http://www.cjmb.org

Open Access Original Article

Crescent Journal of Medical and Biological Sciences
Vol. 2, No. 4, October 2015, 125–129
eISSN 2148-9696

Assessment of Antioxidant Properties of *Allium cepa* on Serum Antioxidants and Spermatogenesis After Consuming Tartrazine in Rat

Manuchehr Hoseinpouran[1*], Arash Khaki[2], Habibollah Nazem[3]

Abstract

Objective: The aim of this study is to assess the antioxidant properties of onion on biochemical serum factors, antioxidants and testicular tissues in Wistar rats after consuming tartrazine.

Materials and Methods: Forty male Wistar were divided into four groups of 10. The first group was used as the control group and were given only water without additives, group 2 were given tartrazine, group 3 were given tartrazine plus onion juice and the fourth group which was given only onion juice through gastric gavage. The experiment was conducted for 60 days, then the antioxidant activities superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and biochemical parameters namely high density lipoprotein (HDL), low density lipoprotein (LDL) and testosterone together with the histopathological studies (sperm count and testicular weight) were measured.

Results: Tartrazine caused a decrease in the activity of antioxidant enzymes (SOD, CAT, GPX) and a decrease in the level of testosterone and HDL and also a decrease in sperm count and testicular tissue weight. Tartrazine caused an increase in the LDL levels.

Conclusion: Results showed that consumption of tartrazine is associated with production of free radicals and in turn causes significant decrease in antioxidant activities and biochemical serum factors which damage the cellular compartments of the testis. Onion as an antioxidant in this study reduces the damaging effects of tartrazine on the enzymatic activities of antioxidant and biochemical serum factors.

Keywords: Antioxidants, Onions, Tartrazine, Testicular tissue, Water

Introduction

Food colorings are among the important and influencing factors enhancing the quality of food appearance and in enticing the public on purchasing such product (1). Innovation in technology and marketing competition lead to the greater use of food coloring in modern food industry (2). Natural food coloring are less stable and in recent years the use of synthetic food coloring have taken its precedence due to its more stable property, cheaper prize, high heat resistance, easy availability (3,4) and for economic purposes (5). The most common artificial food dyes, the azo dyes, contain aromatic azo compounds such as tartrazine and are widely used. Tartrazine is a synthetic lemon yellow azo dye primarily used as a food coloring. It is also known as E number E102, C.I. 19140, FD&C Yellow 5, Acid Yellow 23, Food Yellow 4, and trisodium 1-4-5-pyrazolone-3-carboxylate (6). Many products such as soft drinks, energy and sport drinks, flavored chips, sauces, ice creams, pop corns, instant puddings and gelatin, jams, jellies and chewing gums contain tartrazine (7). Several studies conducted on the use of tartrazine showed behavioral changes in children such as irritability, restlessness, sleep disturbances (8), and mutagenic effects on DNA (9). Metabolites of tartrazine can generate reactive oxygen species (ROS) accelerating oxidative stress (10) and changes in liver and renal biochemical profiles (11) have been reported. Tartrazine in higher dose is dangerous and in fact causes the production of free radicals. Free radicals are atoms or molecules that once losing their electrons begin a chain reaction and once this process is started, it can cascade, resulting in the disruption of a living cell. Free radicals to a large extent can affect the sperm (12). In this study, we used onion as an antioxidant. Onion (*Allium cepa* Liliaceae), also known as the bulb onion or common onion is a vegetable and is the most widely cultivated species of the genus Allium (13). Onion is a biennial plant having a hollow cylindrical leaves. Onion stem to a height of 1 m (14). Results of several researches conducted showed that onion is effective in reducing blood pressure and lipids, prevention and treatment of atherosclerosis,

Received 1 June 2015, **Accepted** 28 September 2015, **Available online** 1 October 2015

