Curcumin (Lrn+UL+Cur) groups respectively. The results indicate a decrease in the average delay time in the second group that was labyrinthectomized and then was learned in comparison with control and other groups. But this decrease in time of intering was not significant ( $p=0.2$ , Figure 2).



**FIGURE 2.** Means  $\pm$  SD of memory retrieval 2 day after learning Ctrl: Control, UL:unilateral labirentectomy, Cur:Curcumin, Lrn: Learning, data shows as means  $\pm$  SD

### Memory retrieval 5 day after learning

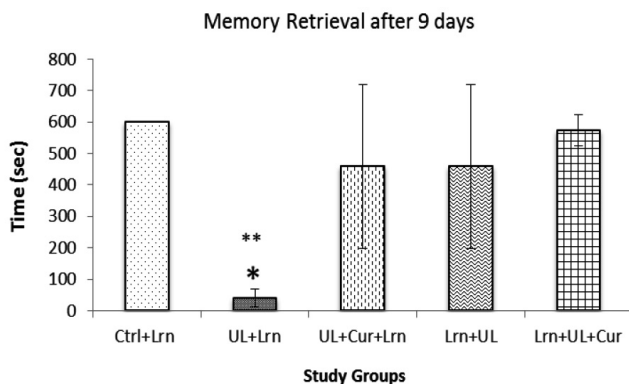
Average latency in entering the dark chamber 5day after was 600, 161 $\pm$ 151, 471 $\pm$ 240, 485 $\pm$ 264 and 473 $\pm$ 236 minute in Control (Ctrl), Unilateral Labyrinthectomy + learning (UL+Lrn), Unilateral Labyrinthectomy + Curcumin + learning (UL+Cur+Lrn), learning + Unilateral Labyrinthectomy (Lrn+UL) and learning + Unilateral Labyrinthectomy + Curcumin (Lrn+UL+Cur) groups respectively. The results show a significance decrease in the average delay time in the second group that was labyrinthectomized and then was learned in comparison with control ( $p<0.01$ ) If it was not significant in comparison to other groups (Figure 3).



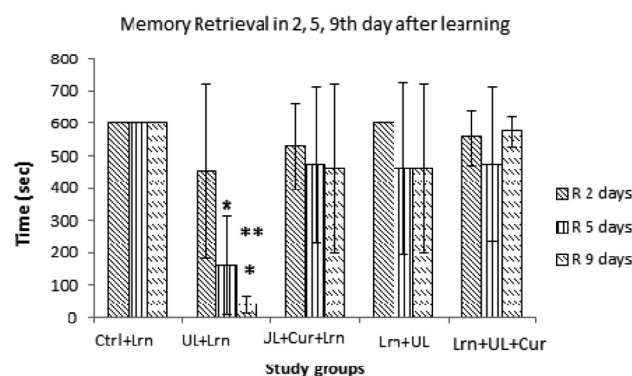
**FIGURE 3.** Means  $\pm$  SD of memory retrieval after 5 day after learning, Ctrl: Control, UL:unilateral labirentectomy, Cur:Curcumin, Lrn: Learning. \* shows the signaficancy whit other groups ( $p\leq 0.05$ )

### Memory retrieval 9 day after learning

Average latency in entering the dark chamber 9day after was 600, 41±29, 459±261, 458±264 and 573±50 minute in Control (Ctrl), Unilateral Labyrinthectomy + learning (UL+Lrn), Unilateral Labyrinthectomy + Curcumin + learning (UL+Cur+Lrn), learning + Unilateral Labyrinthectomy (Lrn+UL) and learning +Unilateral Labyrinthectomy + Curcumin (Lrn+UL+Cur) groups respectively. The results show a significance decrease in the average delay time in the second group that was labyrinthectomized and then was learned in comparison with other studied groups ( $p<0.001$ ) (Figure 4 and Figure 5).



**FIGURE 4.** Means ± SD of memory retrieval after 9 day after learning, Ctrl: Control, UL:unilateral labirentectomy, Cur:Curcumin, Lrn: Learning, \* shows the signficancy whit other groups ( $p\leq0.05$ )

