World Journal of Agricultural Sciences 2 (4): 435-438, 2006
ISSN 1817-3047

Cytogenetic Evaluations of Natural and Synthetic Mixtures of Green-s and Chlorophyll and/or Royal Jelly as Food Additives

Jamal S.M. Sabir

Department of Biological Sciences, Faculty of Science,
King Abdulaziz University, Jeddah 21589, P.O. Box 80141, Saudi Arabia

Abstract: The cytogenetic effect of Green-s was tested in rats bone marrow and germ cells. Green-s was test as a pure synthetic dyes and with natural substances as chlorophyll or royal jelly. The results showed a significant increase in all parameters in animals treated with green-s compared to the control. It is worthy to mention that ingestion of synthetic food colorant alone produce higher influence. The animals treated green-s with chlorophyll and animals treated green-s with royal jelly showed lower numbers of cells with aberrations either in somatic or germ cells compared with control. For study chromosomal aberrations rats were treated subacutely (30 consecutive days) with natural and synthetic colorants and their mixture. Chromosome of somatic and germ cell were studied. Green-s, the synthetic colorant was found to produced highly significant increase in structural chromosomal aberrations in both somatic and germ cells. Treatment with chlorophyll and mixture of natural and synthetic colorant showed lower numbers of cells with aberrations in somatic or germ cells while treatment with royal jelly and mixture with synthetic colorant showed greater lower in numbers of aberrations.

Key words: Food colorants · green-s · chlorophyll · royal jelly · mutagenicity · antimutagenicity · chromosomal aberrations · bone marrow · germ cells

INTRODUCTION

No reliable theoretical model has emerged to predict biological properties of synthetic and natural and its mixture from their chemical structure.

Individual investigation into the metabolic toxicological potential of these products, however has been a routine item in safety evaluation programs.

The available literature as regards their toxicity or metabolism revealed that little work was published. But the magnitude of change is partially dependent on the type of food additives, their mixture, chemical structure, administration dose, duration of treatment, time of sampling after treatment and degree of absorption from the gastrointestinal tract after ingestion among other factors. The safety of natural pigments used with synthetic dyes as food additives is view of their suggested lower toxic effect [1].

Haveland smith and combes (1980 and 1982) studies the genetic effect of 6 major classes of synthetic food colors (anthraquinone, azo dyes, pyrollazone, indigoid, triphenyl methane and xanthane). Abdel Aziz *et al.* [2] studied the cytogenetic effect of sun set yellow (FCF) on the oogenesis of mice.

Roychoudhury and Giri [3] tested the effect 4 permitted food days (indigo carmine, orange, green-s and tartrazine) on cytogenetic assays in male mice the genotoxicity of the food colorants. All those studies showed changes in cytogenetic assays relative to control animals.

The present study is an attempt to evaluate the mutagenic influences of natural with synthetic dyes and their mixture on adult albino rats.

MATERIALS AND METHODS

The synthetic colorant green-s was obtained from Aromisr co. (Egypt) and natural pigment was provided by P. Robertet and co. (France).

Green-s is the monosodium salt of 4,4-bis (dimethylamino) biphenyl methylene-2 naphthol 3,6 disulphonic acid [4].

Corresponding Author: Dr. Jamal S.M. Sabir, Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah 21589, P.O. Box 80141, Saudi Arabia

Chlorophyll (the natural food colorant) is the green coloring matter of leaves of which they occur out photosynthesis, it is a magnesium complex having a porphyrin structure and contain two hydroxyl groups, one of which is esterified with phytyl alcohol and the other with methyl alcohol, it is extracted by alcohol or acetone [5].

The protective royal jelly, was obtained from a bee-keeper. The tested concentration (1 mg kg^{-1} body weight) was dissolved in distilled water. The determined i.p. and oral LD 50 amounted to 5 mg and 50 mg kg^{-1} body weight.

Chlorophyll (the natural food colorant), as well as green-s (the synthetic colorant) (suspended in distilled water) were ingested orally by a dose level 4 mg kg^{-1} body weight or a dose level 8 mg kg^{-1} body weight of their mixture (1:1 W/W) subacutely.

Male rats, weighing 80-100 grams were used in these studies.

