Evaluation of Toxic Impact of Tartrazine on Male Swiss Albino Mice.

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Summary

Tartrazine, commonly known as Lemon Yellow, is one of the commonly used food colourant. Present investigation was undertaken to evaluate the toxic effects of this dye on male reproduction in *Swiss albino* mice. For this study mice were divided in 4 group viz. control group for low dose (LD), experimental group for low dose (0.2 gm/kg b.wt), control group for high dose (HD) and experimental group for high dose (0.4 gm/kg b.wt). Tartrazine was given for 30 days to all the experimental animals mixed with standard mice feed. The body weight of the mice was recorded daily. A significant increase in the body weight was observed in both the experimental groups when compared with the respective control groups. At day 31st, mice were sacrificed, testes and cauda epididymis were removed and their weights were recorded. A decrease in the weight of testes and cauda was observed in both the treated groups which were found to be significant when compared with the respective control. There was a significant decrease in the sperm dynamics i.e. sperm motility and sperm density in a dose dependent manner. Further, an increase in sperms abnormalities was observed in the samples obtained from the cauda in both the tartrazine treated groups when compared to the respective controls.
Key words: tartrazine, sperm motility and density, sperm morphology, Swiss albino mice.

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Introduction

Colour is an important property of food, which helps us to choose the food, and therefore, colourants have been added to food for centuries to enhance its appearance. Many natural and synthetic colourants are currently approved for use in food; however 95% of those used today are synthetic because they are produced easily, cheaper and provide better coloration.

According to the Prevention of Food Adulteration Act, 1954[1] eight synthetic colours are permitted to be added to the eatables. Out of them, the tartrazine is the most widely used food colourant in soft drinks, juices, biscutes, ice-cream, sauces and snacks etc.

Human study indicated that the food colours could induced a wide range of allergic reactions in sensitive individuals[2-3].

Many researchers[4-6] studied the metabolic and toxicological disorders induced by the administration of specific food colourant additives to rats and other mammals. Many azo compounds are found to be genotoxic and carcinogenic in laboratory animals[7-8]. However, there is paucity of literature regarding the toxicity of tartrazine. The present investigation was planned to evaluate the toxicity of tartrazine on the reproductive organs of male albino mice which will help to explore the effects of this commonly used dye on the metabolism.

Materials and methods

Animal model used:

Adult male Swiss albino mice of B-6 strain, 4-5 weeks old, weighing 25±3 gms were used for the present study. They were maintained at standard laboratory conditions in polypropylene cages (12-h light/dark cycle; 25±3°C temperature; 35–60 relative humidity), and were fed on standard mice feed procured from Aashirwad Food Ltd., Chandigarh (India). The water was given *ad libitum*.

Dye Used:

Tartrazine (**EU number** 102; **FD&C Yellow 5; C.I** 19140; **CAS registry number:** 1934-21-0) is a water soluble yellow coloured azo dye. Chemically, the tartrazine is Trisodium (E)-5-oxo-1-(4-sulfonatophenyl)-4-((4-sulfonatophenyl) diazenyl)-4, 5-dihydro-1H-pyrazole-3-carboxylate (C_{16} H_9 N_4 Na_3 O_9 S_2). The dye tartrazine used in present study was procured from the local market. It was manufactured by Malaya Fine-Chem. Pvt. Ltd., Bangalore (India) and it is sold in the Indian markets with the trade name "lemon yellow IH 6597."The other chemicals used in the experimentation were of analytical grade.

Experimental design:

The animals were divided into four groups of 8 mice in each group. Groups I and III served as control respectively for low dose and high dose. Groups II and IV were administered orally 0.2 gm (as low dose–LD) and 0.4 gm (as high dose–HD) per kg b. wt. of tartrazine after mixing with the standard mice feed respectively for 30 days. The animals of groups I and III were served as control for experimental groups II and IV and they were fed with only standard mice feed (daily food consumption data are given in Table 1). The doses were selected on the basis of LD50 calculations.