[1]Department of Biology, Faculty of Science, Payame Noor University, Tehran, Iran. [2]Department of Pathology, Tabriz Branch, Islamic Azad University, Tabriz, Iran. [3]Department of Biology, Faculty of Science, Payame Noor University, Iran.
***Corresponding author:** Manuchehr Hoseinpouran, Department of Biology, Faculty of Science, Payame Noor University, Tehran, Iran.
Tel: +989146111088, Email: mhoseinporan@yahoo.com

reducing systemic infections, the treatment of gastrointestinal disorders, effective in detoxification and in reducing the risk of abortion (15,16). Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Antioxidants by reducing the damaging effects of free radicals in human tissues, inflammation and subsequent muscle and joint pain will be reduced (9,17). Therefore, we can assume that onion reduces the harmful effects of tartrazine on the enzymatic activities of antioxidant and biochemical serum factors.

Materials and Methods

Chemicals

The dye tartrazine used in this present study was made and packed by Mallya-Fine, Bangloor, India and all chemicals and reagents were from Sigma Chemical Co. USA.

Animals

In conducting this study, 40 Wistar rats were purchased from Iran Pasteur Institute; these rats were about 8 weeks old and weighed about 250±10 gms, respectively. Throughout the study, the rats were exposed to light for 10 hours and also 10 hours in the dark (light/dark cycles) from 9 AM to 9 PM, within a room temperature of 23.9-25.3°C and a constant humidity of 55%-60%. Animals used in this study were treated in accordance to the Animal Protection Law (19).

The 40 Wistar rats were divided into 4 groups; 3 groups experimental (n = 30) and one group (n = 10) as a control. The control group (group 1) was given only water without any additives. The experimental group was divided into 3 groups having 10 mice in each group. Group 2 (n = 10) were given a daily dose of 1% tartrazine 2 cc through gavage while group 3 (n = 10) were given a daily dose of 2 units of tartrazine 1% together with 3 cc of onion juice through gavage and group 4 (n = 10) were only given a daily dose of 1 cc of onion juice. It should be noted that the onion juice used in this study was extracted with the use of a juicer. After 8 weeks of the study, blood samples were taken from the retinal area of these rats for the purpose of assessing biochemical serum factors and antioxidants. In order to evaluate the testicular tissue of these mice, pentobarbital sodium 40 mg/kg was administered intraperitoneally as an anesthesia and the peritoneal cavity was incised through a lower transverse abdominal incision and the testes of the control and experimental group were removed from the body and immediately weighed. At the end of the study, cervical dislocation was used for euthanasia and this took about 2 hours (9-11 AM).The obtained testicular tissue were fixed in a 10% neutral buffered Formalin for histopathology and after preparing the section for microscopy with a microtome in a thickness of (5 micro), the hematoxylin-eosin staining was used. In order to obtain microscopic images of the slides the ultra as a 400 Kodak and a light microscope (Olympus-Z) made in Japan were used. Then the tissue extracts were used to estimate the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA). The specimen were then transferred in a small laboratory Petri dish (35 mm) containing culture medium and were cut into small pieces with a scissor and finally transferred in a 24- hole plate with 0.05 ml of culture medium containing bovine serum albumin 4 mg/ml and incubated at 37°C for 20 minutes. Tissues cut into pieces were removed from the culture medium and the resulting sperm suspension was incubated then a sperm suspension with a dilution of 1:100 was obtained and a drop of this diluted sample was placed on a microscope slide and the sperm in terms of its number, percentage of motility and vitality were examined.

Results

Tartrazine were given to the Wistar rats for 2 months and the activity of antioxidant enzymes in the group consuming tartrazine and in the control group were measured. The activities of antioxidant enzymes SOD (Figure 1), CAT (Figure 2), GPx (Figure 3) in all 4 groups were studied. Results showed a significant decrease in enzymatic activity in the group of mice consuming tartrazine.

In addition to the activity of antioxidant enzymes, biochemical factors of low density lipoprotein (LDL) (Figure 4), high density lipoprotein (HDL) (Figure 5), and testosterone (Figure 6) were also measured in four groups. Testosterone and HDL levels of rats consuming tartrazine showed a significant decrease and an increase in LDL levels.

