Original Article

Effect of Watermelon Extract on Nerve Conduction Velocity, Memory, and T4 Level in Rats

Majid Jafari Nejad Bajestani, Mahdi Yousefi, Mousa-Al-Reza Hadjzadeh[1,2,3], Mahmoud Hosseini[1,2,3], Ali Taghipour[4], Shiba Yousefvand[1]

Department of Persian Medicine, School of Persian and Complementary Medicine, Mashhad University of Medical Sciences, [1]Department of Physiology, School of Medicine Mashhad University of Medical Sciences, [2]Division of Neurocognitive Sciences, Psychiatry and Behavioural Sciences Research Center, Mashhad University of Medical Sciences, [3]Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, [4]Department of Epidemiology and Biostatistics, Cancer Research Center, Social Determinants of Health Research Center, School of Health, Mashhad University of Medical Sciences, Mashhad, Iran

Address for correspondence:
Prof. Mousa-Al-Reza Hadjzadeh, Department of Physiology, School of Medicine Mashhad University of Medical Sciences, Mashhad, Iran.
Division of Neurocognitive Sciences, Psychiatry and Behavioural Sciences Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
E-mail: hajzadehmr@mums.ac.ir

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Abstract

Background: Watermelon is a fruit with the thirst-quenching effect and especially is consumed in summer. In this study, the effect of consumption of watermelon extract (in drinking water) on thyroid hormone level (TT4), animals' weight, water and food consumption, nerve conduction velocity (NCV), and memory in Wistar rats were investigated. **Materials and Methods:** Twenty-four male Wistar rats were divided into three groups: control, CT 1500 mg/kg, and CT 3000 mg/kg ($n = 8$, in each group). Watermelon extract was administered for 42 days. The results measured in this study were analyzed with repeated measures or one-way analysis of variance and compared by the least significant difference *post hoc*. $P < 0.05$ was considered as significant difference. **Results:** The results showed that animals' weight in both the treatment groups decreased compared to the control group ($P < 0.05$ and $P < 0.01$, respectively). Water and food consumption and the level of TT4 increased when compared to the control group in both the treated groups (both $P < 0.001$). NCV in both the treated groups decreased in comparison with the control group (both $P < 0.001$). Spatial memory did not change significantly, but passive avoidance memory in both the treated groups significantly decreased in comparison to the control group (respectively, with $P < 0.05$ and $P < 0.01$). **Conclusion:** Based on these finding, in spite of increase in food and water consumption in the treated groups, animal weight did not increase significantly. Impairment in memory and decrease in NCV were also occurred, but TT4 level was increased in animals treated with watermelon extract.

Keywords: *Citrullus, hyperthyroidism, memory, neural conduction*

Introduction

Watermelon is a fruit wildly consumed in Iran nearly for 6 months from April to September. It is the fruit of watermelon, a plant belonging to the Cucurbitaceae family. Watermelon is rich in lycopene, water, and Vitamins niacin, A and C. The red color in fruits such as watermelon is due to lycopene. Lycopene is associated with a reduced risk of cardiovascular diseases and some cancers.[1] Watermelon (*Citrullus lanatus*) is also rich in L-citrulline, a precursor of L-arginine that may reduce the risk of cardiovascular diseases. The results of some studies showed that watermelon and arginine improved cardiovascular risk factors such as lipid antioxidant capacity and inflammation.[2] It was also reported that in humans, watermelon daily intake reduced body weight (BW), body mass index, and blood pressure, and improved blood lipid profile and antioxidant status, and helped to reduce cardiovascular risk factors.[3] For watermelon, several properties have been mentioned, including reducing thirst and fever, diuretic, and laxative effects. There is also evidence of its effects in the treatment of hepatotoxicity.[4] It was also reported that watermelon had protective effects on pancreatic cells and was effective in lowering blood sugar.[5] In another study, watermelon effects on gastric ulcer treatment in rats were demonstrated through its protective role on the stomach.[6] Another study in humans showed that watermelon has antioxidant effects.[7] It was also reported that the consumption of watermelon skin increased thyroxine (T4) levels.[8,9] In Persian Medicine, watermelon is used for the relief of nerve pain.[10] The consumption of high amount of watermelon has sleepy effects and can also cause central nervous system symptoms such as dizziness and drowsiness.[11]

