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Effect of Brilliant Blue FCF Food Colour on Plant and Human DNA

Rubina Gilani*, Shruti Joshi, V J Shiva Shankari**,**

P.Mary Joshna and Dr. Venugopal Rao*****

**B.Sc 3rd year BZC **B.Sc 3rd year GCM, ***Head, Department of Genetics*

St. Ann's College for Women, Mehdipatnam, Hyderabad – 500028, Telangana, India.

Abstract

Food colours are used to make the food look vibrant and play an important role in visually stimulating one's appetite. It also makes one to relate a particular flavor depending on the colour of the food. Food colours are of two types – Natural food colours and synthetic food colours. Natural food colours are derived from plant sources and appear less vibrant compared to synthetic food colours which are chemically synthesized. Synthetic food colours are more commonly used since they are less expensive and small amounts can give an intense and uniform colour. Present study deals with the impact of synthetic food colour brilliant blue FCF on Plant DNA and Human DNA. Plant DNA was extracted from mint leaves and then plant DNA and human DNA were exposed to 1%, 3%, 5% and 7% dilutions of Brilliant blue FCF at 10, 30, 50 and 70 minutes time interval. The stock solution prepared was 1 gram of food colour per 100ml water. Optical density values of treated DNA samples were taken after each time interval at 520nm using a standardized colorimeter. The plant DNA showed a decrease in OD values at all concentrations after 10 min interval showing possible renaturation. However, all the concentrations showed an increase in OD values after 30 minutes interval and thereafter. In case of human DNA the damaging effect of Brilliant blue FCF was seen at all concentrations. The OD values remained almost constant after 10 minutes interval for all the concentrations. After 30 minutes, there was a slight increase in the OD values of all the concentrations. Thereafter at 50 minutes, the OD values of all the concentrations soared high, followed by a peak denaturation at 70 minutes for all the concentrations. The

damaging effect of DNA is seen at all concentrations in human DNA and plant DNA though, the highest effect was seen at 3% for human DNA and at 7% for plant DNA. The overall results suggest that there is a decrease in the optical density of pant DNA followed by a gradual increase whereas the OD values of Human DNA showed slight increase followed by a drastic increase. This clearly demonstrates the toxic effect of Brilliant blue FCF on both plant as well as the human DNA.

Keywords: Food colours, Optical Density, Brilliant Blue FCF, Denaturation, Renaturation.

INTRODUCTION

Food coloring or color additive, is any dye, pigment or substance that imparts color when it is added to food or drink.[1][26] They come in many forms consisting of liquids, powders, gels, and pastes.[8] Food coloring is used both in commercial food production and in domestic cooking. Food colourings are of two types: artificial and natural.[5] Natural colours are a better alternative as they are wholly derived from plants. Natural colouring usually appears less vibrant when compared to artificial colouring. This however, does not affect the taste of your food. Natural food colour is any dye, pigment or any other substance obtained from vegetable, animal, mineral that is capable of colouring foods or drugs. Colours come from variety of sources like seeds, fruits, vegetables, algae and insect. Grass, beet root, and turmeric are some of the natural sources from which colours are extracted.[29] On the other hand synthetic food colours or artificial colours are manufactured by chemical reaction and are commonly used in food and pharmaceutical industries. [5][7][21] Synthetic dyes are commonly found in fruit squash, fruit cordial, coloured fizzy drinks, instant puddings, cake mixes, custard powder, soups, sauces, ice cream, sweets, chewing gum, jams, jellies, yoghurt and many convenience foods together with glycerine, lemon and honey products. It can also be found in the shells of medicinal capsules.[18][36][8] Some of the common food colours are **tartrazine** (IUPAC name - Trisodium (4E)-5-oxo-1-(4-sulfonatophenyl)-4-[(4-sulfonatophenyl)hydrazono]-3-pyrazolecarboxylate), **sunset yellow FCF** (IUPAC name - disodium 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonate), **brilliant blue FCF** (IUPAC name - ethyl - [4 - [[4 - [ethyl -[(3 - sulfophenyl) methyl] amino] phenyl] - (2 - sulfophenyl) methylidene] - 1 - cyclohexa - 2, 5 - dienylidene] - [(3 - sulfophenyl) methyl] azanium.), **amaranth** (IUPAC name - trisodium (4*E*)-3-oxo-4-[(4-sulfonato-1-naphthyl)hydrazono]naphthalene-2,7-disulfonate), **allura red** (IUPAC name - disodium 6-hydroxy-5-((2-methoxy-5-methyl-4-sulfophenyl)azo)-2-naphthalenesulfonate), **quinoline yellow**(IUPAC name - Sodium 2-(1,3-dioxoindan-2-yl)quinolinedisulfonate) and **indigo carmine**(IUPAC name - 3,3′-dioxo-2,2′-bisindolyden-5,5′-disulfonic acid disodium salt) [30][23][35][5][24][26]