Parameters studied:

Body weight and Organ weights:

The treated males were weighed and autopsied after 24 hours from the last dose. The animals were sacrificed by cervical dislocation. The testes and cauda epididymis were carefully dissected out, made free from adherents and weighed on an electronic top pan balance to the nearest mg.

Sperm motility and density:

For sperm motility and density, the cauda epididymis was teased in 0.2 ml of physiological saline, within 5 minute of sacrifice. The percent motility was determined by counting both motile and immotile spermatozoa per unit area. The sperm density (count) was determined by routine procedures and expressed as millon/mm^3 of suspension[9].

Sperm morphology:

Sperm morphology was assessed as described by Kvist and Björndahl[10]. Samples were fixed in 95% ethanol and then stained with haematoxylin– eosin. The stained smears were then observed under a light microscopy (400 × magnifications) and at least 500 sperm cells were observed per animal to assess the morphological abnormalities according to the Feustan *et al.*[11]. The sperms with abnormal in head and tail were recorded. Results are expressed as the percentage of overall abnormalities in a given treatment.

Ethical aspects:

The study was approved by the ethical committee, Centre for Advanced Studies, Department of sZoology, University of Rajasthan, Jaipur, Rajasthan. The guidelines of Indian National Science Academy, New Delhi[12] were followed for maintenance and use of the experimental animals.

Statistical analysis:

Statistical significance between the control and experimental data were subjected to one way analysis of variance (ANOVA).

Results

Effects on Body weight and Organ weight:

The dye tartrazine caused an increase in the body weight at both the experimental groups which was found to be highly significant. However, a highly significant decrease was observed in the weight of testes and cauda epididymis at both the dose levels. (Table 2)

Effects on Sperm motility and density:

The dye tartrazine caused a highly significant decrease in the percentage of caudal sperm motility at both the dose levels. Similarly, the dye caused a decrease in the caudal sperm density which was found to be non- significant at low dose but highly significant at high dose of tartrazine when compared with respective controls. (Table 3)

Effects on Sperm morphology:

The sperm morphology assay of the tartrazine treated mice revealed a dose dependent increase in the incidences of various sperm abnormalities which are exhibited in Table 4. The percentage morphologically normal spermatozoa were significantly reduced in low and high doses of tartrazine. A highly significant increase was recorded in the number of headless sperms and tailless sperms at both the dose levels. The dye caused an increase in the number of hookless sperms which was found to be non-significant at low dose but highly significant at high dose. The increase in the number of double head and double tail sperms was found to be non-significant at both the dose levels.

Table 1: Showing consumption of food in both control and experimental mice

Groups	No. of mice in a group (kept individually)	Amount of food/mice/day (gm)	Dye added/ mice/ day (gm/kg/b.wt)	Food intake/mice/day
Group I (Control for LD)	8	5	nil	all food consumed
Group II (Low dose- LD)	8	5	0.2	all food consumed
Group III (Control for HD)	8	5	nil	all food consumed
Group IV (High dose- HD)	8	5	0.4	all food consumed

Table 2. Showing mean body weight, testes weight and cauda epididymis weight in mice.

Groups	No. of animals	Body weight (gm)		Organ weight (gm /100gm b. wt.)	
		Initial	Final	Testes	Cauda
Group I (Control for LD)	**8**	23.5±0.26	25.5±0.5	0.458±0.011	0.123±8.2
Group II (Low dose- LD)	**8**	23.37 ± 0.26^{ns} (P<0.7)	$30.5\pm0.63^{***}$ (P<0.000)	$0.363\pm0.010^{***}$ (P<0.0000)	$0.056\pm6.7^{***}$ (P<0)
Group III (Control for HD)	**8**	24.37±0.53	25±0.53	0.443±0.008	0.106±5.68
Group IV (High dose- HD)	**8**	24.37 ± 0.5^{ns} (P<1)	$31.37\pm0.70^{***}$ (P<0)	$0.302\pm0.011^{***}$ (P<0)	$0.063\pm0.0004^{***}$ (P<0)

*** = highly significant; ns = non-significant

Table 3: Showing sperm dynamics in both control and experimental mice.

