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RESEARCH ARTICLE

INVESTIGATION OF EFFECT ON E. COLI OF FOOD DYES AND LACZ GENE EXPRESSİON

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ABSTRACT

This study aimed to investigate the effect of Brilliant Blue and Dark Green food dyes on the *Escherichia coli* which is part of the intestinal flora.The food dye stock solution was prepared as 50 mg/ml with serum physiologic solution. Both of the food dyes were added in nutrient broth medium as 5, 2,5 and 1,25 mg/ml concentrations Design bacteria was inoculated inside of the dyes a 0.34 McFarland. After samples werewaitedin shaking incubator during 24 h, streaking was made to agar medium. In addition, RNA of control and sample groups were extracted forusing kits for molecular studying. Isolated RNAs were exposed to real time PCR (polimerase chain reaction) to determine whether or not to be mutation at lacZ gene region of chromosome. That was the result of the study which has been found that bacteria growth decreased in bacteria groupsapplied Brilliant Blue and Dark Green compared to control group especially after 12th day. More and more raising of Brilliant Blue and Dark Green concentration prevented *E. coli* growth. Real time PCR results indicate that both of two food dyes have toxicity on *E. coli* generations were not related to lacZ gene mutation. When effects of Dark Green and Brilliant Blue on growth of *E. Coli* ATCC 10799 strain for 20 generations, a general reduction has been observed in bacterial reproduction in all 3 doses, from the 1st day to the 20th day. While fluctuations existed in the number of bacterial colonies from the 1st day until the 12th day, as of 12th day there is a stable reduction. It has been understood whether the reason of this phenotypic reduction is genotypic or not.

INTRODUCTION

Food dyes are substances that are added to the food with a variety of purposes. In scientific terms, it means "substances that directly or indirectly become a component of the food or that are used to affect the food's character". Food dye should not be masking the spoiled food and should not be deceptive for the customer (Yücecan, 1999). Another issue about the food dye is reliability (FAO, 2003). Reliability and of course a nice quality of the food is a quite important issue both for customer and community health. (Venil,2013, Meggos,1995). Colorant blend in Dark Green: (E102+E132) CI 19340+CI 73015 is a formulation like that, and it gives green color. This colorant is mostly used for sweets (300 mg/kg), dressing and coating materials (500 mg/kg) and soups (50 mg/kg), in the written amount. Brilliant Blue colorant is again used as colorant for sweets (300 mg/kg), dressing and coating materials (500 mg/kg) and soups (50 mg/kg). Besides these usages, it is used in soaps, shampoos, mouth cleaners and in other hygiene products, and also in cosmetic products and various industries. Also, it can be used in textile and leather industry (Mital, 2006). Intestinal microflora are microorganisms that have different useful tasks related to digestion of host organisms living in the digestive system.

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The number of these bacteria in the digestive system is approximately 10-13 times of that is in human body. Bacteria make most of the flora in the large intestine, and make 60% of the stool (Guarner and Malagelada,2003). It is estimated that there 300-1000 types that live in the digestive tract. However, experts report that 99% of them belong to 30-40 types (Beaugerie and Petit 2004). The relation between intestinal flora and human is mutual and it is an example of symbiotic life that is, it is based on mutual benefits (Gibson, 2004). In this study, we researched the effects of food dyes on *Escherichea coli. Escherichea coli* is a member of Enterobacteriaceae (smaller than 1,5µm) family; gram negative and rod shaped bacteria and they grow fast in both aerobic and anaerobic environments. In this thesis study, we have used *Escherichia coli* ATCC 10799 bacteria as the test microorganism. In studies that food dyes effects is being researched, this bacteria is frequently preferred for especially being a member of *Enterobacteriaceae* family and its clinical importance. *E. coli* uses lactose and glucose as carbon sources and firstly, it metabolizes glucose and then lac operon genes stop their development for a while until it gains the ability to metabolize lactose. Although lactose exists since the beginning of the bacterial growth phase, the cell does not induce necessary enzymes for lactose catabolism until it consumes glucose (Lodish, 1997). In this study, we aimed to define the effects of food dyes on development of a member of

our intestinal microflora; *E. coli.* For this reason, Real Time PCR has been used.