Along with enzyme activity and biochemical parameters, histological study was carried from the testicles and sperm count (Figure 7) and testicular weight (Figure 8) was assessed in 4 groups.

Discussion

The aim of this present study was to evaluate the antioxidant properties of onion on the activities of antioxidant enzymes in the blood (GPx, CAT, SOD) and on serum biochemical factors (testosterone, LDL, HDL) and histological assessment of the testis in terms of sperm count, motility and weight after consumption of tartrazine. Based on the results obtained, consumption of food products containing tartrazine generates the production of free radicals

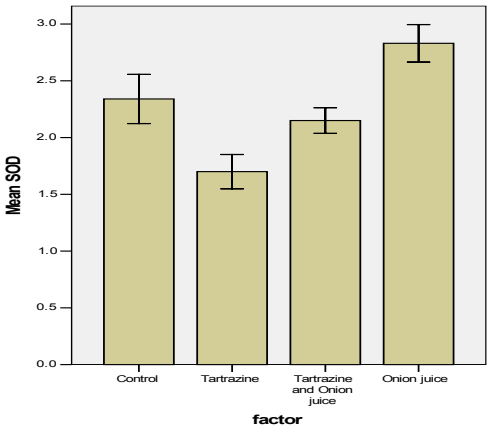

Figure 1. Superoxide dismutase (mmol/ml)

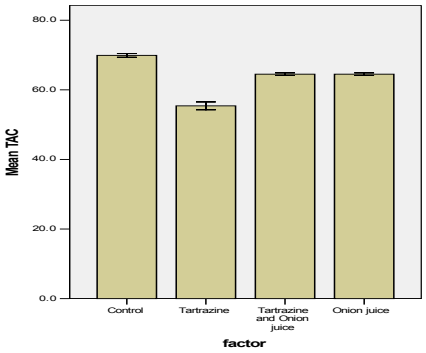

Figure 2. Catalase (mmol/ml).

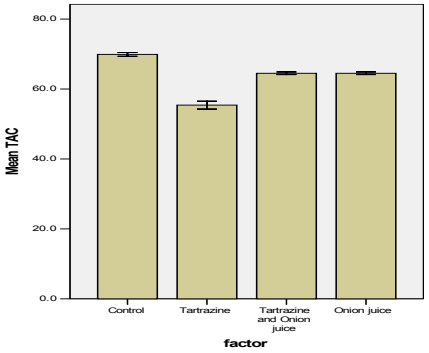

Figure 3. Glutathione Peroxidase (mmol/ml).

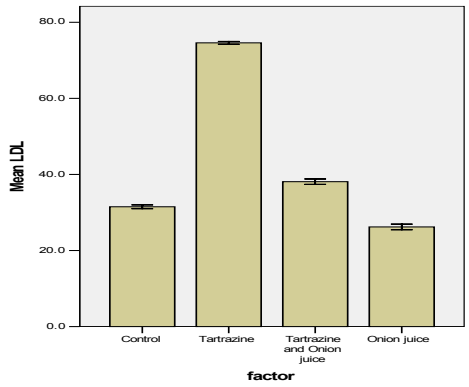

Figure 4. Low Density Lipoprotein (LDL) (µ/L).

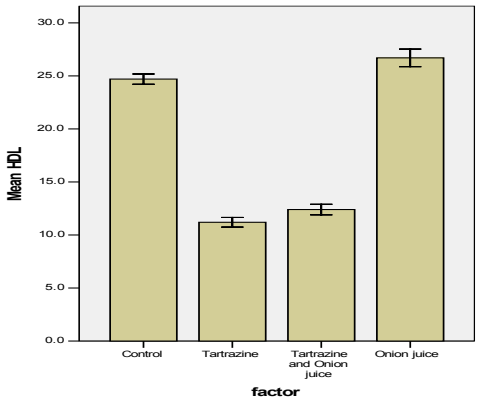

Figure 4. High Density Lipoprotein (HDL) (µ/L).