Groups	No. of animals	Sperm density (million/ml)	Sperm motiliy (%)
Group I (Control for LD)	**8**	16.37±1.39	65.77±1.39
Group II (Low dose- LD)	**8**	13.87±0.76^{ns} (P<0.13)	50.73±2.48*** (P<0.0001)
Group III (Control for HD)	**8**	17.12±1.03	66.82±1.55
Group IV (High dose- HD)	**8**	10.62±1.13*** (P<0.0008)	40.70±2.07*** (P<0)

*** = Highly significant; ns = non-significant

Table 4: Showing percentage frequencies of different types of abnormal sperms in mice.

Groups	Normal	Sperms without tail	Sperms without head	Hookless Sperms	Double head/tailed Sperms
Group I (Control for LD)	89.2725±3.05	5.739±2.14	3.68±1.92	1.5345±0.87	0.1515±0.24
Group II (Low dose- LD)	58.3275±4.97*** (P<0)	22.206±3.76*** (P<0)	16.159±4.52*** (P<0.003)	2.2315±1.51^{ns} (P<0.5308)	0.6625±0.77^{ns} (P<0.3252)
Group III (Control for HD)	86.365±2.98	5.4045±2.14	7.2115±2.4	0.763±0.746	0.25±0.396
Group IV (High dose- HD)	59.977±5.60*** (P<0)	19.6655±4.47*** (P<0.0001)	15.89±3.61*** (P<0.003)	4.511±1.835*** (P<0.0048)	0.595±0.663^{ns} (P<0.4834)

*** = Highly significant; ns = non-significant

Discussion

The toxic effect of a dye could be analyzed by monitoring alterations in the body weight of the animals. In the present study administration of tartrazine caused a significant increase in the body weight. Chatterjea and Shinde[13] reported that an increase in the body weight over 20% above the mean body weight is considered as obesity. In the present study, the increase in the body weight was found to be 30.5% at low dose and 28.7% at high dose. Hence, it can be inferred in the view of above observation that the dye somehow, raised the body weight and caused obesity in the experimental animals. Similar results have also been reported by Osman *et al.*[14] in mice fed with synthetic food colourants; Sharma *et al.*[15] in mice fed with chocolate brown; Chakravarty *et al.*[16] in mice fed with malachite green; Sharma *et al.*[17] in mice fed with orange red; Sharma *et al.*[18] in mice fed with apple green; Chakravarty *et al.*[19] in mice fed with orange G; Sharma *et al.*[20-21] in mice fed with tomato red; Chakravarty *et al.*[22] in mice fed with lead chromate. On the contrary, a decrease in the body weight was reported by Brozelleca *et al.*[23] in Sprague-Dawley rats fed with allura red; Abdel-Aziz *et al*.[24] in rats fed with some synthetic food colourant; Helal *et al.*[25] in rats fed with some synthetic and natural food colourant; and Helal *et al.*[26] in rats fed with sunset yellow and sodium nitrite.

In the male reproductive system weight loss of the gonads, epididymides and accessory sex organs as well as reduced sperm count and epididymal sperm motility are considered standard criteria for the characterization of toxic agents that may cause fertility problems in the treated subject[27- 28].

The present study revealed a highly significant decrease in the weights of testes and cauda epididymis of both the treated groups. The weight of testes largely depends on the mass of the various spermatogenic cells. Hence, the depletion in the spermatogenic elements might be the possible cause of the reduction in the testes weight. This observation finds supports from the reporting of Sherins and Hawards[29]; Takihara *et al.*[30]; Abdel-Aziz *et al.*[24]; Mathur *et al.* [31-34] and Sharma *et al.*[21].

Futher, it was observed that tartrazine at both the dose levels significantly reduces caudal sperm count and decreases sperm motility. These findings are consistent with the studies of Wyrobek *et. al.,* [35]; Abdel-Aziz *et al*.[24]; Krishanmoorthy and Laheri[36]; Mathur *et. al*. [33] and Mathur *et. al*.,[34]. According to Wyrobek *et. al.,*[35] several kind of mutation can lead to abnormal sperm morphology. The abnormal sperm shape can be caused by protein abnormality, as sperm shape is partially imparted by sturtural protein.