MATERIALS AND METHODS

Microbiological studies

Preparation of Bacterial Strains

Stock bacteria culture have been taken from -20 ^{0}C and kept waiting for 1 hour, planted in EMB (Eosin Methylene Blue) agar medium, incubated at 37 ^{0}C for 24 hours. Thus, bacteria revival process has been carried out. Using McFarland device, 10^8 bacteria suspension have been prepared from fresh culture measuring 0.34 value.

Preparation of Food Dyes Solutions

Food dyes solutions have been prepared by adding 500 mg food dyes into 10 ml physiological saline solution as shown that table 1. In the study, control group Nutrienth broth medium and as food dyes Brilliant Blue and Dark Green have been used. By diluting with Nutrient Broth, 3 different concentrations have been prepared as 5 mg/ml, 2,5 mg/ml and 1, 25 mg/ml. *E. coli* ATCC 10799 bacteria strains have been inoculated to the control group and colorant samples in 1 ml 0,34 McFarland value as shown that table 2 .

They have been incubated in agitator incubator at 37 ^{0}C for 24 hours. After 24-hour incubation, in the 2nd day, bacteria culture that grew in the color containing environment and they have been inoculated. This process continued for 20 days. At the same time, each day bacteria numbers in the environment were diluted by physiological saline solution and samples in 3 different concentrations were created in the 10^{-8} - 10^{-5} range and they were planted into PCA (Plate count agar) agar medium and defined as CFU (Colony Forming Unit).

Table 1. Preparation of food dyes solutions

Food dyes	Concentration
E133 Brilliant Blue CI 42090	50 mg / ml
Dark Green CI 19140+CI 73015	50 mg / ml

Table 2. Concentration of food dyes

Food dyes	First concentration	Second Concentration	Third concentration
E133 Brilliant Blue CI 42090	5	2.5	1.25
Dark Green CI 19140+CI 73015	5	2.5	1.25

Molecular studies

E. Coli RNA High Pure PCR RNA Kit(Roche, Germany,Cat. No.11 828 665 001) have been used. Transcriptor first strand cDNA Synthesis Kit have been used for c DNA synthesis. In the study, primer has been prepared for lacz gene expression. Primers have been prepared of oligo name left; TTT CCA TAT GGG GAT TGG TG, and right; CTG GAA TTC CGC CGA TA T series and Roche LightCycler Taqman Master Real Time PCR protocol has been carried out.

RESULTS

In this study, effects of 2 food dyes (Dark Green, Brilliant Blue) at different concentrations on development of *E. coli* which is a member of intestinal microflora. As concentration of Dark Green and Brilliant Blue doses increased, it was seen that *E. coli* reproduction was prevented. Possible effects of two food dyes Dark Green and Brilliant Blue that are used in this study on *E. Coli*ATCC 10799 strain reproduction for 20 generations. 500 mg have been weighed from each colorant and dissolved in 10 ml distilled water. 3 different concentrations have been prepared and increasing dose of food dye effects have been researched. Possible effects of two food dyes Dark Green and Brilliant Blue that are used in this study on E. ColiATCC 10799 strain reproduction for 20 generations. 500 mg have been weighed from each colorant and dissolved in 10 ml distilled water. 3 different concentrations have been prepared and increasing dose of food dyes effects have been researched.

