

The Effects of Seed Extract of Carrot on Memory, Nerve Conduction Velocity, and Serum Thyroxin in Rats

Abstract

Background: Thyroid hormones are essential to maintain the tissue's metabolism throughout the life. Thyroid hormones exert their effects on physiology and almost all body functions. Carrot is a rich source of iodine and carotenoids that can interfere in the synthesis of thyroid hormones. **Materials and Methods:** The present study was designed to investigate the effects of carrot consumption on T4 levels and its effect on memory, nerve conduction velocity (NCV), animal weight, and finally, on water and food intake in Wistar rats. In this study, 24 male rats were used and divided into three groups: Control, Ca 200 mg/kg, and Ca 400 mg/kg. **Results:** The results indicated that carrot consumption at Ca 200 mg/kg treated group increased the level of serum T4 and induced hyperthyroidism. Animal weight in both treated groups did not change compared to the control group ($P > 0.05$). Water and food consumption, and the level of T4 in Ca 200 mg/kg group increased when compared to the control group ($P < 0.05$). NCV in both treated groups was not significantly different in comparison with the control group ($P > 0.05$). Spatial memory and passive avoidance memory in both treated groups significantly decreased compared to the control group ($P < 0.05$). **Conclusion:** Carrot consumption, via increases the synthesis of thyroid hormones, creates hyperthyroidism, but due to induced moderate hyperthyroidism did not impact on weight. Moderate hyperthyroidism induced appetite and memory impairment. Then increased food intake or effect of hyperthyroidism on metabolism increased water intake.

Keywords: Carotenoids, *Daucus carota*, hyperthyroidism, iodine, memory disorders, thyroxin

Introduction

The thyroid gland is one of the most important endocrine glands, which exists in all vertebrates. This gland, with the production of triiodothyronine (T3) and tetraiodothyronine (T4), maintains the tissue metabolism in the ideal situation. Thyroid hormones exert a spectrum of effects on the physiology of almost all body organs and their functions.^[1] Thyroid hormones directly affect on energy homeostasis.^[2] Although the body weight in severe thyrotoxicosis usually decrease, but moderate hyperthyroidism can causes an increase in appetite, so increase food intake.^[3,4] Thyroid hormones also affect on the central nervous system (CNS); it has been reported that thyroid hormones are critical for the development of the CNS from the fetal to the adult life, and these hormones are critical for normal functions of all body systems throughout the life.^[5,6] Disruption of the levels of thyroid hormones causes

impairment in memory and learning. It was reported that hypothyroidism induced by iodine deficiency causes impaired cognitive functions in infants.^[7-9] Iodine is an essential mineral component for thyroid hormone synthesis and is very vital for the normal functioning of the thyroid gland. Iodine deficiency causes hypothyroidism and is associated with stillbirth and abortion. Numerous compounds like seafood and some other compound such as carrot are rich in iodine.^[10-12]

Carrot or *Daucus carota* is a vegetable from *Umbelliferae* family, which is highly used as vegetable.^[13,14] Carrot contains substances such as pyrrolidone, glucose, sucrose, protein, pectin, asparagine, carotenoids, and iodine. Iodine in carrot can be used as is an essential element for the synthesis of thyroid hormones. Furthermore, carotenoids in carrot are the precursor to the synthesis of thyroid hormones. Therefore, it seems that carrot is an effective source of iodine in the function of this gland.^[10-12] It was reported that carrot seeds consumption

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had some effects on the nervous system (NS) according to traditional Persian medicine.^[15,16] Carrot and its juice are used as a foodstuff or beverage. Theoretically, carrot has effects on energy homeostasis and the functions of the CNS and peripheral NS (PNS). One of the valuable tests for assessing the performance of the PNS is the measurement of the nerve conduction velocity (NCV).^[17,18]

Due to carrot is used as one of the most consumed fruits and especially in some cases, to improve liver enzymes. On the other hand, it is one of the warm nature fruits and can be useful in cases of memory loss, and it has been mentioned in the sources of traditional Iranian medicine. In our best knowledge by the time, no study has been conducted to evaluate the effects of carrot on NCV, memory, T4 level, and subsequently food and water intake, and animal body weight in rats. Therefore, this study was designed to evaluate the effects of carrot consumption in the rat.

