

## The Effect of Iranian Shallot or Garlic Aqueous Extracts on Learning, Memory and Serum Biochemical Variables in Fructose-fed Wistar Rats

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### Abstract

#### Objective(s)

We determined the effect of a high fructose diet either alone or in combination with Iranian shallot or garlic extract on cognitive functions, plasma lipid profile, and the intraperitoneal glucose tolerance test (IPGTT).

#### Materials and Methods

Following induction of insulin resistance in fructose-fed rats (Fru-fed), they were randomly assigned to three subgroups. The first subgroup was kept as Fru-fed while the two other subgroups were daily treated by aqueous garlic or shallot extract.

#### Results

Twelve weeks treatment with shallot or garlic significantly prevented the learning and memory deficits induced by fructose-feeding. Administration of garlic, but not shallot extract could significantly diminish the levels of cholesterol and low-density lipoprotein. Treatment with garlic or shallot extract can significantly improve the IPGTT in the Fru-fed rats.

#### Conclusion

The high fructose diet may contribute to spatial memory deficits. Iranian shallot or garlic extracts appear to improve learning and memory impairments in fructose-fed rats.

**Keywords:** Fructose-enriched diet, Garlic, Iranian shallot, Learning, Lipid profile, Memory, Morris Water Maze

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## Introduction

A high fructose diet causes numerous pathological changes, including oxidative stress, insulin resistance, type 2 diabetes, and liver disease (1). It has been shown that the damaging effects of a high fructose diet extend directly to the brain. Specifically, placing male Syrian hamsters on a 60% fructose diet for 6 weeks produced hippocampal insulin resistance. This finding is particularly significant given that the hippocampus is integral to many forms of learning and memory and that converging lines of evidence indicate that neural insulin signaling facilitates hippocampal-dependent memory (2). Learning and memory of a spatial water maze experience are correlated with activation of the hippocampal insulin signaling pathway (3).

The medicinal benefits of *Allium* vegetables, especially garlic, have been noted throughout recorded history. Most findings suggest that garlic decreases plasma cholesterol and triglyceride levels in patients with elevated levels of these lipids. The organosulfur contents of garlic are primarily alliin derivatives which are reported to be the cause of its cholesterol lowering effects. Studies in animal models indicate that dietary supplementation of garlic depresses the hepatic activities of lipogenic and cholesterogenic enzymes (4). Shallot (*Allium hirtifolium*), is a major component of many Asian diets and is widely believed to be beneficial to health. The current knowledge of the properties and constituents of shallot and its analogy with garlic suggests that some biological activities of shallot extracts may be similar to those of garlic extracts. The present experiment tested the effects of feeding rats with a high fructose diet on hippocampal-dependent spatial water maze learning and memory, and sought to determine whether combining fructose with garlic or Iranian shallot aqueous extract would prevent the memory impairments induced by fructose.

## Materials and Methods

### Preparation of shallot and garlic extracts

Fresh Iranian shallot (*Allium hirtifolium*) and garlic (*Allium sativum*) bulbs were obtained

from the local market in Mashhad, Iran. Aqueous garlic and shallot extracts were prepared according to a previously reported method (5). Briefly, the shallot and/or garlic bulbs in good physical shapes were peeled and homogenized in cold, sterile 0.9% saline. The homogenized mixture was filtered 3 times through cheesecloth. The prepared extracts were quickly frozen until used.

### Animals and treatments

Male albino Wistar rats weighing 180-240 g were purchased from the animal house of Razi Research Center in Mashhad, Iran. Animal handling was performed with regard to Iranian animal ethics society and local university rules. The animals were kept under a 12 hr light-dark cycle at room temperature. All animals were allowed to adapt to the environment for two weeks after their arrival before the experiment started. The animals were randomly divided into two groups. Each group of rats was separately housed in standard cages with *ad libitum* access to water and standard pellet diet. The first group (n= 8) was fed with standard rat chow and tap water (control). The second group (n= 24) received normal chow such as control group and 10% w/v fructose (Merck) dissolved in drinking water (Fru-fed) for a period of eight weeks (6). Eight weeks later the fasting blood glucose, insulin, triglyceride, cholesterol, high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) were determined in both groups. The Fru-fed animals were randomly assigned to three subgroups (n= 8). The first subgroup was kept as Fru-fed (sham) while the second (Fru-fed-G) and third (Fru-fed-S) subgroups were daily treated by i.p. injections (500 µl) of garlic and Iranian shallot extracts (500 mg/kg body weight (BW)), respectively, for a period of twelve weeks (7). The control and sham groups received 500 µl of normal saline in the similar manner.