For studying chromosomal aberrations, rats were divided into 4 groups (each of 5 rats) the first group was used as normal control, the second group was orally ingested with green-s (synthetic food colorant), the third group was orally ingested the mixture of green-s with chlorophyll, the four groups were orally ingested with green-s and royal jelly. At the end of experimental period (30 days), the animals of the four were killed by decapitation. Both treated and control animals were injected i.p. with colchicines 3 hours before killing. Bone marrow and spermatocytes cells were prepared according to Yoside and Amano [6]. Slides were stained with phosphate buffered Giemsa. Around 50 metaphases were studied from each somatic and germ cells for each animals, scoring the different types of chromosomal aberrations such as chromosomal gaps, deletion, fragments and centromic attention were studied. The results of chromosomal aberrations were analyzed using the analysis of variance (ANOVA) according to Snedecor and Cochran [7].

RESULTS AND DISCUSSION

The results of synthetic food colorant green-s showed a highly significant increase in chromosome aberrations in both bone marrow and spermatocyte cells. Subacute treatment caused high percentage of aberrant cells. This may be due to the accumulation effect of the dye since the animals received the dye for 30 consecutive days. Deletion is the main type of chromosomal aberrations in both types of examined cells in Tables 1 and 2.

Cytogenetic analysis of animals treated with green-s and chlorophyll mixture illustrated that that a significant decrease ($p \leq 0.01$) in all structural chromosomal aberration

Table 1: Chromosomal aberrations in bone marrow cells of rats treated with the natural and synthetic dyes

			M±S.E				
Treatment	No. of animal	No. of examined	Gap	Deletions	Fragment	Centromic attnution	Total aberration
Control	5	250	0.40±0.24c	0.40±0.24d	0.20±0.20c	0.40±0.24d	1.40±0.24d
Green-s	5	250	3.00±0.77a	14.80±1.46a	5.40±7.36a	7.20±1.32a	30.40±2.11a
Green-s + chlorophyll	5	250	1.80±0.58b	7.80±1.50b	2.20±0.66b	4.60±0.68b	16.40±3.09b
Green-s + royal jelly	5	250	0.80±0.37c	4.00±1.30c	1.00±0.32b	2.00±0.71c	7.80±2.56c

- Data expressed as mean (M) stander error (SE)

- Means of different letters (a, b, c, d) in the same column are significantly different ($p \leq 0.01$)

Table 2: Chromosomal aberration in spermatocyte cells of rats treated with the natural and synthetic dyes

			M±S.E			
Treatment	No. of animal	No. of examined	Chain	Autosomal	x-y univalent	Total aberration
Control	5	250	0.00±0.00	0.40±0.24	0.20±0.20	0.60±0.24
Green-s	5	250	3.20±0.58a	7.60±0.75a	4.60±0.51a	15.40±1.08a
Green-s + chlorophyll	5	250	2.00±0.71b	4.40±0.93b	2.60±068b	9.00±1.92b
Green-s + royal jelly	5	250	0.80±0.37c	2.00±0.45c	1.60±0.51b	4.40±1.21c

- Data expressed as mean (M) stander error (SE)

- Means of different letters (a, b, c, d) in the same column are significantly different ($p \leq 0.01$)

compared with treatment by green-s and control groups (Table 1), the group of animals treated with green-s and royal jelly mixture showed highly significant decrease ($p \leq 0.01$) in total chromosomal aberration compared with treatment by green-s and control group.

The effect of the same colors on germ cells are presented in Table 2, which was significant decrease in the case of green-s with chlorophyll ($p \leq 0.01$) and highly significant decrease in animals treated with the mixture green-s and royal jelly, the aberrations were presented by autosomal our results revealed that green-s (the synthetic food colorant) has mutagenic effects on mammalian somatic and germ cells, but the presence of chlorophyll and royal jelly in mixture with green-s reduced it's effect they inhibited it's toxicity by reducing the up take and the accumulation of green-s via decreasing its absorption rate. Also the present results has agreement those chlorophyll and Ronal jelly are important as antioxidant when they used in food stuff.

Giri *et al.* [8] showed that green-s induced significant increase in sister chromatid exchanges and chromosomal aberration in mice and induced a significant increase in the mitotic index when compared with the control.

Agreement with results Abdou *et al.* [9]. Chromosomes of somatic and germ cells were studied. Green-s, the synthetic colorant was found to produced highly significant increase in structural chromosomal aberrations in both somatic and germ cells. The treatment with by it chlorophyll as natural dye recorded lower numbers of cells with aberrations either in somatic or germ cells.