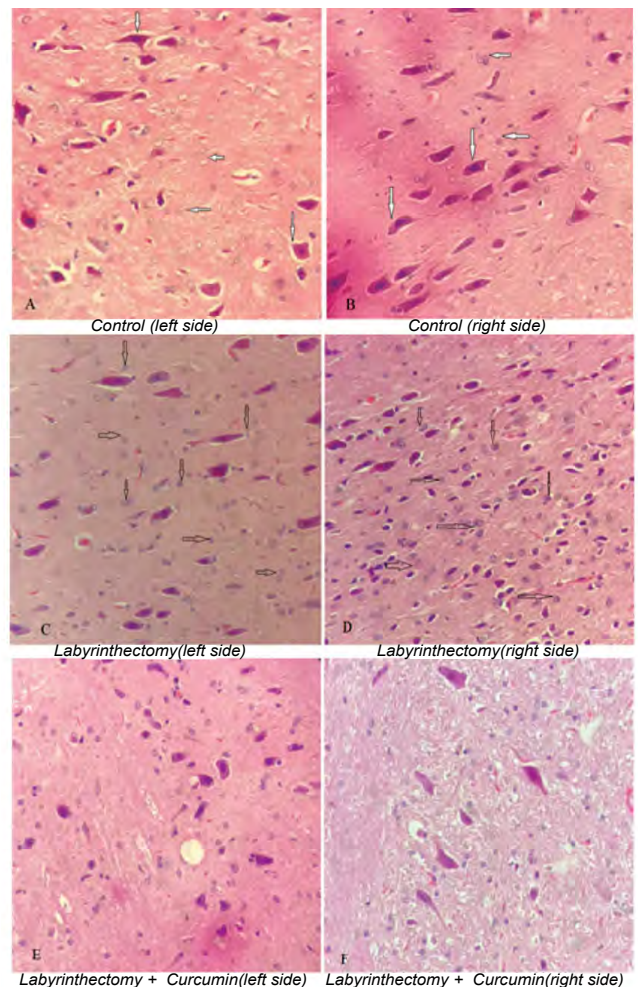


**FIGURE 5.** Comparison of retrieval memory at 3, 6, and 10th of study period, Ctrl: Control, UL:unilateral labirentectomy, Cur:Curcumin, Lrn: Learning, \* shows the signficancy whit other days ( $p\leq0.05$ )

### Vestibular nucleus histological changes

The morphological findings of vestibular nucleus neurons in the control group after staining with H&E showed that the shape of the neurons was dis-

tinct and polygonal and also nucleus of the cells and long dendrites was visible. The high number of neurons, as well as low neuroglial cells, was evident (Figure 6 A and B). But after unilateral labyrinthectomy, at the side of damage, vestibular nucleus neurons were degenerated and the number of neurons decreased. Also, the dendrites were shorter and the size of the cells was smaller in comparison with control. But neuroglia had increased (Figure 6D). While on the healthy side of the same group with no labyrinthectomy, Neurons were healthy and the number of neuroglia was higher than that of the control group but the neuroglia was less than the side of the damage, and some cells appeared small in size (Figure 6 C). As shown in figure 6 E and F curcumin administration reduced the damage of vestibular nucleus.



**FIGURE 6.** Photomicrograph of right and left vestibular nucleus (H&E staining). A and B are control groups (left and right side respectively), C and D labyrinthectomy group, E and F are curcumin treated group. Arrows show the polygonal and microglia cells ( $\times 400$ ).

## DISCUSSION

The results of this study showed that unilateral labyrinthectomy impairs learning and memory recall. The vestibular system plays a significant role in the production of situational, ocular and motor reflexes. It also plays a role in memory and even recognition (3) vestibular disorders not only causes reflexes impairment but also causes anxiety, memory and attention disorders (14,15). The results of present study, in accordance with previous studies, showed that destruction of the inner ear caused impairment causes impairment in memory consolidation and retrieval. Our results showed that the memory and retrieval process was impaired on the 6th and 10th day after labyrinthectomy. While, if learning was initially done, memory and retrieval impairment caused by labyrinthectomy will be less. This finding confirms the vestibular systems role in the onset of the learning process. These findings confirm the results of previous studies on the role of the vestibular system in the learning process (15). The results of the present study showed that there was no memory retrieval impairment when using curcumin following labyrinthectomy. Soukhak-Lari et al. investigated the effect of curcumin on the passive avoidance learning test, and found that administration of curcumin improved memory (16).

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The results of this study confirm the results of previous studies that emphasize the role of curcumin on memory improvement (17,18). The effects of memory recovery following treatment with curcumin may be due to anti-inflammatory properties of curcumin (19) or antioxidant effects and neuroprotection of this agent (17,20,21). In present study, the protective effect of curcumin on vestibular nucleus cells is evident. It confirms the results of previous studies (22).

## CONCLUSION

Finally, the results of this study showed that unilateral labyrinthectomy causes learning process disruption, particularly when labyrinthectomy was occurred before learning. Also, the results showed curcumin has a protective effect on the learning process and memory retrieval after labyrinthectomy. This effect can be due to its anti-oxidant, anti-inflammatory properties and neuroprotective properties.

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