According to the abovementioned properties, the aim of this study in part was to evaluate the effects of consumption of watermelon extract on memory, if any, and

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also on nerve conduction velocity (NCV), T_4 levels, food and water intake, and BW in rats.

Materials and Methods

Animals

In this study, 24 Wistar male rats with an average weight of 180–220 g were used. The rats were taken from animal house at the Faculty of Medicine, Mashhad University of Medical Sciences, Iran. 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 (22°C–24°C, 12 h of dark/light cycle) throughout the study and had free access to food (Javaneh Khorasan, Iran) and tap drinking water. The BW of rats and their 24-h water and food intake (1 day in a week) were measured and recorded. This study was conducted with three experimental groups (n = 8 per each group): control group received ordinary drinking water. The second group (CT 1500) received water-soluble extract of watermelon at a dose of 1500 mg/kg BW, and the third group (CT 3000) received water-soluble extract of watermelon at a dose of 3000 mg/kg BW.

Preparation of extracts

Watermelon grooved in Chabahar weighing about 7 kg was purchased from a local market in Mashhad and used for all experiments. In brief, after removing the skin, pulp of the watermelon was grinded with a mixer; 3930 g of the collected watery product was taken and was filtered with filter paper and vacuum pump. 3167 g of watermelon juice was obtained; then, watermelon juice was evaporated at 45°C–54°C and yielded 271.3 g of extract. Finally, the watermelon extract was stored at 4°C until use.

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 (22°C–24°C). The surface of maze imaginary is divided into four equal quarters, and a circular platform (28 cm height and a 10 cm diameter) is hidden 2 cm below the water level. It is placed in the center of the southwest quarter (the target quarter). The maze is located in a room with several different spatial symptoms which are visible to the animals. This collection is monitored through a detector camera at a height of 180 cm above the center of the water maze, connecting to the computer. Before the experiment, each animal was released from one of the four positions (North [N], East [E], South [S], and West [W]) of the maze. The choice of location was started randomly by experimenter. Each animal had a maximum period of 60 s at each test to find and stand on the platform and stay 15 s on it. Time spent to find the platform and the distance traveled to find the platform were recorded by the camera. In the 5th day, spatial memory test was carried out. The platform was removed, and the rats swimmed for 60 s. The time spent in the target quarter (Q1) and the distance traveled in the target quarter (Q1) in comparison with the other quarter were calculated.

Passive avoidance test

The passive avoidance is usually used to explain the experiments in which animals learn to avoid a painful stimulus. The shuttlebox was used for passive avoidance test. The shuttlebox is a box with two rooms, separated from each other by a guillotine door. There are a darkroom and a bright room, and the bottom of the box is made of metal bars. The animals' training stage was performed for 3 days before the main test. Each animal was placed in the bright room for 20 s. Then, the door was 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 through the rods below the animal's feet in the darkroom. Twenty-four hours 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 darkroom were recorded for 300 s. During the experiment, no electric shock was applied to the animal in the darkroom.

Nerve conduction velocity measurement

Measurement of NCV is a major laboratory technique for the study of peripheral nerve function. It seems that 42 days is a relevant time for developing NCV alteration. Therefore, in this study, 42 days were chosen for treatment of watermelon extract. 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, The Netherlands). 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 was stimulated by 10 V through inserting a needle-induced stimulation electrode in the sciatic hole. 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 points along the nerve was calculated.[12]

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 was measured for 24 h in every week during the experiment. Animals were also weighed weekly.

