INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

POSSIBLE CARCINOGENIC POTENTIAL OF VARIOUS MARKETED DYES

ST. Jadhav*, CG. Kulkarni, MC. Shinkar and KD. Lokhande TA

Government College of Pharmacy,Vidyanagar, Karad 415124, Satara, Maharashtra, India.

ABSTRACT

Colour is a visual perceptual property corresponding in humans to the categories called red, yellow, blue, etc. Science tells us that the way your food looks is almost as important as how it tastes but this is true for the foods that are naturally colourful or the foods coloured with natural dyes. Considering the aesthetic sense, foods have been coloured naturally as well as artificially since long. Ultimate acceptance of artificial colour loaded foods by consumers has favoured tremendous increase in utilisation of synthetic dyes in the market. The bad side of their toxicity is however forgotten many a times. Millions of people are also using hair dyes to improve their appearance and colourful festivals like *'Holi'*(festival of colours in India) has no limits on sprinkling and colouring the body, thus leading to health and ecological problems.In present study, the possible carcinogenicity of the synthetic food colours, hair dyes, *and 'Holi'* colour is tested through genotoxicity on *Salmonella typhimurium* TA100 strain. In our findings,hair dyes and food colours after S9 metabolic activation were proved to be mutagenic and statistically significant at $p<0.05$.

Keywords: Ames test carcinogenicity, mutagenicity, genotoxicity, *Salmonellatyphimurium.*

INTRODUCTION

Various food colours are widely added to edible preparations all over the world to alter their colour and enhance the aesthetic value. During early days of civilisation coloursof only natural origin were in use. However, since last two centuries, the use of synthetic food dyes has been increased tremendously without the concern of health and safety issues. Because of their low cost tinctorial powerthe great bulk of artificial colourings used in food are synthetic dyes[1,2]. Over many decades synthetic food dyes have been suspected of being toxic and many have been banned whenever possible. However, many food items with the banned dyes may be seen around in rural as well as urban market.Many people also use blends of dyes, the toxic effects of which may be unknown.

The use of hair dyes also can be traced back over thousands of years and today millions of people use various hair dyes to improve their appearance. Over the years hair dyes have been examined as a risk factor[3] for possibility of their potential toxicity including acute, subchronic, reproductive and genetic toxicity.Even after *in-vitro* and *in-vivo* risk studies, wide use of hair dyes continues all over the world.

The festival of colours in India, viz., *'Holi'*is proving to be a health risk due to toxic colours used to colour the body during the celebrations.Unlimited and uncontrolled use of these dyes can lead to grave consequences in terms of human health and ecological balance[4].

This study was taken up with an initiative to checkthe possible carcinogenicity of various synthetic food, hair and body colouring dyes that are available in the local market of Karad (India) and popularised through advertisements.

MATERIALS AND METHODS

Materials

All solvents and materials used were of analytical grade.

Test bacterial strain

Mutant strain *S.typhimurium* TA100 was provided by Bioera Life Sciences,Pvt. Ltd. and was maintained on nutrient agar at 7-8^{0}C. Reviving of culture was done by inoculating bacteria in nutrient broth & incubating it at 37^{0}C for 24 hrs prior to its use.

Samples

Food colours, Hair dyes and *'Holi'* colour were collected from local marketof Karad,India.(Brand names not disclosed)

Methods

Preparation of samples for mutagenicity assay

Samples for checking their mutagenicity were prepared by mixing them in distilled water by adjusting concentrations 50µg/µl and sterilised prior to their use.

Preparation of S9 mixture

S9 components were prepared by mixing $Mgcl_2$ & KCl,Glucose-6-Phosphate, Nicotinamide adenine dinucleotide phosphate ,Phosphate buffer, Sterile distilled water & Rat liver extract in sterile tubes[5].

Genetic Analysis

Histidine dependency of *Salmonella typhimurium*TA100 was checked by streaking a loopful of culture across Glucose Minimal Agar(GMA) plate supplemented with an excess of biotin and were observed for growth[6].