Martials and Methods

Animals

This study was conducted on 24 male Wistar rats with an average weight of 180–220 g. The rats were taken from animal house at the Faculty of Medicine, Mashhad University of Medical Science, Iran. All experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The rats were divided into three groups randomly ($n = 8$ per each group). Animals were kept and treated in accordance with the ethical protocols of laboratory animals approved by Mashhad University of Medical Sciences. The rats were kept and treated under standard conditions (20°C–24°C, 12 h of dark/light cycle) and had free access to food (Javaneh Khorasan, Iran) and tap drinking water. The bodyweight of rats and their 24-hour water and food intake (1 day in a week) were measured. This study was conducted for 6 weeks and in three groups: Control group received ordinary drinking water. The second group (Ca 200) received hydroalcoholic extract of carrot seeds at a dose of 200 mg/kg, and the third group (Ca 400) received hydroalcoholic extract of carrot seeds at a dose of 400 mg/kg.

Preparation of extracts

In this study, 1458 g of carrot seeds were prepared from a local market in Mashhad, verified by the research center of medicinal plants of Ferdowsi University, Mashhad, Iran, and used for all experiments. Carrot seeds were powdered by a grinder. Then, the powder was soaked in 70% ethanol for 72 h at 40°C–45°C. After that, this solution was filtered through a filter paper, and the volume was reduced with vacuum pump. The solvent was removed by an oven at 40°C–45°C. Finally, the carrot extract was stored at 4°C until use.^[13]

Measurement of spatial learning and memory

Morris Water Maze is a water tank with a diameter of 136 cm, a height of 60 cm, and a depth of 30 cm; almost half that height is filled with water (20°C–24°C). The surface of the maze imaginary is divided into four equal quarters, and a circular platform (28 cm height and 10 cm diameter) is hidden 2 cm below the level of water in the center of the southwest quarter (the target quarter). The maze is located in a room where there are several different spatial symptoms, and these symptoms are constant during the experiments and are visible to the animals in the maze. This collection is monitored through a detector camera hanging at the height of 180 cm above the center of the water maze, connecting to the computer. Before the experiment, the animals are trained for 4 days. On the 5th day, spatial memory test was carried out. The platform was removed, and the rats were swimming 60 s. The time spent in the target quarter (Q4) and the distance traveled in the target quarter (Q4) in comparison with other quarters were calculated.^[19]

Passive avoidance test

The shuttlebox was used for passive avoidance test. The shuttlebox is a box with two rooms and two rooms separated from each other's by a guillotine door. There is a dark room and a bright room, and the bottom of the box is made of metal bars. The animals training stage was performed 3 days before the main test. Each animal was placed in the bright room for 20 s. Then, the door opened and the animal due to the desire to enter the darkroom entered it. On the arrival of the animal into the darkroom, an electric shock with a frequency of 50 Hz and a 0.5 mA for 5 s was applied via the rods below the animal's feet in the darkroom. 24 h after the last training, the avoidance memory test was performed. In the experiment day, 2 s after the presence of the animal in the bright room, the guillotine door was opened, and the time of the animal's delay to enter the darkroom and also the duration of its presence in the dark room were recorded for 180 s at 3, 24, and 48 h after shock. During the experiment, no electric shock was applied to the animal in the darkroom.

Nerve conduction velocity measurement

At the end of the experiment (day 42), the animals were anesthetized with intraperitoneal injection of a combination of xylazine and ketamine (a dose of 80 mg/kg ketamine and 8 mg/kg Xylazine) (Alfasan, Nederland). After ensuring complete anesthesia, the animals were fixed on the laboratory board. To determine the motor NCV, first, the right sciatic nerve of the animal by inserting a needle-induced stimulation electrode in the sciatic hole, with 10V was stimulated. Then, the tibial nerve was stimulated in the animal's knee. To record the motor response, needle surface stability electrode was placed in the animal's claws. Sciatic-Tibial motor neural conduction

velocity using two stimulation point along the nerve was calculated.

The measurement of T4

In this study, serum T4 level was measured using a radioimmunoassay kit (Poua Patan Goster Co, Iran), and was read by a gamma counter.