### Biochemical assays

Blood samples from 16 hr fasted rats were collected from retro-orbital plexus using

heparinized micro-hematocrit tubes at baseline (the day before fructose-feeding), 8 weeks after fructose-feeding, and at twelfth week of shallot or garlic extract treatment. Serum glucose, triglyceride, cholesterol, HDL-C, and LDL-C were determined by enzymatic colorimetric assays using standard kits (Parsazmun Company, Iran). The level of serum insulin was estimated by the immunoradiometric assay kit (Immunotech, Marseille, France).

#### **Insulin resistance index calculation**

Fasting insulin resistance index (FIRI) was calculated according to the formula (8):

$$\text{FIRI} = \frac{\text{Fasting insulin (mU/ml)} \times \text{Fasting glucose (mg/dl)}}{25}$$

#### **Intraperitoneal glucose tolerance test**

Intraperitoneal glucose tolerance test was done for all animals after eight weeks of fructose feeding and at twelfth week of shallot or garlic extract treatment. The IPGTT was performed by intraperitoneal injection of a 40% glucose solution (2 g/kg BW) after the overnight fasting. Blood samples were collected from retro-orbital plexus using heparinized microhematocrit tubes before (zero time) and 30, 60, and 120 min after the glucose loading. Blood glucose level was measured with a standard glucose/triglyceride/cholesterol meter (Accutrend GCT, Roche, Germany).

#### **Morris Water Maze test**

Animals were tested in a spatial version of Morris Water Maze test as described previously (9). The Morris Water Maze consisted of a circular water tank (120 cm diameter, 50 cm height) that was partially filled with water (25 °C). The animals were acclimated to the tank environment with 1 day of free-swimming in the pool with no platform. Each session lasted for 2 min. The pool was divided virtually into four equal quadrants, labeled N-S-E-W. A platform was placed in one of the four maze quadrants (the target quadrant) and submerged 1.5 cm below

the water surface. The platform remained in the same quadrant during the entire experiment. The rats were required to find the platform using only distal spatial cues available in the testing room. The cues were maintained constant throughout the testing. The rats received four consecutive daily training trials in the following 4 days, with each trial having a ceiling time of 60 sec. The rat had to swim until it found and climbed onto the platform. After climbing onto the platform, the animal remained there for 30 sec before the commencement of the next trial. The escape platform was kept in the same position relative to the distal cues. If the rat failed to reach the escape platform within the maximally allowed time of 60 sec, it was gently placed on the platform and allowed to remain there for 30 sec. The time to reach the platform (latency in seconds) was measured.

#### **Statistical data analysis**

All data were expressed as mean±SEM. The intergroup variation was statistically evaluated by one-way analysis of variance (ANOVA) followed by Duncan's test. The difference between the control and Fru-fed groups were analyzed by Student's t-test. The statistical analysis was done using the SAS Statistical Software version 9.0. The *P* values less than 0.05 were considered statistically significant.

#### **Results**

Rats which were fed fructose-enriched diet over a period of eight weeks, developed hypertriglyceridemia associated with insulin resistance. A significant increase in blood triglyceride level by approximately 43% was observed (105.09±5.41 vs. 73.47±1.49; *P*< 0.05). Serum levels of cholesterol (116.22±3.94 vs. 100.56±6.63) and LDL (71.25±2.87 vs. 61.64±4.89) were increased by 15.6% and 15.7%, respectively compared with the control rats (*P*< 0.05). Serum levels of HDL-C (18.9±0.80 vs. 20.6±0.72) were not significantly different among the control and Fru-fed groups. Also, the FIRI (24±4 vs. 20±5) and the area under the glucose tolerance curve (23.21±0.82 vs. 19.96±0.51) were significantly greater in the Fru-fed group than that of the control group (*P*< 0.05).

Table 1. The area under the intraperitoneal glucose tolerance curve and serum lipid profile in the control, Fru-fed, Fru-fed-G, and Fru-fed-S rats.

	Control	Fru-fed (sham)	Fru-fed-G	Fru-fed-S
Area under curve X 10 <sup>3</sup> (min.mg/dl)	23.99±0.76*	31.72±2.54	24.18±1.8*	25.23±1.96*
Triglyceride (mg/dl)	66.75±3.63*	90.67±5.64	77.75±8.14	86.14±6.51
Cholesterol (mg/dl)	76.37±3.42*	98.57±3.56	81.14±5.08*	95.43±5.76
LDL-C (mg/dl)	47.37±2.48*	62.62±2.24	51.25±3.8*	62.7±3.62
HDL-C (mg/dl)	24.8±2	27.25±2.8	26.25±2.97	26.14±2.61

TG, triglycerides; Chl., cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Fru-fed, fructose-enriched diet; Fru-fed-G, Fru-fed rats treated with garlic extract; Fru-fed-S, Fru-fed rats treated with Iranian shallot extract. \* $P < 0.05$ , control, Fru-fed-G, and Fru-fed-S versus Fru-fed (sham). Each value is expressed as the mean±SEM (n= 7).

