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Brief Communication

Effects of lemon essential oil aroma on the learning behaviors of rats

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Essential oils (EO) are volatile odors obtained from various plants. Their usage dates from ancient India and Egypt, and they have been used for more than 5 thousand years. The EO can be produced by distillation, expression, and CO_2 high-pressure methods. Today, they are widely used for esthetic and therapeutic purposes by inhalation, oral administration, or through the skin. In the fields of medicine and psychiatry, under the name of "aromatherapy," they are used in various fields, such as in emotion and control of mood, anxiolytics, antidepressants, alertness, improvement of memory, and in the treatment of cognitive disorders. Besides these usages, it is known that aroma is used to increase labor efficiency and to decrease labor errors.[1] In addition to esthetic and therapeutic usages; their potential usages in the fields of behavioral attributes and learning has started to attract attention in recent years. Research in this field shows that aroma has important effects on attention level, cognitive performance, creativity, mathematical success and writing skills, task performance, robust perception, and memory. Moreover, it is stated that aroma may have important effects on learning efficiency, learning speed, and learning permanency by positively affecting the mood, which have an important place in directing human behavior. These effects occur through the connection of the olfactory nerve to the hypothalamus and limbic system.[2] Lemon essential oil is produced by the cold expression method, and is a volatile fragrant oil. In traditional medicine, lemon oil is used for colds and to calm. In addition, recent studies have shown that lemon oil has anxiolytic, sedative, anti-spasmodic, and antidepressant effects. A study performed on humans showed that it improved creativity and mood and affected heart rhythm.[3] The aim of this study is to investigate the effects of lemon essential oil as an aromatic stimulus on the learning behaviors of male rats.

Fourteen adult male Wistar rats, from the Experimental Animal Center of Firat University, Medical Faculty, Elazig, Turkey between January and February 2006, weighing 250-280 g, comprised the study material. All the protocols in the present study were performed according to the guidelines of the local ethics committee. Throughout the experiment, the rats were housed in polycarbonate cages (50x30x20 cm) with grid coverings. Inside the experiment room, the rhythm was kept on a 12-h light:12-h dark cycle. To avoid the effects of different odors, we paid attention to the air and odorless condition of the experiment room. Since sawdust on the ground could lead to unwanted odors, it was changed once a day. There was no restriction on drinking water, and the rats were fed once a day. A 120x60x60 cm open, glass labyrinth in the shape of bathtub with 4, 40x60 cm divisions was used. The labyrinth was filled with tap water to the height of 40 cm, and its temperature was kept at 25±1°C. The starting (start position) and the end points (goal position) were determined. The rats were released into the labyrinth at the starting point, and they were directed to find the target once a day for 5 days for familiarization. At the end of 5 days, the 14 rats were randomly divided into 2 groups: Group I, the controls (n=7), and Group II (lemon oil) (n=7). At the end of the familiarization period, the rats in both groups were placed at the starting point in the labyrinth and directed to find their targets once a day for 10 days. A chronometer recorded their time to reach the target. Before testing each rat, anything that may produce odor such as urine, excrement, and so forth, was eliminated from the water by filters. During the experiment period, the rooms of the 2 groups were separated to isolate the rats in the control group from the lemon essential odor. When the rats of the control group were under atmospheric conditions, the rats of the lemon oil group were exposed to lemon essential oil (150 ml), which was dropped into the cage of the experimental group once a day. The rats were exposed to lemon essential oil in the mornings, and every rat inhaled it for 5 minutes. The labyrinth test was carried out in a different room. During the experimental period, the rats in both groups were located inside the labyrinth and a chronometer recorded the duration taken to locate the target. For the duration of the experiment the rats were monitored for weight, food, and water. The Student t test was used to analyze the data. The results were shown in the form of mean ± standard deviation. For this analysis, the Statistical Package for Social Sciences Version 11.0 for Windows (SPSS Inc., Chicago, IL, USA) was used, and a value of $p<0.05$ was assumed to be significant.