The food colouring history dates back to early Egyptians and Romans civilization, when people used saffron, various flowers, carrots, mulberries, beets, and so forth to

put colour to their foods suggesting use of coloring agents from prehistoric times. Later during the middle of the nineteenth century people had started using synthetic colors in place of natural colors. Since then the extensive use of synthetic food azo dyes (–N=N–) has become very common due to increasing canned and fast food culture, despite their legislative ban. [13][5]

Moreover these dyes have no nutritional value, they have no health benefits, they are not preservative.[9] They only make food attractive to meet new consumer demand, since the visual aspect is considered to be an important factor for the selection of products by final consumers.[5]

As per norms of international research and the recommendations of the Codex Committee on Food Additives and Contaminants (CCFAC), intake of dye is under the control of ADI (acceptable daily intake).[20][17] Nowadays, food industries are ignoring the guidelines provided by these regulatory agencies to sell their products in a large scale.

There are some potential problems with synthetic food dyes, specifically: Carcinogenicity – leading to cancer development, genotoxicity – leading to mutations or damaging chromosomes in bacteria, fungi, insects and mammalian cells in vivo and in vitro and neurotoxicity – leading to the damage of nerve tissue. [27][32][33][31][14][6][11][15][10][26]

Brilliant Blue FCF (Blue 1) is an organic compound classified as a triarylmethane dye, reflecting its chemical structure. Its IUPAC name is ethyl - [4 - [[4 - [ethyl -[(3 - sulfophenyl) methyl] amino] phenyl] - (2 - sulfophenyl) methylidene] - 1 - cyclohexa - 2, 5 - dienylidene] - [(3 - sulfophenyl) methyl] azanium. [30] Known under various commercial names as FD&C Blue No.1, Acid Blue 9, D&C Blue No. 4, Alzen Food Blue No. 1, Atracid Blue FG, Blue #1 Lake, Erioglaucine, Eriosky blue, Patent Blue AR, Xylene Blue VSG, C.I. 42090 Basacid Blue 755, Sulfacid Brilliant Blue 5 J, Neolan Blue E-A, it is a colorant for foods and other substances. It is denoted by E number E133. The individual response varies not only according to dose, age, gender, nutritional status and genetic factors, but also according to long term exposure to low doses (Sasaki et al., 2002).[4][22][16]

MATERIALS AND METHODS

Extraction of plant DNA from mint leaves

Mentha (also known as mint) is a genus of plants in the family Lamiaceae. Mint leaves are easily available as they can tolerate a wide range of conditions and can also be grown in the full sun. They are fast growing, extending their reach along surfaces through a network of runners.

The kit used is HIMEDIA HiPer Plant Genomic Kit (solution based). [2]
100mg of finely cut young and tender mint leaves are grinded in 400µl lysis buffer and is transferred to collection tube containing RNase solution. The sample is incubated for 10mins at 65˚c. To the mixture130µl of precipitation buffer is added and is incubated on ice for 5mins followed by centrifiguation at 14,000rpm for 5 minutes. Then lysate is added onto the HiShredder and is centrifuge for 2 mins at 14,000rpm.The supernatant is removed and binding buffer is added following continuous mixing. The mixture is added to mini prep spin column and is centrifuged for 1 min at 8,000rpm and the supernatant is discarded. The sample is transferred to new tube and 500µl of wash solution is added followed by centrifugation at 8,000rpm for 1 min. The supernatant is discarded. Again 500µlof wash solution is added followed by centrifugation at 8,000rpmfor 1 min. The supernatant is discarded. The sample is centrifuged for additional 2 min at14,000rpmto remove traces of wash solution. To the sample 100µl of elution buffer is added directly and is incubated for 1 min at room temperature, centrifugation is carried out for 1 min at 10,000rpm to elute the DNA. White cloudy ppt is obtained after adding isopropyl alcohol or ethanol.