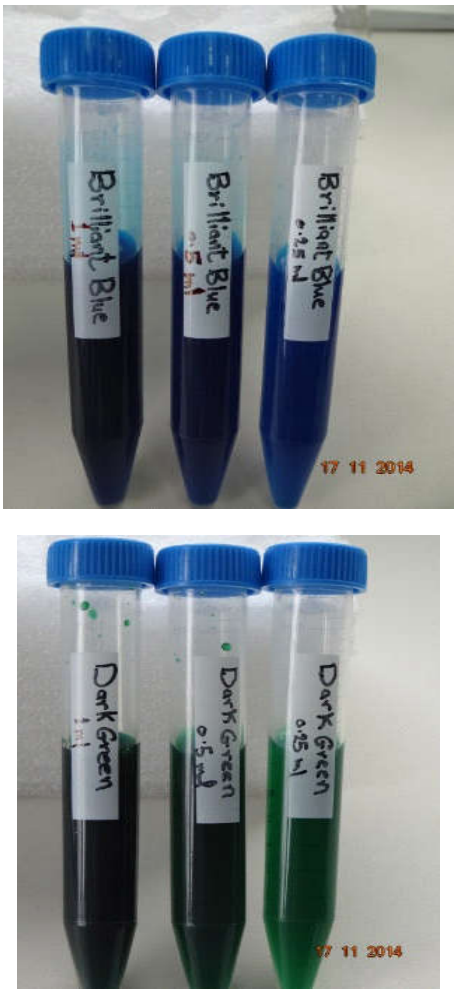

Figure 1. Food dyes concentrations

Effects of Dark Green food dye on E.coli bacteria reproduction for 20 days

From the 1st day to 20th day, a general reduction in bacterial reproduction is seen in all 3 doses. While fluctuations existed in the number of bacterial colonies from the 1st day until the 12th day, as of 12th day there is a stable reduction. We see that the minimum growth amount is in D.G.1 curve (where the color concentration is the most intense). Likewise, it is seen

that the maximum growth is in D.G.3 curve (where the color concentration is the least). In line with this information, it is seen that as the Dark Green food dye concentration increases, it causes a reduction in E.coli bacteria growth as Figure 3.

Effects of Brilliant Blue food dye on E.coli bacteria reproduction for 20 days

From the 1st day to 20th day, a general reduction in bacterial reproduction is seen in all 3 doses. While fluctuations existed in the number of bacterial colonies from the 1st day until the 12th day, as of 12th day there is a stable reduction. We see that the least growth is in B.B.1 curve (where the color concentration is the most intense). Likewise, it is seen that the maximum growth is in B.B.3 curve (where the color concentration is the least). In line with this information, it is seen that as the Brilliant Blue food dye concentration increases, it causes a reduction in E.coli bacteria growth as shown that figure 4. That is, it is seen that Dark Green food dye has more inhibition effects on E.coli reproduction when compared with Brillant Blue color.

Due to increasing doses, it is seen that Dark Green and Brilliant Blue prevents E. coli reproduction. If we put the antimicrobial activities of the used food dyes on E. coli in order, it can be as Dark Green>Brilliant Blue. No significant differences have been found in the RT-PCR amplification curve of Dark Green food dyes. No significant differences have been found in the RT-PCR amplification curves of the generation that is exposed to Control and Brilliant Blue food dyes.

DISCUSSION

Food dyes are widely used in our daily life. As no toxic effects were noticed in people until 1950, there was no emphasis on the reliability of the colorants that are used in food. However, as some toxic effects have been observed after this date, interest in harmful effects of food dyes have also increased. Significantly, chronic toxicity tests have started to be carried out in Food and Drug Administration (FDA) toxicology labs (Safeway, 1987). Number and the type of the food dyes that are allowed to be used in food show variety according to countries. For example, from the Nordic countries, Norway, in 1978 and Sweden in 1980 banned the participation of all kinds of certified (synthetic) dyes in food stuffs completely. Austria allows 27 dyes in total, 8 being synthetic (Yaman 1996, Karaali 1993). In our country, "Colours for use in foodstuffs disclosure" that Ministry of Food, Agriculture and Livestock published in 2002, and which is still in effect, has defined the usage amounts of these materials in foods.