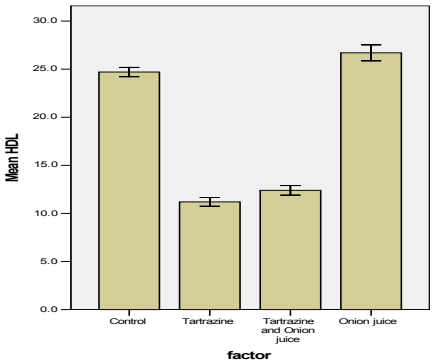

Figure 6. Testosterone (ng/ml)

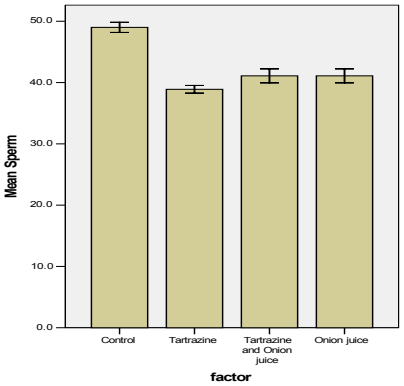

Figure 7. Sperm count (Million cells/ml).

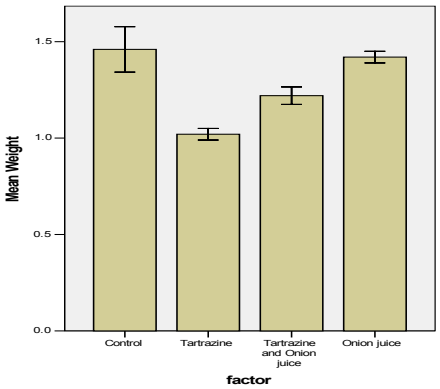

Figure 7. Testicular Weight (g)

such as H_2O_2 resulting to the suppression of the activity of the antioxidant enzymes in the blood. Studies conducted by Arjuman et al (20) and Bray et al (21) showed similar results. In our study, tartrazine caused a decrease in the weight of testis in rats and also a significant reduction in motility and sperm count. In studies conducted by Assmann et al (22), Coskun et al (23), and Sharma et al (24) achieved similar results with our study. In this study, the use of tartrazine as an artificial food coloring has been observed to cause significant decrease in biochemical factors (testosterone and HDL) while increasing the serum cholesterol (LDL). Similar results were achieved in the study conducted by Saxena et al (25) in 2014 of which throughout this study, tartrazine caused a reduction in the levels of

testosterone, HDL, total protein and albumin and on the other hand caused an increase in the density of alkaline phosphatase, SGPT and bilirubin. Prolong consumption of this artificial dye would lead to detrimental effects on human health. Similar results were reported by Gautam et al (26), Hasan (27), Chatterjee et al (28), Osman et al (29) and Sharma et al (30).

The study conducted by Amin et al (31) indicated that tartrazine and carmoisine caused some changes in biochemical markers of the vital organs such as the liver and the kidneys not only in higher doses but in lower levels as well. Similar results have been obtained in study conducted by Mehedi et al (32) in 2013. In a study conducted by Kikelomo et al (33) results indicated that tartrazine reduced the enzymatic activity of antioxidant and stimulates the production of free radicals which in turn causes damage to the testicular protective cells. In our present study, the onion has been used as an antioxidant and it seems that it has a promising effect in the reduction of the damaging effects of tartrazine on biochemical factors and serum antioxidants and in the quality of testicular tissue. In this study, it has been observed that, consumption of onion resulted in a significant decrease in the side effects of tartrazine. In the study conducted by Calomme et al (34) results indicated that administration of onion extracts reduced oxidative stress, prevented carcinogenic action and supported the body's antioxidant defense system which is effective in improving prostate cancer. Also, results of the study conducted by Obioha et al (35) showed that oral administration of onion reduced the toxic effects of cadmium on testicular tissue and improved the process of spermatogenesis. Still in this regard, Izawa et al (36), have reported that onion extract can reduce the toxic effects of some environmental pollutants on spermatogenetic tissue. Park et al. reported that rats given diet containing onion has increased the level of serum antioxidant and decreased the level of malondialdehyde in the liver and isoprostane in the brain. In this study, onion consumption resulted in significant decrease in the toxic effects of tartrazine.