Estimation of food and water consumption

Food and water intake in rats were measured for 24 h in every week during the experiment; animals were also weighed weekly.

Data analysis

The results were expressed as mean \pm standard error of the mean. Water maze data were collected with Radyab (Detector) software. Delay time and the distance traveled to find the platform, and the data from the passive avoidance test were analyzed with repeated measure analysis of variance (ANOVA). The time spent in the target quarter and the distance traveled in this and other quarters, and the other parameters measured in this study were analyzed with one-way ANOVA, and compared by LSD *posthoc*. $P < 0.05$ were considered as significant difference.

Results

In this study, the effect of carrot extract on T4 level, animal weight, food and water intake, NCV, and finally, spatial and avoidance memories were studied, and the results are shown in Figures 1-7.

The results showed that [Figure 1] T4 level in Ca 200 mg/kg treated group was significantly higher than the control group ($P < 0.001$), but in Ca 400 mg/kg group, the T4 level did not change compared to the control group ($P > 0.05$) [Figure 1] (mean: Control = 4.12; Ca 200 = 6.317; Ca 400 = 4.635; $F = 22.086$).

Figures 2-4 indicate, the effect of carrot extract on body weight, and the amount of food and water intake. The results showed that, both (200, 400 mg/kg) treated group had not any change in their weight compared to the control group during the 6 weeks of treatment ($P > 0.05$) [Figure 2 and Table 1]. The amount of food and water consumption in 400 mg/kg group, were not different when compared to the control group ($P > 0.05$), but in 200 mg/kg carrot group, food and water intake was significantly increased compared to the control group (both $P < 0.001$) [Figures 3 and 4]. (Mean food intake: Control = 89.5714; Ca 200 = 179; Ca 400 = 83.2857; $F = 110.774$) (mean water intake: Control = 171.86; Ca 200 = 329.29; Ca 400 = 144.43; $F = 95.686$).

Figure 5 shows that, NCV in both treated groups (Ca 200 mg/kg and Ca 400 mg/kg) did not change in comparison to the control group ($P > 0.05$) [Figure 5]

(mean: Control = 10; Ca 200 = 9.6667; Ca 400 = 11.30; $F = 8.970$).

As Figure 6 shows, the time of finding the platform and the distance traveled to find the platform in neither of the training days did not differ in both treated groups compared to the control ($P > 0.05$). On the probe day, in the Ca 400 mg/kg group, there was no change in the time spent in the target quarter (Q4) compared to the control group ($P > 0.05$). In Ca 200 mg/kg group, the time spent in target quarter (Q4) ($P < 0.05$), and non-target group significantly decreased in comparison to the control group (respectively by $P < 0.001$, $P < 0.05$). Furthermore, the distance traveled in the target quarter (Q4) in both doses of Ca (200, 400 mg/kg) had no change in comparison to the control group ($P > 0.05$), but in Ca 200 mg/kg the distance traveled in non-target quarter (Q3) significantly decreased compared to the control group ($P < 0.01$) [Figure 6 and Table 2]. Figure 7 shows the delay time to enter the darkroom, the number of the entrance to the darkroom, the duration of stay in the darkroom, and the duration of stay in the bright room in Ca 400 mg/kg group, did not change compared to the control group ($P > 0.05$). In Ca 200 mg/kg group, the delay time to enter the darkroom at 3 ($P < 0.001$), 24 ($P < 0.05$) and 48 ($P < 0.01$) hours after the shock, significantly decreased compared to the control group. The number of the entrance to the darkroom in Ca 200 mg/kg group significantly increased at 3 ($P < 0.001$), 24 ($P < 0.05$) and 48 ($P < 0.05$) hours after the shock in comparison with the control group. The duration of stay in the darkroom in Ca 200 mg/kg group at 3 ($P < 0.001$), 24 ($P < 0.05$) and 48 ($P < 0.01$) hours after shock were significantly higher than the control group. Finally, the

Table 1: Mean and F value presented for body weight

Mean	Control	Ca 200	Ca 400	F
Day 0	216.95	255.33	212.17	2.533
W1	232.32	254	222.67	1.190
W2	261.17	278.33	244.5	1.301
W3	282.22	284	254	1.26
W4	289.02	301.67	264.5	1.614
W5	292.83	305.67	272.5	1.398
W6	321.6	309.17	289	1.247