Presence of abnormal sperm is an another useful indicator of chemical toxicity on the reproductive cells. In the present study, statistically significant a increase was observed in the number of abnormal sperms at both the dose levels. It indicates that the dye exerted toxic impact during the process of sperm differentiation and caused sperm abnormalities.

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References

1. PFAA. Prevention of Food, Adulteration Act, Rule 55. Indian Eastern Book Company Lucknow. 1994, 16th edition.
2. Miller K. Sensitivity to tartrazine. Br. Med. J. 1982; 285:1597-1598.
3. Babu S and Shenolikar S. Health and Nutritional implications of food colours. Ind. J. Med. Res. 1995; 102: 245-249.
4. Reyes FG, Valim MF, Vercesi, AE. Effect of organicsynthetic food colours onmitochondrial respiration. Food Additives and Contaminants.1996; 13(1): 5.
5. Tanaka T. Reproductive and neurobehavioural toxicity study of tartrazine administration to mice in the diet. Food and Chemical Toxicology.2005; 5: 16-25.
6. Zraly Z, Pisarikova B, Trckova M, Herzig I, Juzl M, Simeonovova J. Effect of lupin and amaranth on growth efficiency, health and carcass characteristics and meat squality of market pigs. Acta Veterinaria Brunenssis.2006; 75(3):363-372.
7. Combes RB, Haveland – Smith, A. A review of the genotoxicity of food, drug and cosmetic colours and other azo, triphenylmethane and xanthenes dyes. Mutation Research.1982; 98: 101-148.
8. Sasaki YF, Kawaguchi S, Kamaya A, Ohshima M, Kabasawa K, Iwama K, Taniguchi K, Tsuda S. The comet assay with 8 mouse organs: Results with 39 currently used food additives. Mutation Research. 2002; 519: 103-119.
9. W. H. O. (1999). W. H. O. Laboratory manual for examination of human semen and sperm cervical mucus interaction. New York: Cambridge University Press.
10. Kvist U, Björndahl L, editors. In: Manual on basic semen analysis.ESHREMonographs 2. Oxford: Oxford University Press; 2002.
11. Feustan MH, Bodnai KR and Kerstette SL. Reproductive toxicity of 2-methoxy ethanol dermally to occluded and non-occluded slides in male rats. Toxcol. Appl. Pharmacol. 1989; 100: 145-65.
12. Indian National Science Academy. Guidelines for care and use of animals in scientific research reported by Indian National Science Academy, New Delhi. 2000.
13. Chatterjea MN and Shinde R. Diet and nutrition. In: Textbook of medical biochemistry. 5th ed. Jaypee Brothers Medical Publishers (P) Ltd., New Delhi. 2002; pp 683-685.
14. Osman MA, Afifi A, Hussien RM, Kamilia B, Abdle Aziz and Salah SH. Long term biochemical and genotoxicity studies of four synthetic food and drug colourant in mice. Bull. Fac. Pharm. 1995;33 : 12-13.
15. Sharma A, Goyal. RP, Chakravarty G and Sharma S. Haemotoxic effects of chocolate brown. A commonly used blend of permitted food colour on Swiss albino mice. Asian J. Environ. Sci. 2005 a; 19:93-103.
16. Chakravarty G, Goyal. RP, Sharma A and Sharma S. Haematolgical changes induced by the common non-permitted food colours, Malachite green in Swiss albino Mice. Ind. J. Environ. Sci. 2005; 9(2): 113-117.
17. Sharma S, Goyal. RP, Chakravarty G and Sharma A. Orange red, a blend of permitted food colour induced haematological changes in Swiss albino mice, *MUS MUSCULUS*. Bull. Pure & App. Sci. 2005 b; 24(A):2. 99-103.
18. Chakravarty G, Goyal. RP, Sharma S and Sharma A. Haematological and Serological toxicity of Orange G in Swiss albino mice, *Mus musculus.* Nat. Envi. and Poll. Tech. 2006; 5 (1):95-99.
19. Sharma A, Goyal. RP, Chakravarty G and Sharma S. Toxicological studies of apple