The total area under the glucose tolerance curve between 0 and 120 min significantly decreased in the Fru-fed rats after twelve weeks treatment with garlic or shallot extract ( $P < 0.05$ ). Administration of garlic aqueous extract could significantly decrease the serum cholesterol and LDL-C levels in the Fru-fed rats ( $P < 0.05$ ), whereas shallot extract could slightly diminish the levels of cholesterol and LDL-C in the Fru-fed group. Compared with the sham group, the Fru-fed-G and Fru-fed-S rats had no significant decreases in triglyceride and HDL-C levels (Table 1).

Fru-fed group showed a lower ability to find the platform and learned its location in the fourth day of training ( $P < 0.05$ ) (Figure 1). Treatment with Iranian shallot or garlic significantly prevented the learning and memory deficits induced by fructose-feeding by 38.5% and 32.2%, respectively.

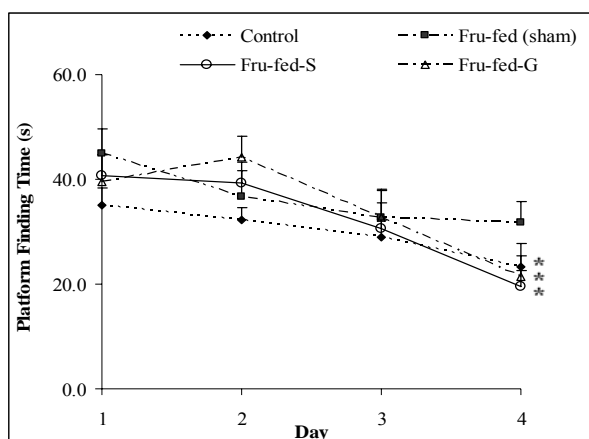


Figure 1. Effect of a high fructose diet either alone or in combination with Iranian shallot or garlic aqueous extract on the performance of spatial memory acquisition phase in rats (platform finding time). \* $P < 0.05$ , control, Fru-fed-G, and Fru-fed-S versus Fru-fed (sham); on the 4th day of the training sessions. Each value is expressed as the mean±SEM (n= 7).

## Discussion

Our results show that fructose-fed group has a higher blood glucose and triglyceride levels than that of control group. The FIRI and the area under the glucose tolerance curve were significantly greater in fructose-fed group compared to the control group throughout the investigation period. Thus, the fructose-fed animals displayed characteristics typical of insulin resistance. Our data demonstrate that fructose consumption increases plasma cholesterol and LDL-C concentrations. There is evidence that the metabolic effects of fructose occur through rapid utilization in the liver due to the bypassing of the regulatory phosphofructokinase step in glycolysis. This in turn causes the activation of pyruvate dehydrogenase subsequent modifications favoring esterification of fatty acids, and again leading to increase in very low density lipoprotein (VLDL) secretion (10). The increase in VLDL secretion can then lead to chain reactions in other lipoproteins and lipids such as LDL.

Our results show that consuming a 10% fructose diet for 20 weeks impairs retention performance in a spatial water maze probe test. The present data indicated that high dietary fructose significantly increased plasma TGs and glucose concentrations. As TG concentrations increased, the latency to reach the target increased. Our hypothesis is that fructose impairs memory by producing hippocampal insulin resistance. Supporting our hypothesis are previous studies showing that application of TGs to liver cells decreases the ability of insulin to activate its signaling cascade and TGs can penetrate the blood brain barrier (11).

Evidence from several investigations suggests that some of the sulfur compounds

such as allicin and S-allyl-L-cysteine (SAC) may be responsible for the therapeutic properties of garlic (12). Many studies, mostly short term, have investigated the hypolipidemic effect of garlic. Results were conflicting, ranging from a non-detectable to a statistically significant hypocholesterolemic effect. These differences may be attributable to various causes, such as differences in study design and in characteristics of the participants. Another possible confounder is the type of garlic preparation used, which could yield different amounts of the active compound. Yeh and Liu (13) indicated that the cholesterol-lowering effects of garlic extract stem in part from inhibition of hepatic cholesterol synthesis by water-soluble sulfur compounds, especially SAC. Several studies have been carried on shallot extracts reported the presence of flavonols, polyphenolic derivatives, and sulfur compounds. It has been reported that alliin and allyl groups are absent in shallot and the concentration of S in the shallot is smaller than that in the garlic (14).