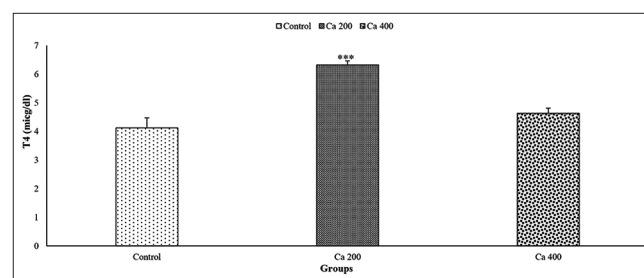


Figure 1: The effect of seed extract of carrot (Ca 200 mg/kg and Ca 400 mg/kg) on the T4 level (mg/dl) in treated rats. Data are expressed as mean \pm standard error of the mean. * $P < 0.001$. ($n = 6$, in each group, and 6 weeks = the duration of the experiment)**

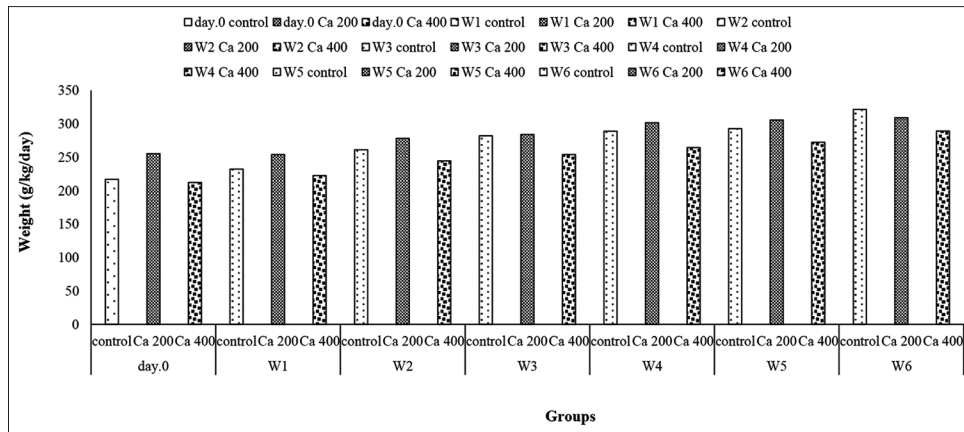


Figure 2: The effect of different doses of seed extract of carrot on weight (g) in treated rats. Data are expressed as mean \pm standard error of the mean. ($n = 6$, in each group, and 6 weeks = the duration of the experiment)

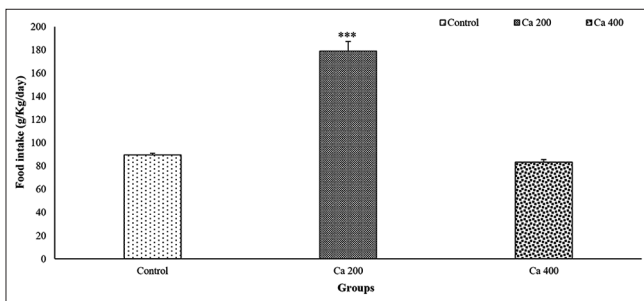


Figure 3: The effect of seed extract of carrot (Ca 200 mg/kg and Ca 400 mg/kg) on food intake (g/J/kg/day) in treated rats. Data are expressed as mean \pm standard error of the mean. $***P < 0.001$. ($n = 6$, in each group, and 6 weeks = the duration of the experiment)

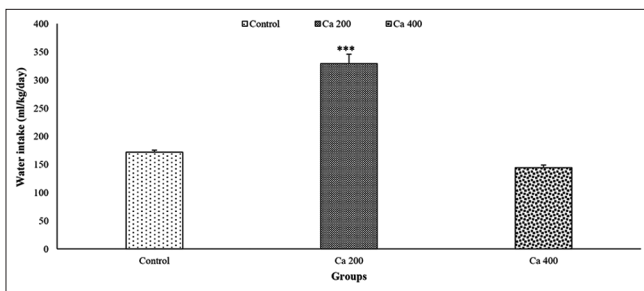


Figure 4: The effect of seed extract of carrot (Ca 200 mg/kg and Ca 400 mg/kg) on water intake (ml/J/kg/day) in treated rats. Data are expressed as mean \pm standard error of the mean. $***P < 0.001$. ($n = 6$, in each group, and 6 weeks = the duration of the experiment)

duration of stay in the bright room in Ca 200 mg/kg group, at 3 ($P < 0.001$), 24 ($P < 0.05$) and 48 ($P < 0.01$) hours after the shock significantly decreased when compared to the control group [Figure 7 and Table 3].