Conclusion

In this present study, we can conclude that tartrazine enhances the production of free radicals which in turn decreases the activity of antioxidant enzymes and serum biochemical factors and also, create a negative effect on spermatogenesis such as reduced sperm motility and sperm count, respectively.

Ethical issues

Animals used in this study were treated in accordance to the Animal Protection Law.

Conflict of interests

The authors declare no conflict of interests.

Financial support

The researchers received no financial support or grant from any funding agency in the public and commercial sectors.

Acknowledgments

I would never have been able to finish my dissertation without the guidance of my supervisor Dr. Arash Khaki, help from friends, and support from my family. I would like to express my deepest gratitude to my advisor, Dr. Habibollah Nazem for his excellent guidance, caring, patience and providing me with an excellent atmosphere for doing my research. I would like to thank Dr. Fatemeh Moghanizade, who as a good friend was always willing to help and give her best suggestions.

References

1. Singh S. Impact of color on marketing. Management Decision 2006;44(6):783-789.
2. Kiple KF, Ornelas KC. Contemporary food related issues. UK: Cambridge University Press; 2000:1667-1676.
3. Pratima R, Sudershan RV. Risk assessment of synthetic food colors: a case study in Hyderabad, India. International Journal of Food Safety, Nutrition and Public Health. 2008;1(1):68-87. doi:10.1504/IJFSNPH.2008.018857.
4. Soltan Dalal MM, Vahedi S, Najarian A, et al. To measure unpermitted used colors in presented black cherry and barberry juice in Tehran city. Payavard Salamat J Tehran Univ Med Sci. 2008;2(1):55-62.
5. Ozaki A, Kitano M, Itoh N, et al. Mutagenicity and DNA damaging activity of decomposed products of food colors under UV irradiation. Food Chem Toxicol. 1998;36:811-817.
6. Mittal A, Kurup L, Mittal J. Freundlich and langmuir adsorption isotherms and kinetics for the removal of tartrazine from aqueous solutions using hen feathers. J Hazard Mater. 2007;146(1-2):243-248.
7. Walton K, Walker R, van de Sandt JJ, et al.The application of in vitro data in the derivation of the acceptable daily intake of food additives. Food Chem Toxicol. 1999;37(12):1175-1197.
8. Rowe KS, Rowe KJ. Synthetic food coloring and behavior: a dose response effect in a double-blind, placebo-controlled, repeated-measures study. J Pediatr. 1994;125(5 Pt 1):691-698.
9. Sasaki YF, Kawaguchi S, Kamaya A, et al. The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat Res. 2002;519(1-2):103-119.
10. Bansal AK, Bansal M, Soni G, Bhatnagar D. Modulation of N-nitrosodiethylamine-induced oxidative stress by vitamin E in rat erythrocytes. Hum Exp Toxicol. 2005;24(6):297-302.
11. Himri I, Bellachen S, Souna F, et al. A 90-day oral toxicity study of tartrazine, a synthetic food dye, in wistar rats. Int J Pharm Pharm Sci. 2011;3(Suppl 3):159-169.
12. Thomas MJ. The role of free radicals antioxidants.