In a research, intestinal conditions have been found in 44.4% out of 81 patients between the ages of 16-73 that used food dyes. 25% of these patients could be treated with a diet (without dyes and protectives), and drug treatment was needed for the rest (13). Food dyes that are in the form of aromatic azo have incurred reductive disintegration by being under the influence of acid, digestive enzymes in the intestine and intestine flora and aromatic amines are formed (Chung,1992). Having been absorbed, these amines become metabolize, mutagenic and promutagenic metabolites are formed (Liener, 1996). As a result of the observations that show Brilliant Blue and Patent Blue colorants are resistant to in vitro metabolic activities by rat intestinal microflora, there is a common view that these colorants lose their metabolisms in the stomach-intestin way. Moreover, it is not expected for this colorants to lose their mutagenity features. Because if there are colorants in the environment due to any detoxification caused by test microorganism, no bleaching is seen (Hess and Fitzhugh 1955). In various systems where there is no mikrosomes, in situations where there is non-specific DNA damage and there is no mutagenity, Brilliant Blue colorant loses its transformation ability in mammalian cells.

Brilliant Blue and Tartrazine colorants are both inactivate in this type of transformation and bacterial mutation systems. For further research, mutagenity studies of these two colorants are needed (Price, 2006). Studies have shown that food dyes have harmful effects in gastrointestinal cancer, in those who have allergen and in kidneys. It is seen that food colorants are toxic in vitro human lymphocytes and that it is attached to the DNA directly in these cells. Reductive and hydrolytic activities of two food colorants named metanil yellow and indigo karmin have been tested by different strains of enteric bacteria. While *E.coli* and Vibrio spp. types had visible reducing activity after 3 hours, other enteric bacteria had increased activity since the beginning of incubation. Following *Enterobacter cloacae* that has maximum reducing ability, *Enterobacter aerogenes and E. coli* had also reducing power. During the 10-hour incubation process, enteric bacteria races gave better responses in obtaining hydrolytic activity of indigo karmin. Normally, intestinal microflora can cause intestinal health problems in consumers (Singh, 1997). In the study carried out by Swaroop et al. in 2011, toxic effects of synthetic food dyes especially of additives such as Eritrozin, Tartrazin, Ponceau 4R, Sunset Yellow FCF, Brilliant Blue FCF, Fast Green FCF, Karmozin, Indigo Karmin on genes with sitokinesis blocking micronucleus technique in human lymphocytes have been researched. As a result, it is found that groups that contain synthetic dyes are more genotoxic when compared to control group and it is highlighted that the ADI (Acceptable Daily Intake) value should be used as base in food dyes usage (Swaroop, 2011). Kuş and Halil Erhan researched the genotoxic and cytotoxic activities on lymphocyte cell cultures of food additives using mitotic and replication index, mikronükleus test. They found that the mitotic index frequency and replication index value is decreased and micronucleus frequency is increased and concluded that Sunset Yellow and Brilliant Blue food additives may have cytotoxic and genotoxic potential (Kuş and Eroğlu 2014). Possible effects of two food dyes Dark Green and Brilliant Blue that are used in this study on E. ColiATCC 10799 strain reproduction for 20 generations. From the 1st day to the 20th day, a general reduction in bacterial reproduction is seen in all 3 doses. While fluctuations existed in the number of bacterial colonies from the 1st day until the 12th day, as of 12th day there is a stable reduction. It has been studied whether the reason of this phenotypic reduction is genotypic or not. With this aim, within the bounds of possibility, it has been researched whether there is a genotypic change in lacz gene which is one of the most effective genes in bacterial development. It is seen that there is no genotypic change in terms of lacz gene. However, gene areas that are effective in bacteria development such as rpoB, lacA, lacy can also be studied. Even genotypic changes that

cause inhibition in bacteria development of food dyes can be detected by doing whole genome sequencing.