Discussion

In this study, the effect of carrot extract on the level of serum T4, animal weight, water and food intake, NCV, and memory were investigated.

In the present study, consumption of carrot extract induced hyperthyroidism, the T4 blood level was increased. Carrot

is one of the most consuming vegetables from *Umbelliferae* family.^[13,14] In carrot, there are several compounds such as: Iodine and carotenoids. Iodine is a vital element for the synthesis of thyroid hormones. Carotenoids are also important precursor compounds for the synthesis of thyroid hormones. Ultimately, thyroid function is dependent on the adequate supply of iodine to the gland. In hyperthyroid status, the biosynthesis of both monoiodotyrosine (MIT) and diiodotyrosine (DIT), in particular DIT, has been increased due to iodine excesses. As a consequence, MIT/DIT has decreased. In addition, thyroidal type 1 deiodinase and Dio1 mRNA expression were significantly impaired by iodine overload. As a result, the thyroid hormone levels shift, inducing hyperthyroidism.^[20] It seems that carrot consumption, due to the presence of iodine and carotenoids, creates hyperthyroidism,^[11,21,22] these reports are in line with our results and confirm that, consumption of carrot seeds extract enhanced the level of T4. The results of our study showed that the consumption of carrot extract has no effect on animal weight. Along with our results, it was reported that carrot consumption has no effect on animal weight.^[23] Severe hyperthyroidism usually caused weight loss,^[11] but carotenoids increased weight,^[3,4] so this may be the reason for not losing weight in our study. However, severe and long-term hyperthyroidism may lead to weight loss, but in our study, no weight loss was observed. This may be due to mild hyperthyroidism or the short duration of treatment in the current study. Thyroid hormones directly affect on energy homeostasis.^[2] It was reported that moderate hyperthyroidism can cause an increase in appetite, so increase food intake.^[3,4] The result of these reports confirm our results in the present study. In our study, the amount of food and water intake was increased. In the present study, with increased food intake, water intake also was increased. The reason for this result maybe tendency to drink water, when the food consumption increased,^[24] or increased drinking water maybe due to an increase in body metabolism; which is usually present in any form of hyperthyroidism. The results of this study

indicated that consumption of carrot extract has no effect on NCV. Nonaligned with our results it was shown that the consumption of carrot in humans, have harmful and negative effects on CNS,^[15,16] this difference in the results of these studies with our study may be due to the difference in the

race; because our study was performed on Wistar rats and those studies on humans. In this study, spatial and passive avoidance memories impairment (decreased memory) has arisen. Thyroid hormones are critical for maintaining the hippocampal functions.^[25] The hippocampus is one of the important brain areas for spatial learning.^[26] Changes in thyroid hormone levels (hyperthyroidism) cause changes in cognitive functions such as concentration, spatial and avoidance memory. In fact, hyperthyroidism causes learning and memory impairment.^[25,27] These findings are in line with our results. Indeed, in our study, the treatment of animals with carrot caused a hyperthyroidism. It may be, suggested that hyperthyroidism resulted in animals impaired spatial memory and passive avoidance. In line with our study, it was reported that the efficacy of spatial learning in hyperthyroid rats was decreased.^[26] Some studies are against our findings, it was reported that the consumption of carrot seeds by reducing acetyl cholinesterase activity improved