At the end of the experiment, there was no weight loss in the rats, and there was no decrease in the amount of water and food consumption. However, we observed a notable increase in the motor activities of the rats in the experiment group. There were no differences between the groups in finding the target point before aroma application. However, during the aroma application, the lemon oil group required a shorter time to find the target point than the control group (**Table 1**).

The cognitive potential of odor has been disregarded for many years because of the dominant role of visuality in education. However, in recent years, research on the

Table 1 - The distribution of the groups' durations taken to find the target during the impartiality provision test according to days (days 1-10 and days 11-20).

Day	Control (n=7) Mean ± SD	Experiment (n=7) Mean ± SD	*P*-value
Before application of aroma			
1	41.3±11.4	38.9±9.8	NS
2	37.1±11.7	35.2±10.6	NS
3	37.7±9.4	36.1±8.3	NS
4	29.6±7.8	30.2±7.1	NS
5	27.1±6.0	24.5±7.3	NS
6	25.4±6.1	24.3±9.9	NS
7	20.7±5.1	22.3±6.3	NS
8	22.0±5.2	16.4±7.3	NS
9	18.7±5.8	16.8±6.9	NS
10	17.0±3.7	15.9±5.5	NS
During application of aroma			
11	19.3±5.1	14.4±8.9	0.032
12	17.4±4.9	9.4±5.6	0.009
13	18.0±6.1	8.1±3.1	0.001
14	14.3±1.9	8.9±5.2	0.020
15	14.1±3.1	6.4±4.7	0.004
16	11.6±2.1	6.8±3.2	0.012
17	12.3±2.0	5.2±1.7	0.000
18	13.6±2.0	5.5±2.5	0.000
19	11.1±2.2	4.8±1.4	0.000
20	11.6±1.4	5.0±1.1	0.000

sense of smell has increased and extended. This research, which is mostly carried out on humans and animals for experimental purposes, shows that various aromas have a positive effect on attention level and concentration, perception, cognitive performance, memory, and mood during the learning process.[2] Ceccarelli et al[1,3] observed that there are effective changes in the robust perception, anxiety, and mood conditions of the rats exposed to lemon aroma for a long time. These effects occur through the ability of olfactory stimulation to affect olfactory-hippocampal and limbic system pathways. In another study, Aloisi et al[4] stated that the lemon aroma may regulate the behavioral and neural reactions in the rats and may be effective in learning to escape from aversive conditions. It is known that the Japanese use odor to increase labor efficiency. They found that worker errors decrease by 21% when lavender is used, by 33% when jasmine is used, and 54% when a lemon odor is used. In similar research by psychologists from the Russian Science Academy, it was observed that lemon, jasmine, and eucalyptus aromas sprayed into the environment increased productivity in computer operators.

In this research, the keyboard errors decreased by 30% when a jasmine aroma was sprayed into the environment, and 50% when lemon aroma was used. The experimental research carried out in this field by Akpinar[5] determined that the lemon aroma sprayed into the classroom increases attention level, enhances cognitive success, and memory of the students.

In this study, the durations to locate the target by the rats were compared, and the results were found to be in favor of the experiment group. This result shows that the rats exposed to the lemon aroma spent less time finding the target than the ones in the control group. The results show that lemon oil can affect learning. These effects occurred by a connection of the olfactory tract with the hippocampus. In addition, this condition can be attributed to an increased attention level resulting from the stimulated CNS of the rats exposed to lemon oil. Attention is very effective on working memory and visual concentration, and it has a determining role in directing cognitive learning. In the labyrinth test, the rats in the experiment group found the target more quickly than the ones in the control group. This may be a result of the fact that increased attention level enhances memory. Other research in this field states that memory and attention are related to each other, and attention is a determining factor in storing information into the memory.

In conclusion, our results indicate that the lemon aroma may have some effects on learning. The findings obtained from this study are consistent with the findings of similar research on the behavioral properties of the lemon aroma.

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