Human DNA
The human DNA sample was provided by PARAM SCIENCE AND TECHNOLOGY.[3] Pure plant DNA and human DNA samples were exposed to the food color with variable concentrations at different time intervals.

Preparation of food colour solution:
A stock solution of 1gram% i.e.1gram of food colour dissolved in 100ml of distilled water is prepared. From the stock solution various dilutions were prepared.

RESULTS AND DISCUSSION

Optical density values of treated DNA samples taken at 520nm using colorimetry revealed variation in different concentrations of food colour diluted with water.[24] The increase in OD values over time may indicate the damage of DNA.

Effect on Plant DNA :
The damaging effect of Brilliant Blue FCF food color was highest at 7% concentration. However it was observed that after 10 minutes all the concentrations (1%, 3%, 5% and 7%) showed a drastic decrease in the OD values indicating possible renaturation or absence of damaging effect. After 30 minutes, all the concentrations showed a rise in the OD values till 70 minutes with an exception of 3% concentration whose OD value again showed a decrease at 70 minutes. The increase in the OD values at 1%, 5% and 7% indicate denaturation of the DNA (figure 1).

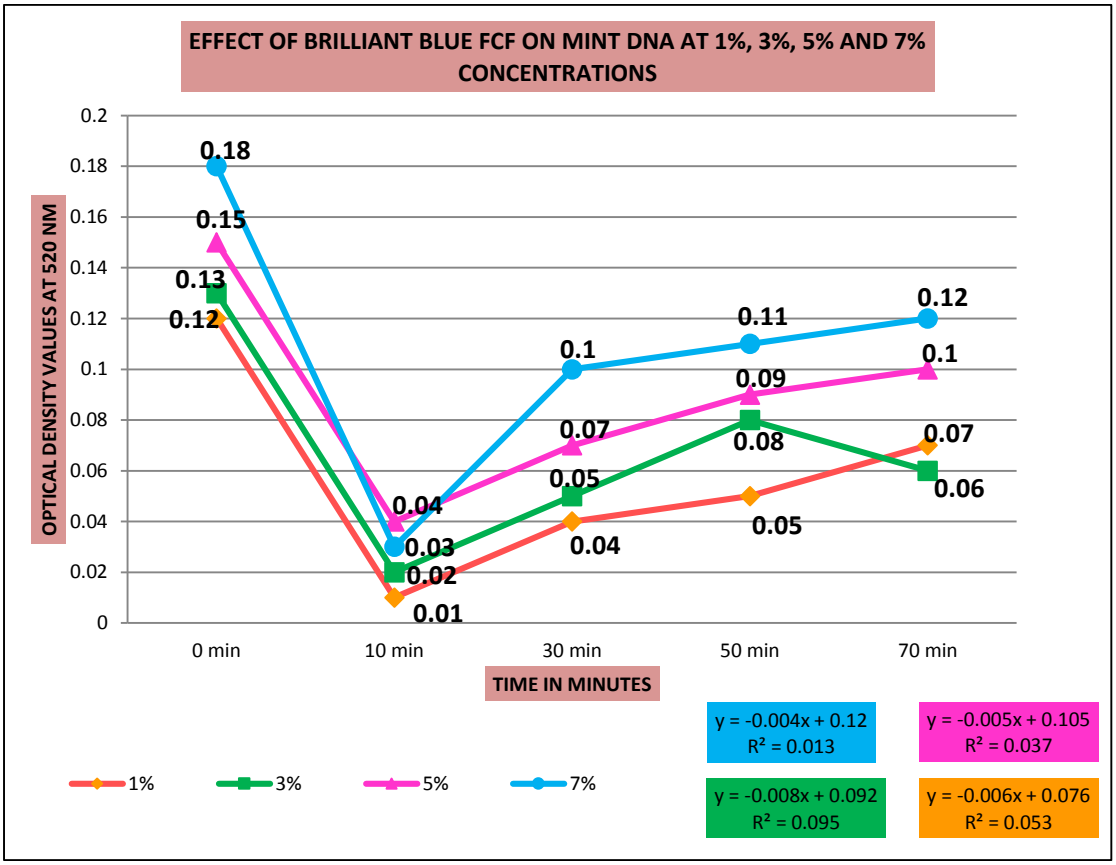

Figure 1

Effect on Human DNA:

The damaging effect of Brilliant Blue FCF was seen at all concentrations. After 10 minutes, all the OD values remained almost constant. When observed after 30 minutes, there was a slight increase in the OD values of all the concentrations. Thereafter, at 50 minutes, the OD values of all the concentrations soared high. A further minimal increase was noted at 70 minutes for all the concentrations. This therefore suggests that as the time period increased, the damaging effect of the food colour increased with a dramatic increase at 50 minutes followed by a peak denaturation at 70 minutes for all the concentrations (figure 2).