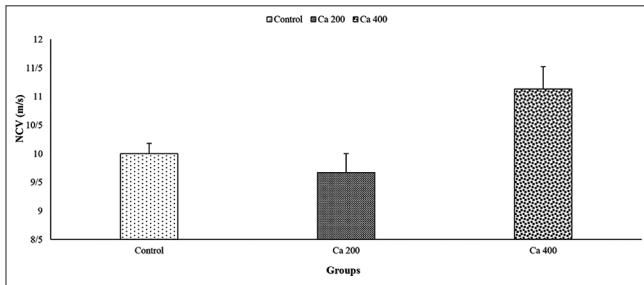


Figure 5: The impact of different doses of seed extract of carrot (Ca 200 mg/kg and Ca 400 mg/kg) on nerve conduction velocity (m/s) in treated rats. Data are expressed as mean ± standard error of the mean. * $P < 0.05$. ($n = 6$, in each group, and 6 weeks = the duration of the experiment)

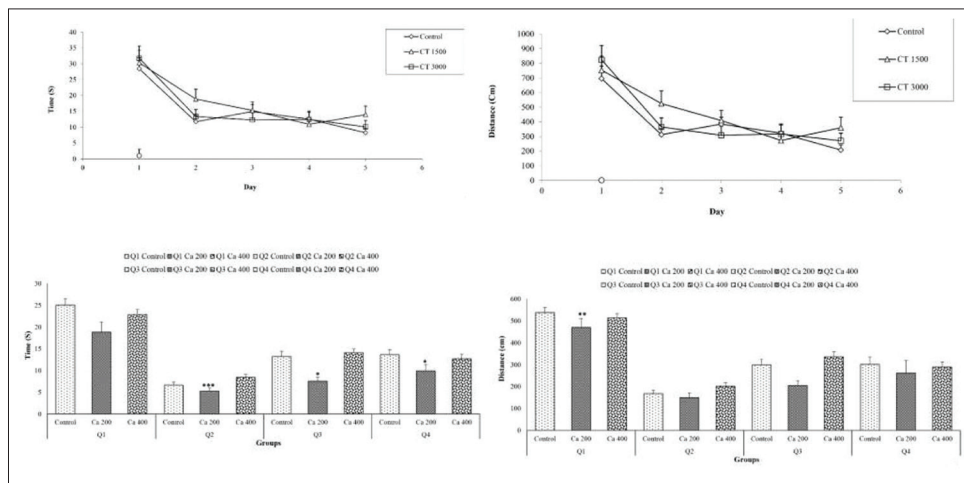


Figure 6: The effect of different doses of seed extract of carrot (Ca 200 mg/kg and Ca 400 mg/kg) on spatial learning and memory in treated rats. Data are expressed as mean ± standard error of the mean. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. #Target quarter. ($n = 6$, in each group, and 6 weeks = the duration of the experiment)

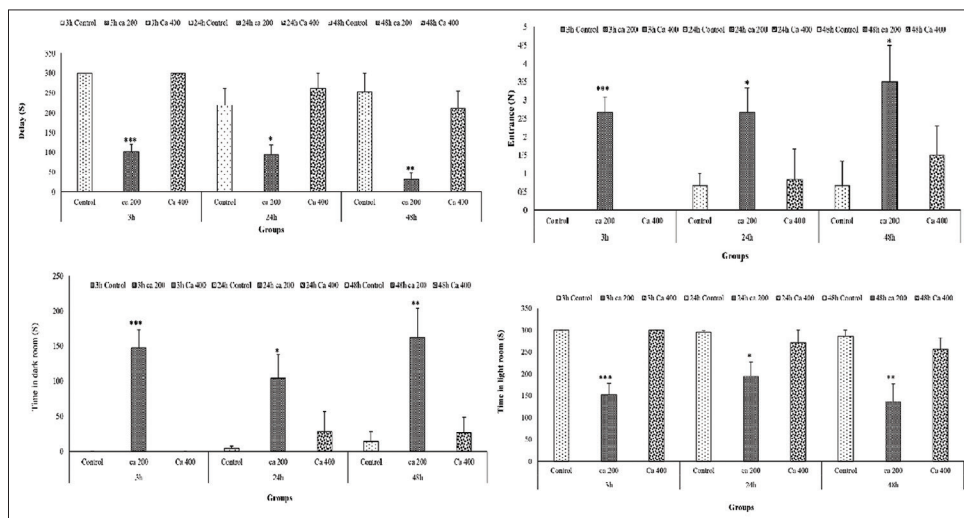


Figure 7: The effect of different doses of seed extract of carrot (Ca 200 mg/kg and Ca 400 mg/kg) on passive avoidance memory in treated rats. Data are expressed as mean ± standard error of the mean. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. ($n = 6$, in each group, and 6 weeks = the duration of the experiment)