Mutagenicity assay

Standard plate incorporation method was used for mutagenicity assay. Two ml soft agar tubes were maintained at temperature 43^{0}C. These tubes were mixed with 0.5ml of metabolic activation (S-9) mixture or buffer, 50µl overnight culture of the S.typhimurium TA100, 5µl positive control chemical /test sample(having concentration 5µg/µl). The net contents of tubes were mixed & poured onto surface of minimal agar plates. After hardening of top agar, the plates were inverted & incubated at 37^{0}C for 48hrs. Samples were run in six parallel sets[6, 7-9].One µg/µl Sodium azide was used as a positive control mutagen.Colonies were counted & results expressed as number of mean of histidinerevertant colonies per plate.

Statistical Method

One way ANOVA, followed by Bonfferoni test was applied at 5 % level of significance to compare samples with standard & control, respectively. Effect of S9 activation was tested by using the test at 5 % level of significance ($p<0.05$).

RESULT AND DISCUSSION

Genetic analysis of *Salmonella typhimurium* TA100 confirmedhistidine dependence of the organism as evident by absence of growth onGMAwith excess of biotin and luxurious growth on GMA with Histidine.Our findings in mutagenic activity test using *Salmonella typhimurium* TA100 withoutS9 metabolic activation indicated mutagenicity of food colour no.1, hair dye no.1&hair dye no.2 which was evident from number of histidinerevertants(47, 34 &51 mean number of coloniesper plate respectively as compared to 22 colonies in control plate). (Refer Fig.no-1). The traces of histidine in the top agar allowed all bacteria on the plate to undergo several divisions to produce a faint background lawn which could be examined with a convex lens. The same test with S9 metabolic activation led to formation of histidinerevertants in presence of food colour no.1,food colour no.2, hair dye no.1,hair dye no.2 as evident by 85, 81and 154,170 mean number of colonies, respectively, as compared to 55mean number of colonies in control plate(Refer Figure no-2).However,*'Holi'*colour was nonmutagenic irrespective of S9 metabolic activation as evident by just 3 mean number of colonies which were very few as compared to spontaneous revertants(Refer Figure no-2). Above findings are found to be statistically significant at $p<0.05$ by using one way ANOVA followed by Bonferroni

test[10,11]. The use of metabolic activation system, i.e., S9 microsomal fraction was proved to be statistically significant by t test at $p<0.05$[12].

CONCLUSION

The safety of cosmetic products and food colours is of prime concern and is regulated by respective health ministries. Regulatory schemes for hair dyes, food colours, tattoos, etc and their ingredients are also developed in many countries. However, the local markets of the areas under study were found loaded with the colouring products without any trademarks, risk assessment approval labels, risk warning labels etc.

Our findings statistically proved the possible carcinogenicity of tested food colours and hair dyes as judged by genotoxicity studies on *Salmonella typhimurium* TA100[13].The public awareness towards safety versus utility of colouring cosmetic and food products needs to be spread among the people to control the possible epidemics of health hazards.

ACKNOWLEDGEMENTS

Authors are thankful to S.Salunkhe, NIPER, S.A.S Nagar (Mohali) for applying statistical methods and Dr.K.B.BuradePrincipal Government College of pharmacy, Karad (India), for providing necessary support to carry out this work.