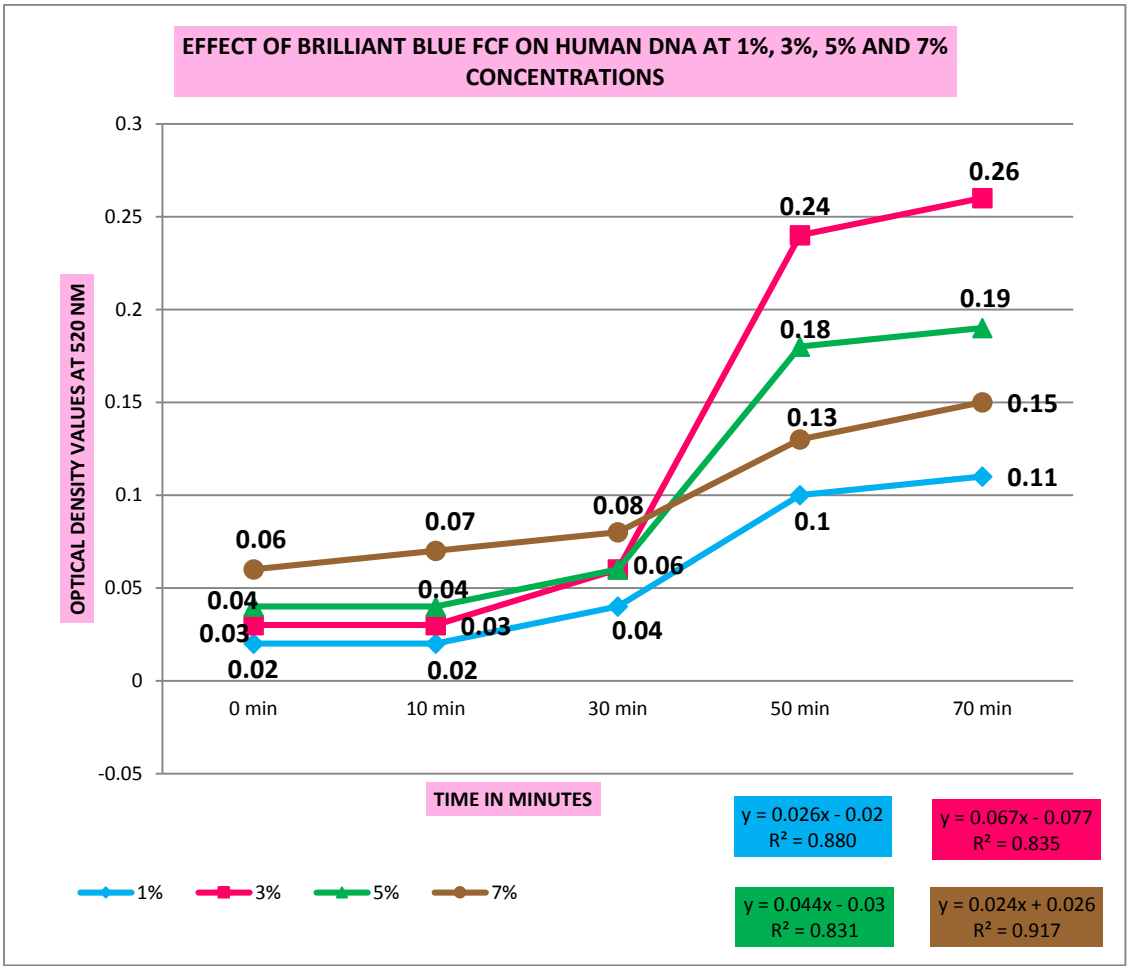
EFFECT OF BRILLIANT BLUE FCF ON HUMAN DNA AT 1%, 3%, 5% AND 7% CONCENTRATIONS
OPTICAL DENSITY VALUES AT 520 NM
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
0 min
10 min
30 min
50 min
70 min
0.26
0.24
0.19
0.18
0.15
0.13
0.11
0.1
0.08
0.07
0.06
0.06
0.04
0.04
0.03
0.03
0.04
0.02
0.02
TIME IN MINUTES
y = 0.026x - 0.02
R² = 0.880
y = 0.067x - 0.077
R² = 0.835
y = 0.044x - 0.03
R² = 0.831
y = 0.024x + 0.026
R² = 0.917
1%
3%
5%
7%