Data analysis

The results were expressed as mean ± standard error of the mean. Delay time and the distance traveled to find the platform and the data from passive avoidance test were analyzed with repeated measures analysis of variance (ANOVA). The time spent in the target quarter and the distance traveled in this and other quarters, T4 level, water and food intake, and finally, the weight of rats measured in this study were analyzed with one-way ANOVA and compared by the least significant difference *post hoc*. $P < 0.05$ was considered as significant difference.

Results

In this study, the effect of watermelon extract on spatial and avoidance memories, NCV, thyroxin, food and water intake, and animal weight were investigated, and the results are shown in Figures 1-6.

Figure 1 shows the level of T4 after 42-day treatment with the watermelon extract. The results showed that the T4 level in both the CT 1500 mg/kg and CT 3000 mg/kg treated groups was significantly higher than the control group (both $P < 0.001$) [Figure 1].

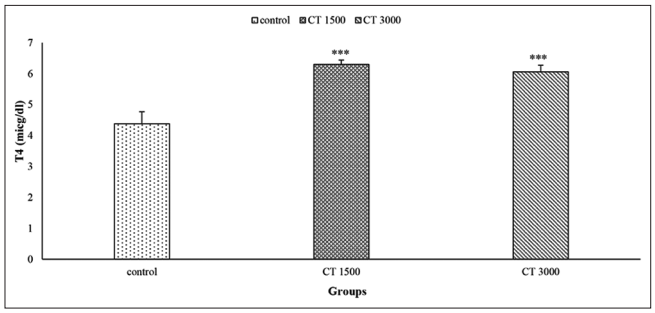

Figure 1: The effect of watermelon extract (CT 1500 mg/kg and CT 3000 mg/kg) on the T4 level in rats. Data are expressed as mean ± standard error of the mean ***$P < 0.001$ ($n = 8$, in each group)

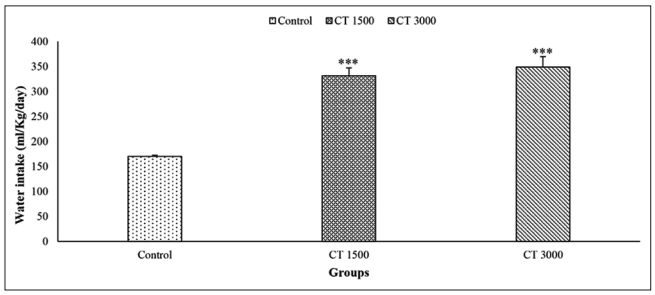

Figure 3: The effect of watermelon extract on water intake (CT 1500 mg/kg and CT 3000 mg/kg) on water intake in rats at the last day of experiment. Data are expressed as mean ± standard error of the mean ***$P < 0.001$ ($n = 8$, in each group)

Figures 2-4 indicate the effects of watermelon extract on BW and the amount of water and food intake at the last day of experiment. The results showed that the BW of animals at the beginning of the experiment (0 day) in the CT 1500 mg/kg and CT 3000 mg/kg treated groups was slightly higher than the control group, while in the 3rd and the 6th weeks after treatment, BW in the CT 3000 mg/kg group was significantly decreased in comparison to the control group (both $P < 0.05$). In the CT 1500 mg/kg group, the BW of animals at the 3rd ($P < 0.05$) and the 6th ($P < 0.01$) weeks significantly decreased when compared to the control group [Figure 2]. Water and food intake at the last day of experiment in CT 1500 mg/kg and CT 3000 mg/kg treated groups significantly increased when compared to the control group (both $P < 0.001$) [Figures 3 and 4].

Figure 5 shows that NCV in both the treated groups (CT 1500 mg/kg and CT 3000 mg/kg) was significantly decreased in comparison with the control group (both $P < 0.001$) [Figure 5].