REFERENCES

Albertini, R.J., Anderson, D., Douglas, G.R., Hagmar, L., Hemminki, K., Merlo, F., Natarajan, A.T., Norppa, H., Shuker, D.E.G., Tice, R., Waters, M.D. And Aitio, A. 2000. IPCS Guidelines for the Monitoring of Genotoxic Effects of Carcinogens in Humans. Mutat. Res. 463, 111-172.

Beaugerie, L. and Petit, JC. 2004. Antibiotic-associated diarrhea. Best Practice and Research. *Clinical Gastroenterology*. 18, 337-352.

Chung, K.T. Stevens S.E.J.R. and Cernıglıa, C.E. 1992. The reduction of azo dyes by the intestinal microflora. *Crit. Rev. Microbiol.* 18.(3), 175-190.

FAO. Food and Agriculture Organization of the United Nations. 2003. The Stateof Food Insecurity in the World (SOFI).5th. Ed. Viale delle Terme di Caracalla. Roma.

Gibson, RG. 2004. Fiber and effects of probiotics (the probiotic concept), *Clinical Nutrition Supplements.* 1, 25-31.

Guarner, F., and Malagelada,R. 2003. Role of bacteria in experimental colitis, Best Practice and Research Clinicl Gastroenterology. 7 .15.

Hess, S. M. and Fitzhugh,O. G.1955. Absorption and excretion of certain triphenylmethane colors in rats and dogs. *Journal of Pharmacology and Experimental Therapeutics*. 114(1), 38-42.

Karaali,A. and Özçelik,B. 1993. Gıda Katkısı Olarak Doğal ve Santetik Boyları. İstanbul Teknik Üniversetesi Kimya-Metalurji Fakültesi, GıdaMühendisliği Bölümü. İstanbul.18(6), 389-396.

Kuş, E.and Eroğlu, H.E. 2014. Genotoxic and cytotoxic effects of sunset yellow and brilliant blue, colorant food additives, on human blood lymphocytes. *Pak. J. Pharm. Sci.* 28 (1), 227-230.

Liener E.I. 1996. Toxic constituents of plant food stuffs. Food Sci. and Techn. A Series monograph. 357–359.

Lodish H., Baltimore D., Berk A., Zipursky S.L., Matsudair P., Darnell J. 1997. Molecular Cell Biology, 3ed. Ed., Scientific American Books, 407-411

Meggos, H. 1995. Food colors: an international perspective.The Manufacturing Confectioner. 59, 65.

Mital, A. 2006. Use of hen feathers as potential adsorbent for the removal of a hazardous dye, Brilliant Blue FCF, from wastewater. *Journal of Hazardous Materials*. B128. 233–239.

Price, P.J., Suk, W.A., Freeman, A.E., Lane, W.T., Peters, R.L., Vernon, M.L. and Huebner, R.J. 2006. In vitro and in vivo indications of the carcinogenicity and toxicity of food dyes. *International Journal of Cancer*. 21(3), 361–367.

Safeway H.L. 1987. Additives why do we need them.Issved by safeway Nut. Adv. Ser.London.

Singh, S., Das, M. and Khanna, S.K. 1997. Reductive and hydrolytic activities of enteric bacteria on food colours. *Indian J Exp Biol*. 35 (8), 912-4.

Swaroop, V.R, Roy, D.D., Vijayakumar, T. 2011. Genotoxicity of synthetic food colorants. *Journal of Food Science and Engineering*.1:128-134.

Venil, Ch., Zakaria, Z. and Ahmad, W. 2013. Bacterial pigments and their applications, *Scince Direc*t. 48, 1065–1079.

Yaman M. 1996. Bazı Gıda Maddelerine Katılan Sentetik Boyaların Miktarlarının Arastırılması.Gazi Üniversitesi Sosyal Bilimler Enstitüsü, Ankara

Yücecan, S. 1999. Nşaat isçilerinin enerji harcamaları, beslenme ve sağlık durumları. Sağlık Teknolojisi Yüksekokulu Beslenme ve Diyetetik Bölümü. Ankara.

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