Figure 2

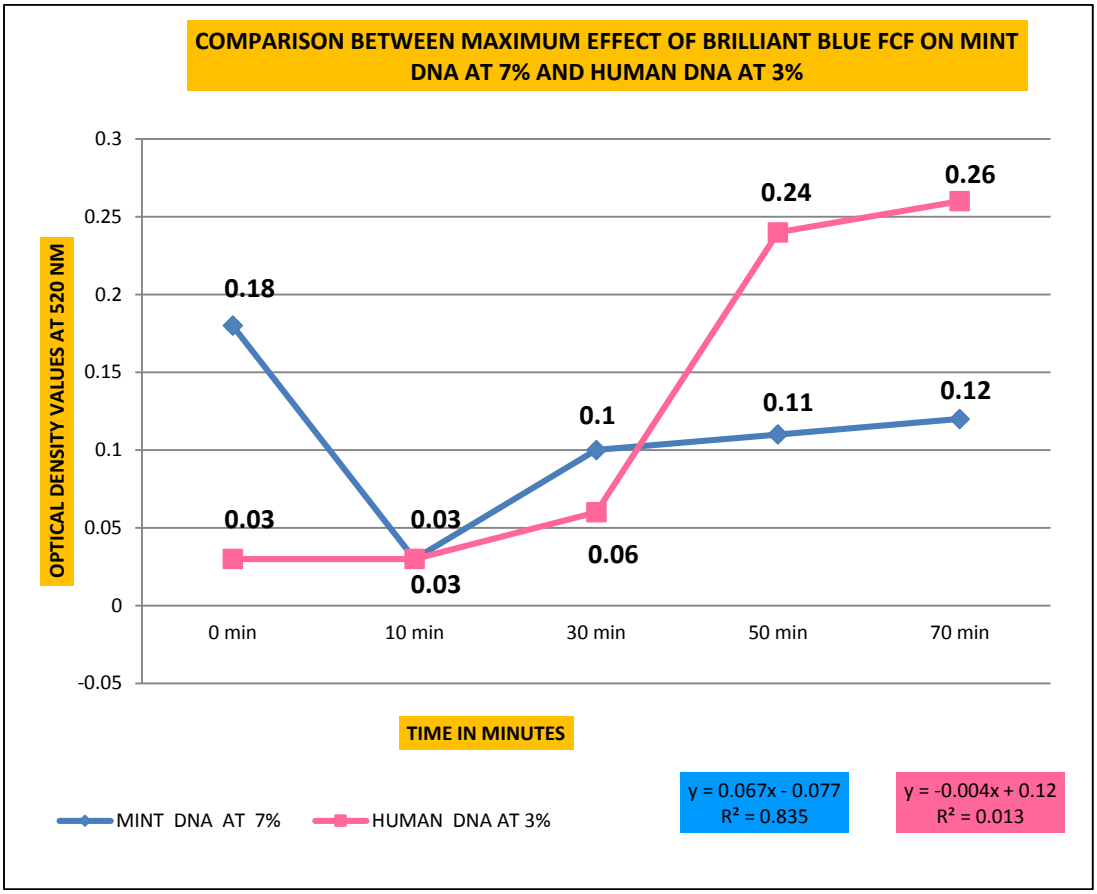

Figure 3

The damaging effect of DNA is seen at all concentrations in human DNA and plant DNA though, the highest effect was seen at 3% for human DNA and at 7% for plant DNA. The overall results suggest that there is a decrease in the optical density of pant DNA followed by a gradual increase whereas the OD values of Human DNA showed slight increase followed by a drastic increase.

From the above graph it can be concluded that the effect of Brilliant blue FCF on human DNA is more when compared to that of Mint DNA (fig 3).

CONCLUSION

Artificial food colors like Brilliant blue FCF which is a commonly used colouring agent causes DNA damage and hence poses high risk to humans, thus promotion of natural colouring substances is needed and recommended.[30][5]

REFERENCES

[1] http://www.precisionnutrition.com/all-about-food-additives

[2] HIMEDIA HiPer Plant Genomic DNA Extraction Kit (Solution Based).