Nutrition. 2000;16(7/8):716-717.
13. Shaath NA, Flores FB. Egyptian onion oil. Dev Food Sci. 1998;40:443-453.
14. Zargari A. Medicinal plants. 5th ed. Tehran: Tehran University; 1993:623.
15. Jakubowski H. On the health benefits of Allium sp. Nutrition. 2003;19(2):167-168.
16. Nuutila AM, Puupponen-Pimia R, Aarni M, Oksman-Caldentey KM. Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. Food Chem. 2003;81(4):485-493.
17. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol. 2009;7(1):65-74. doi:10.2174/157015909787602823.
18. Sies H, Stahl W. Vitamins E and C, B-carotene and other carotenoids as antioxidants. Am J Clin Nutr. 1995;62(6 Suppl):1315S-1321S.
19. Pfeifer H, Conrad M, Roethlein D, et al. Identification of a specific sperm nuclei selenoenzyme necessary for protamine thiol cross-linking during sperm maturation. FASEB J. 2001;15(7):1236-1238.
20. Arjuman A, Nair V, Gopalakrishna HN, Nandini M. Evaluation of the antioxidant potential of NR-ANX-C (a polyherbal formulation) and its individual constituents in reversing haloperidol-induced catalepsy in mice, Indian J Pharmacol. 2007;39(3): 151-154.
21. Bray RC, Cockle SA, Fielden EM, Roberts PB, Rotilio G, Calabrese L. Reduction and inactivation of superoxide dismutase by hydrogen peroxide. Biochem J. 1974;139(1):43-48.
22. Assmann G, Schriewer H, Schmitz G, Hagele EO. Qualification of high density lipoprotein cholesterol by precipitation with phosphotungstic acid/Mg/Cl2. Clin Chem. 1983;29(12):2026-2030.
23. Coskun O, Kanter M, Korkmaz A, Oter S. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and beta-cell damage in rat pancreas. Pharmacol Res. 2005;51(2):117-123.
24. Sharma A, Goyal RP, Chakravarty G, Sharma S. Haemotoxic effects of chocolate brown. A commonly used blend of permitted food colour on Swiss albino mice. Asian J Exp Sci. 2005;19(2):93-103.
25. Saxena B, Sharma S. Serological changes induced by blend of sunset yellow, metanil yellow and tartrazine in swiss albino rat. Toxicol Int. 2014;21(1):65-68. doi:10.4103/0971-6580.128798.
26. Gautam D, Sharma G, Goyal RP. Evaluation of toxic impact of tartrazine on male Swiss albino mice. Pharmacologyonline. 2010;1:133-40.
27. Hasan GM. Effects of some synthetic coloring additives on DNA damage and chromosomal aberrations of rats. Arab J Biotech. 2010;13(1):13-24.
28. Diet and Nutrition. In: Chatterjee MN, Shinde R, eds. Textbook of Medical Biochemistry. 5th ed. New Delhi: Jaypee Brothers Medical Publishers Ltd; 2002:683-685.
29. Osman MA, Afifi A, Hussien RM, Kamilia B, Aziz A, Salah SH. Long term biochemical and genotoxicity studies of four synthetic food and drug colorant in mice. Bull Fac Pharm. 1995;33(1):13-21.
30. Sharma G, Gautam D, Goyal RP. Tartrazine induced haematological and serological changes in female swiss albino mice, Musmusculus. Pharmacologyonline. 2009;3:774-88.
31. Amin KA, Abdel Hameid H 2nd, Abd Elsttar AH. Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food Chem Toxicol. 2010;48(10):2994-2999. doi:10.1016/j.fct.2010.07.039.
32. Mehedi N, Mokrane O, Alami, O, et al. A thirteen week ad libitum administration toxicity study of tartrazine in Swiss mice. Afr J Biotechnol 2013; 12(28):4519-4529.
33. Kikelomo OM, Stephen MS, Michael AF, Udoka EO, Toyin YF. Protective roles of onion and garlic extracts on cadmium-induced changes in sperm characteristics and testicular oxidative damage in rats. Food Chem Toxicol. 2008;46(12):3604-3611.
34. Calomme M, Pieters L, Vlietinck A, Vanden Berghe D. Inhibition of bacterial mutagenesis by citrus flavonoids. Planta Med. 1996;62(3):222-226.
35. Obioha UE, Suru SM, Ola-Mudathir KF, Faremi TY. Hepatoprotective potentials of onion and garlic extracts on cadmium-induced oxidative damage in rats, Biol Trace Elem Res. 2009;129(1-3):143-156. doi:10.1007/s12011-008-8276-7.
36. Park J, Kim J, Kim MK. Onion flesh and onion peel enhance antioxidant status in aged rats. J Nutr Sci Vitaminol (Tokyo). 2007;53(1):21-29.