green, a permitted food colour on Swiss albino mice, *Mus musculus*. Ind. J. Environ. Sci. 2006 a; 10(1): 21-24.
20. Sharma S, Goyal. RP, Chakravarty G and Sharma A. Tomato red toxicity: haematolgical and serological changes in the blood of Swiss albino mice *Mus musculus*. Ind. J. Environ. Sci. 2006 b; 10(2): 145-148.
21. Sharma S, Goyal. RP, Chakravarty G and Sharma A. Toxicity of tomato red, a popular food dye blend on male albino mice. Exp. and Toxi. Path. 2008; 60: 51-57.
22. Chakravarty G, Goyal. RP, Sharma S and Sharma A. Haematolgical and Serological studies on Swiss albino mice fed with Lead chromate. J. Ecot. and Enviro. Monit. 2007; 17(1): 61-66.
23. Brozelleca JF, Olson JW and Reno FE. Life time toxicity carcinogenicity study of F D & C Red no. 40 (allura red) in Spragu-Dawely rats. Food Chem. Toxicol. 1989; 27: 701-706.
24. Abdle Aziz, AH, Shouman, Attia AS and Saad, SF. A study on the reproductive toxicity of erythrosine in male mice. Pharmacol. Res. 1997; 35: 457-62.
25. Helal EGE, Zaahkouk SAM and Mekkawy HA. Effects of some food colourants (synthetic and natural product) on young albino rats: liver and kidney function Egyptn. J. Hosp. Med. 2000; 1: 100-113.
26. Helal EGE. Progressive effect of the interaction of sodium nitrite and sunset yellow on different physiological parameters in albino rats. Egyptn. J. Hosp. Med. 2001;2: 23-46.
27. Ban Y, Komatu KM, Inagaki S and Nakatsuka MH. Testicular spermatid and epididymal sperm head count as an indicator for reproductive toxicity in rats. Exp. Anim.1995; 44: 315-22.
28. Queiroz-Neto A, Mataqueriro MI, Santana AE and Alessi AC. Toxic effects of Annona squamosa seed extract in rats and swine. Revista Brasileira de Toxicologia.1997; 10:11-15.
29. Sherins RJ and Hawards SS. Male infertility. In Campbell's Urology. 4th ed. Harrison JH, Gittes RF, Perlmulter AD, Stamey TA and Walsh PC (eds) Co., Saunders Co., Philadelphia 1978; 715.
30. Takihara H, Cosentino MJ, Sakotoku J and Cockett ATK. Significance of testicular size measurements in andrology II. Correlation of testicular size with testicular function. J. Urol. 1987; 137: 416-419.
31. Mathur N, Krishnatrey R, Sharma S, and Sharma KP. Dysfunction of certain organs in albino rats following exposure to textile dye effluent. Ind. J. compd. Anim. Physiol. 2001; 19: 5-10.
32. Mathur N, Krishnatrey R, Sharma S, and Sharma KP. Toxic effects of textile printing industry effluent on liver and testes of albino rats. Bull. Environ. Contam. Toxicol. 2003; 71: 453-456.
33. Mathur N, Chaudhary V, Mehta M and Krishnatrey R. Effect of Sunset Yellow on testes in rats. J. Ecolphysiol. Occup. Hlth. 2005 a; 5: 1-3.
34. Mathur N, Mehta M and Chaudhary V. Sperm Abnormality Induction by Food Colour Metanil Yellow. J. Ecolphysiol. Occup. Hlth. 2005 b; 5:157-160.
35. Wyrobek AJ, Gordon LA, Burkhart JG, Farncis MW, Kapp RW. Jr., Litz G, Malling HV, Topham JC, Whorton MD. An evaluation of human sperm as indicators of chemically induced alterations of spermatogenic. Mutat. Res. 1983; 115: 73-148.
36. Krishanmoorthy M, Laheri S. Sperm abnormality induction assay of the drug dicofenac sodium. Proc. Nat. acad. Sci. India. 2001; 71(B): 21-25.