[3] Param Science and Technology

[4] Dilek Pandir, 2016, DNA damage in human germ cell exposed to the some food additives in vitro, Cytotechnology, Volume 68, Issue 4, pp 725- 733.

[5] Sunday N. Okafor, Wilfred Obonga, Mercy A. Ezeokonkwo, Jamiu Nurudeen, Ufoma Orovwigho, Joshua Ahiabuike, 2016, Assessment of the Health implications of Synthetic and Natural Food Colourants – A Critical Review, UK Journal of Pharmaceutical and Biosciences (UKJPB), DOI : http://dx.doi.org/10.20510/ukjpb/4/i4/110639

[6] Esra Kus and Halil Erhan Eroglu, 2015, Genotoxic and cytotoxic effects of sunset yellow and brilliant blue colorant food additives on human blood lymphocytes, Pakistan Journal of Pharmaceutical Sciences, 28(1):227-30

[7] Márcio Carocho, Maria Filomena Barreiro, Patricia Morales, Isabel C.F.R. Ferreira**,** 2014**,** Adding Molecules to Food, Pros and Cons: A Review on Synthetic and Natural Food Additives**,** Institute of Food Technologists**,** Volume 13, Issue 4**,** Pages 347–769**,** DOI : 10.1111/1541- 4337.12065

[8] Naveena Shanmugam, Jacob Grotton, Daniel Huang, and Jeeva Jacob, 2013, Food Additives ; "Got Food? Recent Advances in Food Science and Technology"; An e- book co-authored by the Class of 2013 Massachusetts Academy of Math and Science.

[9] Sumita Dixit , S.K. Purshottam , S.K. Khanna & Mukul Das , 2011, Usage pattern of synthetic food colours in different states of India and exposure assessment through commodities preferentially consumed by children, Journal – Food Additives and contaminants, Pages 996-1005, Part A Volume 28, 2011 – Issue 8 ; http://dx.doi.org/10.1080/19440049.2011.580011

[10] V. R. Swaroop, D. Dinesh Roy and T. Vijayakumar, 2011, Genotoxicity of Synthetic Food Colorants School of Environmental Sciences, Journal of Food Science and Engineering pg - 128-134

[11] EFSA European Food Safety Authority, 2010, Scientific Opinion on the re-evaluation of brilliant blue FCF (E 133) as a food additive , EFSA Journal, 8(11):1853 [36 pp.]**,** DOI : 10.2903/j.efsa.2010.1853

[12] G.M. Hassan, 2010, Effects of some synthetic coloring additives on DNA damage and chromosomal aberrations of rats, Arab J. Biotech., Vol. 13, No. (1) :13-24.

[13] Shimada C, Kano K, Sasaki YF, Sato I, Tsudua S, 2010, Differential colon DNA damage induced by azo food additives between rats and mice ; The Journal of Toxicological Sciences, PMID:20686341

[14] Mikyung Park , Hee Ra Park , So Jung Kim , Min-Sun Kim , Kyoung Hye Kong , Hyun Soo Kim , Ein Ji Gong , Mi Eun Kim , Hyung Sik Kim , Byung Mu Lee & Jaewon Lee, 2009, Risk Assessment for the Combinational Effects of Food Color Additives: Neural Progenitor Cells and Hippocampal

Neurogenesis, Journal of Toxicology and Environmental Health, Pages 1412-1423, Part A Current Issues Volume 72, Issue 21-22.

[15] Takaharu Mizutani, 2009, Toxicity of Xanthene Food Dyes by Inhibition of Human Drug-Metabolizing Enzymes in a Noncompetitive Manner, Journal of Environmental and Public Health, Volume 2009, Article ID 953952, 9 pages, http://dx.doi.org/10.1155/2009/953952

[16] Pintea A. M., 2008, Synthetic colorants in Food Colorants. Chemical and Functional Properties, ed. C. Socaciu, CRC Press. 603-615.

[17] Meenakshi Tripathi, Subhash K. Khanna, Mukul Das, 2007, Surveillance on use of synthetic colours in eatables vis a vis Prevention of Food Adulteration Act of India, Food Control ; Volume 18, Issue 3, Pages 211–219

[18] UK Food Guide. http://www.ukfoodguide.net/e102.htm. Retrieved 2007FDA, 2007

[19] Neveen H. Mahmoud, 2006, Toxic effects of the synthetic food dye brilliant blue on liver, kidney and testes functions in rats, Journal of The Egyptian Society of Toxicology, Vol 34:77–84.