The time of finding the platform and the distance traveled to find the platform in the training days did not change significantly compared to the control group ($P > 0.05$). On the probe day, the time spent in target quarter for both the treated groups was 85% of the control group (ns), but the time spent in nontarget quarter (q3) in the CT 3000 mg/kg group was significantly higher than the control group ($P < 0.05$). Furthermore, the distance traveled in the nontarget quarter, in CT 1500 mg/kg ($P < 0.05$), and CT 3000 mg/kg ($P < 0.001$) groups was significantly increased in comparison to the control group.

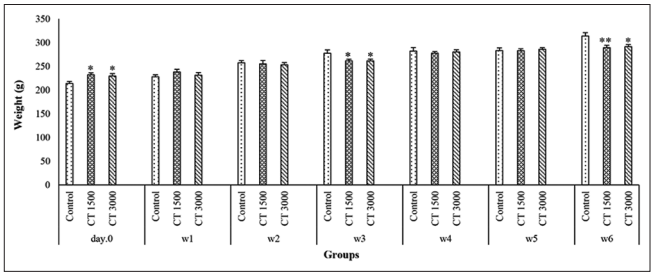

Figure 2: The effect of different doses of watermelon extract on weight in rats. Data are expressed as mean ± standard error of the mean *$P < 0.05$, and **$P < 0.01$ ($n = 8$, in each group)

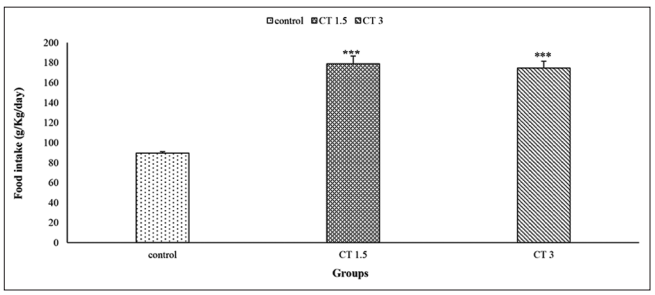

Figure 4: The effect of watermelon extract in water intake (CT 1500 mg/kg and CT 3000 mg/kg) on food intake in rats at the last day of experiment. Data are expressed as mean ± standard error of the mean ***$P < 0.001$ ($n = 8$, in each group)

As Figure 6 shows, the delay time to enter the darkroom in the CT 1500 mg/kg group at 3 ($P < 0.001$), 24 ($P < 0.05$), and 48 ($P < 0.001$) h after the shock decreased significantly compared to the control group. The number of entrances to the darkroom in the CT 1500 mg/kg group increased significantly at 3, 24 ($P < 0.01$), and 48 ($P < 0.05$) h after the shock in comparison with the control group. The duration of stay in the darkroom in CT 1500 mg/kg group at 3 ($P < 0.001$), 24, and 48 ($P < 0.01$) h after the shock was significantly higher than the control group. Finally, the duration of stay in the bright room in CT 1500 mg/kg group at 3 ($P < 0.001$), 24, and 48 ($P < 0.01$) h after the shock significantly decreased when compared to the control group [Figure 6].

Discussion

In this study, the effect of watermelon on the level of thyroid hormone (T4), animal weight, water and food intake, and memory and NCV were investigated.

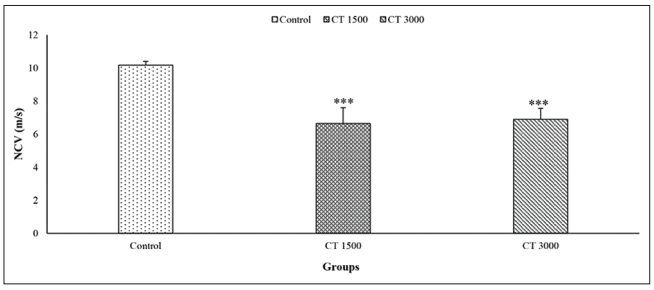

Figure 5: The impact of different doses of watermelon extract (CT 1500 mg/kg and CT 3000 mg/kg) on nerve conduction velocity in rats. Data are expressed as mean ± standard error of the mean ***$P < 0.001$ ($n = 8$, in each group)