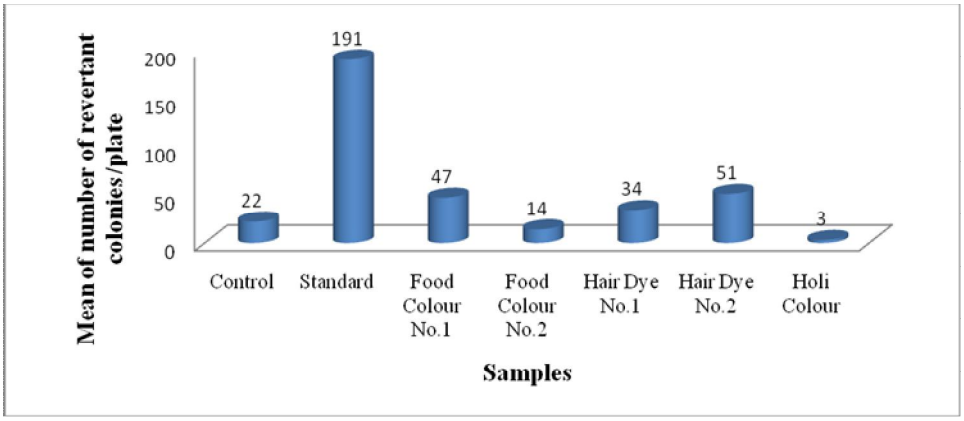

Fig. 1: Possible carcinogenicity of test samples without S9 metabolic activation

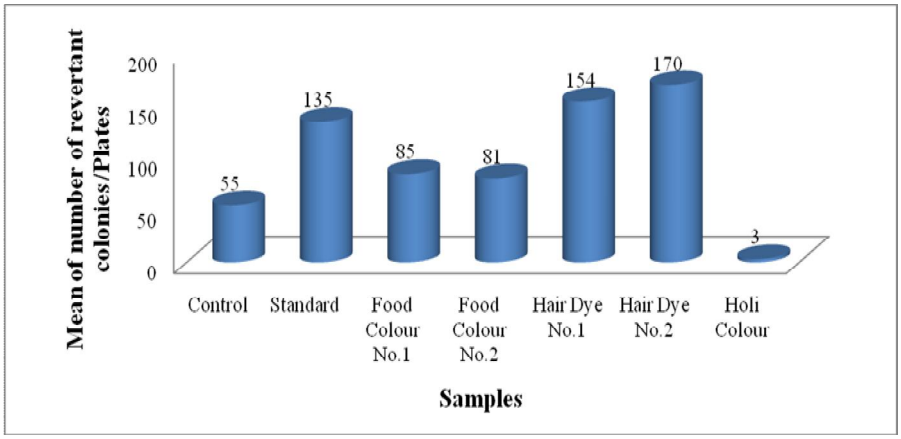

Fig. 2: Possible carcinogenicity of test samples with S9 metabolic activation

REFERENCES

1. Kaur M, Arora S and Katnoria JK. Evaluation of mutagenic potential of food dye (Apple green). Indian J Sci Technol. 2010;3(12):1208-1209.
2. AL-Shinnawy MS. Physiological effect of a food additive on some haematological and biochemical parameters of male albino albino rats. Egypt Acad J biolog Sci. 2009;2(1):143-151.
3. Rauscher GH, Shore D and Sandler DP. Hair dye use and risk of adult acute leukemia. Am J Epidem. 2004; 160(1):19-25.
4. Gardner JJ and Lal D. Impact of 'Holi'' on the environment : A scientific study. Archives of Applied Sciences Research. 2012;4(3):1403-1410.
5. Razak MA, Aidoo KE and Candlish AG. Mutagenic and cytotoxic properties of three herbal plants from Southeast Asia. Tropical Biomedicine. 2007;24(2):49-59.
6. Mortelmans K and Zeiger E. The Ames Salmonella/microsome mutagenicity assay. Mutation Research. 2000;455:29-60.
7. Dorothy MM and Bruce NA. Revised methods for the Salmonella mutagenicity test. Mutation research. 1980;113:173-215.
8. Ames B, McCann J and Yamasaki E. Methods for detecting carcinogens and mutagens with Salmonella/ Mammalian microsome mutagenicity test. Mutation research. 1975;31:347-364.
9. Maron DR and Ames BN. Revised methods for the Salmonella mutagenicity test. Mutation research. 1980;113:173-215.
10. Durham TA and Turner JR. Introduction to statistics in pharmaceutical clinical trials, USA. 2008; 1stEdn: 160.
11. Bolton Sanford, Bon Charles, Pharmaceutical statistics practical and clinical application, New York. 2010;5th Edn:182
12. Mahajan BK and Khanal AB. Methods in Biostatistics for medical students and research workers, New Delhi. 2010; 7thEdn :129-133.
13. Das A and Mukherjee A. Genotoxicity testing of the food colours Amaranth and Tartrazine. Int J Hum Genet. 2004;4(4):277-280.