[20] Committee on Food Chemicals Codex,2003, Food Chemicals Codex -5th edition, National Academy Press, Washington DC, p.463.

[21] Özgür MÜ and Koyuncu İ, 2002, The simultaneous determination of quinoline yellow (E-104) and sunset yellow (E-110) in syrups and tablets by second derivative spectrophotometry, Turkish Journal of Chemistry, 26: 501- 508.

[22] Yu F Sasaki, Satomi Kawaguchi, Asako Kamaya, Miyuki Ohshita, Kazumi Kabasawa, Kayoko Iwama, Kazuyuki Taniguchi, Shuji Tsuda, 2002, The comet assay with 8 mouse organs: results with 39 currently used food additives, Mutation Research/Genetic Toxicology and Environmental Mutagenesis ; Volume 519, Issues 1–2, Pages 103–119http://dx.doi.org/10.1016/S1383-5718(02)00128-6

[23] Shuji Tsuda,Makiko Murakami, Naonori Matsusaka, Kiyoshi Kano, Kazuyuki Taniguchi and Yu F. Sasaki DNA, 2001, Damage Induced by Red Food Dyes Orally Administered to Pregnant and male mice ; Toxicological Sciences, 61 (1): 9299. doi: 10.1093/toxsci/61.1.92

[24] Paul Wight, 2000, Xanthene Dyes, DOI: 10.1002/0471238961.2401142023090708.a01

[25] Kiple KF, Ornelas KC, 2000, Contemporary food related issues, The Cambridge University Press, pp 1667–1676.

[26] Mekkawy HA, Ali MG and El-Zawahry AM, 1998, Toxic Effect of Synthetic and Natural Food Dyes on Renal and Hepatic function in Rats, Toxicology Letters. 1998, 95(1), 155

[27] Aboel-Zahab H, el-Khyat Z, Sidhom G, Awadallah R, Abdel-al W, Mahdy K, 1997, Physiological effects of some synthetic food colouring additives on rats, Bollettino Chimico farmaceutico, 136(10):615-27. PMID:9528169

[28] Babu SB and Shenoliker IS, 1995, Health and Nutritional Implications of Food Colors. Indian Journal of Medical Research, 102(3): 245-255.

[29] Hallagan JB, Allen DC and Barzelleca JF, 1995, The Safety and Regulatory Status of Food, Drug and Cosmetic Color Additives Exempt from Certification, Food and Chemical Toxicology, 33(6): 515-528.

[30] J.F. Borzelleca, K. Depukat, J.B. Hallagan, 1990, Lifetime toxicity/carcinogenicity studies of FD & C blue No. 1 (Brilliant blue FCF) in rats and mice, Food and Chemical Toxicology Volume 28, Issue 4, Pages 221-234

[31] R.D. Combes, R.B. Haveland-Smith, 1982, A review of the genotoxicity of food, drug and cosmetic colours and other azo, triphenylmethane and xanthene dyes, Mutation Research/Reviews in Genetic Toxicology, Volume 98, Issue 2, Pages 101-243 ; DOI:10.1016/0165-1110(82)90015-X

[32] R.B. Haveland-Smith *, R.D. Combes, 1980, Screening of food dyes for genotoxic activity, Food and Cosmetics Toxicology Volume 18, Issue 3, Pages 215 - 221

[33] R.B. Haveland-Smith *, R.D. Combes , 1980, Genotoxicity of the food colours red 2G and brown FK in bacterial systems; use of structurally-related dyes and azo-reduction, Food and Cosmetics Toxicology Volume 18, Issue 3, Pages 223-228

[34] J. P. Brown, A. Dorsky, F. E. Enderlin, R. L, Hale, V. A. Wright, and T. M. Parkinson, 1980, Synthesis of 14C-labelled FD & C blue no. 1 (brilliant blue FCF) and its intestinal absorption and metabolic fate in rats , Food and Cosmetics Toxicology, vol. 18, no. 1, pp. 1–5.

[35] K. Sune Larsson, 1975, A teratologic study with the dyes amaranth and ponceau 4R in mice Toxicology Volume 4, Issue 1, 1975, Pages 75-82, DOI: 10.1016/0300-483X(75)90023-2

[36] https://www.efsa.europa.eu/en/press/news/ans091112