In the present study, watermelon extract was used for 42 days; this time is considered to be a medium interval of time for the induction of the effect of cold temperament to induce neural impairment in animal models. Watermelon caused the thyroid gland hyperactivated, and thus, the T4 blood level was increased. In line with our study, it was reported that exposure to cold temperature affects the activity of the thyroid gland and thyroid hormone secretion in rats and increased the secretion of T4.[13] In favor of the results of this study, it has been shown that the consumption of watermelon in rats increased the level of T4.[14] According to the results of various studies, and our study, watermelon increases T4 level in rats. Thyroid hormones directly affect energy homeostasis.[15] Moderate hyperthyroidism can cause an increase in appetite, so increase food intake, although the BW in severe thyrotoxicosis usually decreases.[16,17] The result of these reports confirms our results in the present study. In our study, although food intake increased, the rate of BW gain was too little, which may be due to elevation of T4 level for a long period (6 weeks) that can increase metabolic rate and energy expenditure. The amount of food and water intake has increased, but the BW of the animals was decreased. In the present study, with increased food intake, water intake also was increased. The reason for this result maybe the tendency to drink water when the food consumption increases.[18]

In this study, the NCV in both the groups treated with watermelon extract was decreased. Watermelon is a plant with a cold nature.[10] NCV reduces in rats through treatment with watermelon.[9] The results from traditional medicine sources indicated that materials with cold nature