In our previous work it was found that neither shallot nor garlic extracts had a significant effect on the glucose tolerance at 4th weeks of treatment. Shallot extract administration, but not garlic extract, for a period of eight weeks can significantly improve the intraperitoneal glucose tolerance (15). In the current study, treatment of Fru-fed rats with aqueous Iranian shallot or garlic extract for a period of twelve weeks could

significantly improve the IPGTT in Fru-fed rats. Twelve weeks garlic administration could significantly reduce the total cholesterol (17.7%) and LDL-C (18.2%). We have found that garlic could not significantly change the HDL-C and triglyceride concentration in Fru-fed rats. The effect of Iranian shallot extract on lipid metabolism in fructose-fed rats has not been investigated before. The present study has been demonstrated that twelve-week Iranian shallot extract administration has no significant effect on the serum lipid levels (TG, Chl, LDL-C, HDL-C) in Fru-fed rats.

## Conclusion

In conclusion, spatial memory decreased in Fru-fed rats compare with the control group. Both garlic and Iranian shallot treatment improved learning and memory impairments in fructose-fed rats. They had a different hypocholesterolemic activity and their hypoglycemic activity is evident from our present study. We studied only one dosage level, high doses and longer duration may be needed to assess the long-term benefit of Iranian shallot and garlic extracts on memory impairment and lipid metabolism in high fructose feeding animals.

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## References

1. Elliott SS, Keim NL, Stern JS, Teff K, Havel PJ. Fructose, weight gain, and the insulin resistance syndrome. *Am. J Clin Nutr* 2002; 76:911–922.
2. Park CR. Cognitive effects of insulin in the central nervous system. *Neurosci Biobehav Rev* 2001; 25:311–323.
3. Dou JT, Chen M, Dufour F, Alkon DL, Zhao WQ. Insulin receptor signaling in long-term memory consolidation following spatial learning. *Learn Mem* 2005; 12:646–655.
4. Qureshi AA, Abuirmeileh N, Din ZZ, Elson CE, Burger WC. Inhibition of cholesterol and fatty acid biosynthesis in liver enzymes and chicken hepatocytes by polar fraction of garlic. *Lipids* 1983a; 18:343–348.
5. Homayounfar H, Baluchnejad Mojarad T, Roghani M, Hosseini M, Kamalinejad M. Effect of Aqueous Garlic (*Allium sativum* L.) Extract on Acetylcholine and Isosorbide-Induced Relaxation of Isolated Aorta in Rat. *Iran Biomed J* 2003; 7:23–27.
6. Dai S, McNeill JH. Fructose-induced hypertension in rats is concentration- and duration-dependent. *J Pharmacol Toxicol Methods* 1995; 33:101–107.
7. Gorinstein S, Leontowicz M, Leontowicz H, Najman K, Namiesnik J, Park Y-S, et al. Supplementation of garlic lowers lipids and increases antioxidant capacity in plasma of rats. *Nutr Res* 2006; 26:362–368.

8. Duncan MH, Singh BM, Wise PH, Carter G, Alagband-Zadeh J. A simple measure of insulin resistance. *Lancet* 1995; 346:120-121.
9. Morris RG, Garrud P, Rawlins JN, O'Keefe J. Place navigation impaired in rats with hippocampal lesions. *Nature* 1982; 297: 681–683.
10. Mayes PA. Intermediary metabolism of fructose. *Am J Clin Nutr* 1993; 58:754S-765S.
11. Kim DS, Jeong SK, Kim HR, Kim DS, Chae SW, Chae HJ. Effects of triglyceride on ER stress and insulin resistance. *Biochem Biophys Res Commun* 2007; 363:140–145.
12. Koch HP, Lawson LD. Garlic: the science and therapeutic application of *Allium sativum* L. and related species. 2nd ed. Williams and Wilkins; Baltimore; 1996.
13. Yeh YY, Lin RI, Yeh SM, Evens S. Garlic reduces plasma cholesterol in hypercholesterolemic men maintaining habitual diets. In: Ohgashi H, Osawa T, Terao J, Watanabe S, Toshikawa T, editors. *Food Factors for Cancer Prevention*. Springer Tokyo, Japan: 1997. p. 226-230.
14. Leelarungrayub N, Rattanapanone V, Chanarat N, Gebicki JM. Quantitative evaluation of the antioxidant properties of garlic and Iranian shallot preparations. *Nutrition* 2006, 22:266-274.
15. Jalal R, Bagheri SM, Moghimi A, Behnam Rasuli M. Hypoglycemic effect of aqueous Iranian shallot and garlic extracts in rats with fructose-induced insulin resistance. *J Clin Bioche Nutr* 2007; 41:218-223.