Table 2: Mean and *F* value presented for spatial learning and memory

Mean	Control	Ca 200	Ca 400	<i>F</i>
Time (day 1)	29.10	33.03	33.87	0.547
Time (day 2)	11.5	21.61	16.10	2.697
Time (day 3)	14.40	20.81	15.61	1.104
Time (day 4)	12.78	13.31	11.10	0.177
Time (day 5)	8.27	12.25	11.53	0.839
Distance (day 1)	714.58	872.33	750.89	0.742
Distance (day 2)	306.02	649.03	412.36	4.741
Distance (day 3)	369.67	569.06	378.66	2.01
Distance (day 4)	329.83	379.54	281.48	0.525
Distance (day 5)	208.50	333.95	291.12	1.234
Speed (1)	25.62	26.78	23.38	3.864
Speed (2)	31.12	30.42	29.96	0.142
Speed (3)	28.10	28.50	28.03	0.042
Speed (4)	27.17	29.31	29.35	1.242
Speed (5)	27.11	27.96	25.93	0.950
Time (Q1)	13.62	9.88	12.70	2.340
Time (Q2)	6.63	5.24	8.44	4.99
Time (Q3)	13.22	7.52	14.08	11.88
Time (Q4)	25.05	18.82	22.83	3.421
Distance (Q1)	301.43	261.68	287.620	0.495
Distance (Q2)	167.74	149.41	201.71	2.262
Distance (Q3)	299.18	204.69	355.50	8.106
Distance (Q4)	537.33	469.16	512.74	0.755
Speed (Q1)	23.01	22.04	24.30	0.744
Speed (Q2)	25.65	23.16	24.02	0.588
Speed (Q3)	23.11	24.48	23.21	0.425
Speed (Q4)	21.46	27.12	24.17	6.34

Table 3: Mean and *F* value presented for the passive avoidance memory

Mean	Control	Ca 200	Ca 400	<i>F</i>
Delay 3	300	101.17	300	108.385
Entrance 3	0	2.67	0	40
Dark room 3	0	147.5	0	32.717
Light room 3	300	152.5	300	32.717
Delay 24	218.67	93.67	260.17	5.783
Entrance 24	0.67	2.67	0.83	2.956
Dark room 24	4.33	104.17	28.33	4.175
Light room 24	295.67	194.17	271.67	4.462
Delay 48	253.67	31.67	211.17	9.604
Entrance 48	0.67	3.50	1.50	3.061
Dark room 48	14.17	162.17	26.83	8.316
Light room 48	285.83	136.17	256.50	7.343

diazepam-induced forgetfulness.^[28-30] In these studies, the effect of carrot on forgetfulness has been studied, while in our study, the effect of carrot on normal rats was investigated. So these differences can be attributable to differences in tested statuses. Since in our results, carrot consumption shows hyperthyroidism and causes impairment in spatial learning memory and passive avoidance memory. According to Traditional Persian medicine, carrot and

other foodstuffs have a special temperament.^[31-33] Carrot has a warm and wet nature, meaning that it can generally imping upon the rate of metabolism. Elevation of T4 can be considered as a pathway to warm the body.

In this study, it was shown that the dose of 400 mg/kg showed fewer effects than the dose of 200 mg/kg in the investigated parameters. Numerous studies have been shown that sometimes a lower dose is effective for a measured parameter, while a higher dose is not effective. This can be due to the occupation of all active positions, in which case, it creates a situation similar to insensitivity to that active substance. Therefore, it is possible that in the present study, the dose of 400 mg/kg was ineffective or had fewer effects than the 200 mg/kg.

Conclusion

In summary, in accordance with the results of this study, carrot by containing iodine and carotenoids may cause the animals rendered hyperthyroid. Hence, due to hyperthyroid state the animal, increased food and water intake. In this study also, impairment in memory occurred, and this may be due to the effects of high T4 level on memory. Perhaps, the validity of these results may need more investigation to find out cellular and molecular mechanisms.

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Conflicts of interest

There are no conflicts of interest.

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