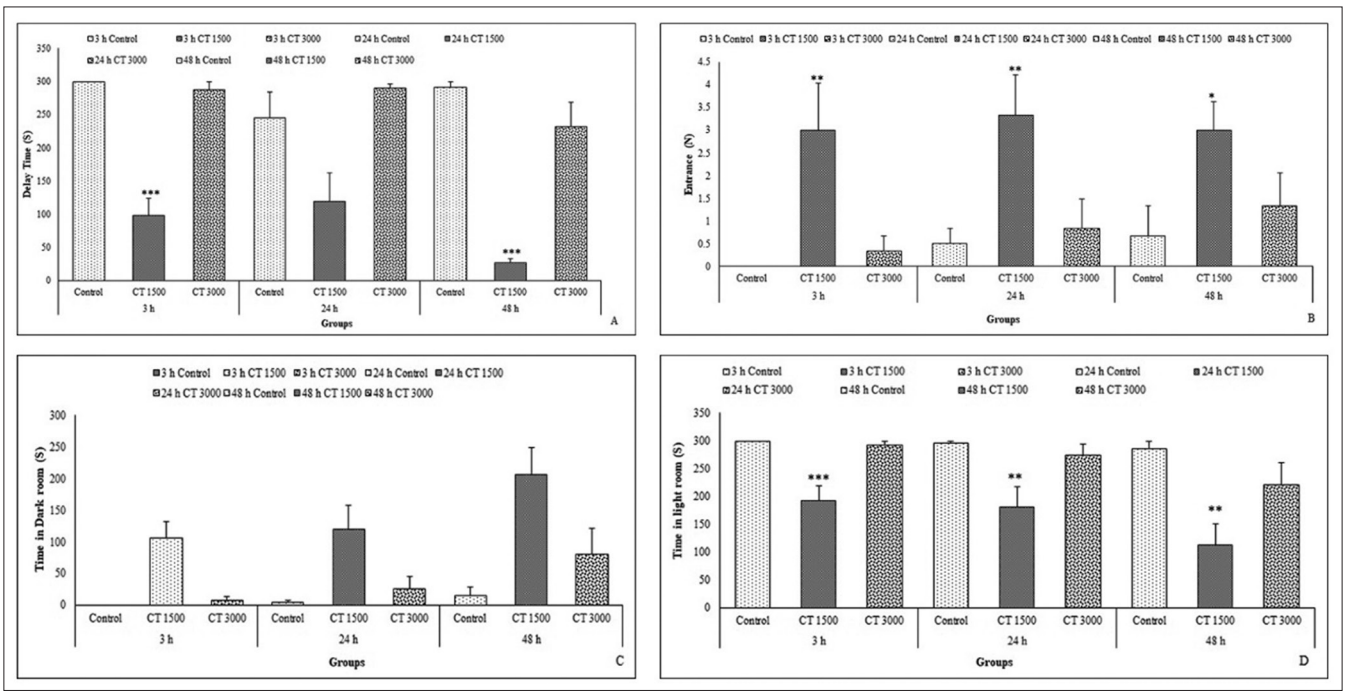

Figure 6: The effect of different doses of watermelon extract (CT 1500 mg/kg and CT 3000 mg/kg) on passive avoidance memory in rats. Data are expressed as mean ± standard error of the mean *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ ($n = 8$, in each group)

reduce the NCV.[19] In confirmation of the results of the current study, there is a positive relationship between NCV and temperature. The NCV decreases with a reduction in temperature. These changes may be associated with inactivation of axons at lower temperature.[20,21] The coldness affects sensory and motor neuronal conduction, but it has a stronger effect on the sensory nerve.[22] Cold induction to the skin in a physiological state reduces the NCV,[22,23] which confirms and is in favor of our results. Not aligned with our study, in patients with diabetic neuropathy, NCV was increased by consumption of watermelon.[10] This study has been done on patients, so not unexpected the contradiction in their results with our study.

In the current study, spatial and passive avoidance memory impairment has arisen. Thyroid hormones are critical for normal hippocampus function.[24] The hippocampus is one of the important brain areas for spatial learning.[25] Changes in thyroid hormone levels (hyperthyroidism or hypothyroidism) cause changes in cognitive functions such as concentration and spatial and avoidance memory. In fact, hyperthyroidism causes learning and memory impairment.[24] Bitiktaş Soner *et al*. showed that acquisition of learning was delayed in rats with hyperthyroidism. They showed that escape latencies and distance traveling were increased on training days in water maze, but no difference was found during prob day which is in favor of our study. They concluded that these findings may be due to an increase in phosphorylation of p38-MAPK in the hippocampus of hyperthyroid rats[26] and also confirm our results. Indeed, in our study, treatment of animals with watermelon causes a hyperthyroxinemia. It may be suggested that hyperthyroxinemia resulted in impaired spatial memory and passive avoidance in animals. In line with our study, it was reported that the efficacy of spatial learning in hyperthyroid rats was decreased.[27] It was reported that hypothermia (swimming in cold water) reduces memory and learning and shows symptoms similar to Alzheimer's disease in rats.[27] According to traditional medicine, cold-nature materials, such as watermelon, reduces memory.[19] These studies confirm our results.

Maifitrianti *et al*. administered watermelon juice at doses of 0.92 g/kg BW and 1.85 g/kg BW and showed that watermelon improves the spatial memory in a rat model of dementia. The results of this study are nonconsistent with our study. In this study, the effect of watermelon on dementia was studied, while in our study, the effect of watermelon on normal rats was investigated. It seems that these differences can be attributable to the model of animals, which in our study was a normal rat, but in Maifitrianti *et al*. study, the effect of watermelon was investigated on rats with dementia.[28]

According to the results of numerous reports and our study, *Citrullus* due to hyperthyroidism causes impairment in spatial learning memory and passive avoidance memory.

Conclusion

In accordance with traditional medicine documents, watermelon has a cold nature, and in the classical studies, hypothermia increases T4 levels in animals. Hence, due to hyperthyroid state the animals, lose weight, and increase in food and water consumption. Watermelon extract consumption in rats for 6 weeks brings about impairment in memory and a decrease in NCV. Validity of these results may need more investigation to find cellular and molecular mechanisms.

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Conflicts of interest

There are no conflicts of interest.

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