Other similar results Sarkar *et al.* [10] tested the protection effect of several type of chlorophyll extracts against mutagenic and clastogenic activity of genotoxicants.

Chlorophyll alone, whether extracted was clastogenic and could reduce the effects of the chromium salt to its own level.

Sarkar *et al.* [11] tested the anticlastogenic activity of sodium-copper- chlorophyllin against two known clastogens.

The results indicated that clastogenic effects induced by chromium in an aqueous solution (20 mg kg^{-1} body weight) were reduced to a significant level by chlorophyllin (1.5 mg kg^{-1} body weight), when administered both before and simultaneously with the toxicant.

Royal jelly has been analyzed into frach fractions by Young and Cho [12]. The first fraction consists of organic acid (phenolic material and bees wax) sterol, phospholipids and saponifiable substance. The second fraction is rich with sugar, unidentified acid, inorganic salt and nitrogen compound. The third fraction is protein in nature, including amino acid aspartic acid, arginine, thryrosine, tryptophan and histidine, the first fraction contains the physiologically active material responsible for sexual development of the queenbees. It contains phenolic, materials that may be responsible for the antimutagenic potential of royal jelly observed in the present studies. It may be mentioned in this connection that naturally occurring plant phenols has substantial antimutagenic and anticarcinogenic effects against a variety of polycyclic aromatic hydrocarbons.

In conclusion, chlorophyll and or royal jelly (natural additive) in the mixture with green-s have less mutagenic effect than green-s (synthetic dye) as food additive.

REFERENCES

1. Abdel-Rahim, E.A., O.A. Shaban, A. Shallan and A.A. Ragab, 1995. The hypointesive effect of natural pigments on synthetic dyes toxicity as food additives: sugars and lipid fractions of blood and liver glycogen as well as growth rate and thyroid function of albino rats. J. Agric. Sci. Mansoura Univ., 20: 517.
2. Abdel Aziz, K.B., E. El-Nahass, H.O. Ali and M.T. Fahmy, 1989. Cytogenetic effects of sunset yellow (FCF) on the oogenesis of mice. Egypt. J. Anat., 12: 117.
3. Roychoudhury, A. and A.K. Giri., 1989. Effect of certain food dyes on chromosomes of *Allium cepa*. Mutat. Res., 223: 313-319.
4. FAO/WHO, 1964. Food agricultural organization World Health Organization, experts, Committee on food additives. Eigth report, Fech, Rep. Ser. Wild Health Org. 309, Geneva.
5. FAO/WHO, 1979. Expert committee on food additives: Eighteenth report "Toxicological evaluation of some food colors, enzmes, Flavor, Enhances, Thickening Agents and certain food additives" Tech. Rep. Ser. Wild Health Org., 557.
6. Yosida, T.H. and K. Amano, 1975. Autosomal polymorphism in laboratory bred and wild norway rats, Rattus norvegicus, found in Misimo Chromosoma, 16: 658-690.
7. Snedecor, G.W. and W.G. Cochran, 1980. Statistical methods, 7th ed. Lowa State Univ., Press, Lowa, USA., pp: 393.

8. Giri, A.K., S.S. Sivam, K.A. Khan and N. Sethi, 1992. Sister chromatid exchange and chromosome aberrations in mice after in vivo exposure of Green-s, a food colorant. Environ. Mol. Mutagen., 19: 223-226.
9. Abdou, H.S., S.H. Salah and E.A. Abdel-Rahim, 1997. Cytogenetic and biochemical evaluations of the hypointensive influence of chlorophyll and green-s food additives. Al-Azhar Bull. Sci., 8: 563-578.
10. Sarkar, D., A. Sharma and G. Talukder, 1996. Clastogenic activity of pure chlorophyll and anticlastogenic effects of equivalent amounts of crude extract of Indian spinach leaf and chlorophyllin following dietary supplementation to mice. Environ. Mol. Mutagen., 28: 121-126.
11. Sarkar, D., A. Sharma and G. Talukder, 1993. Differential protection of chlorophyllin against clastogenic effects of chromium and chlordane in mouse bone marrow in vivo. Mutat. Res., 301: 33-38.
12. Young, T. and M.D. Cho, 1977. Studies on royal jelly and abnormal cholesterol and triglycerides. American Bee J., pp: 36-38.