Effects of selected food dyes on the reproductive system of male albino rats

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ABSTRACT

Background: Chronic consumption of food dyes may be harmful to humans and may be a cause of infertility in men.

Objectives: The study aimed to evaluate the effects of brilliant blue, carmoisine, and tartrazine on reproductive indicators in male albino rats.

Methods: The dyes were administered individually to twenty male albino rats (four groups of five animals each) via oral gavage for 28 days.

Results: Brilliant blue showed a decrease in rapid progressive sperm cell motility (RPSC) at 250 mg/kg, none was observed for tartrazine and carmoisine. Tartrazine showed an increase in non-progressive sperm cell motility (NPSCM) at 500 mg/kg, and progressive sperm cell motility (PSCM) at 100 mg/kg was significantly different from the control at $p<0.005$. Tartrazine (500 mg/kg), Carmoisine (250 mg/kg) and Brilliant blue (250 mg/kg and 500 mg/kg) all caused different degrees of atrophy and necrosis in the seminiferous tubules of the testes. No effect was observed for carmoisine and brilliant blue. Follicle Stimulating Hormone (FSH) in male rats treated showed a significant decrease compared to control at a dose 100 mg/kg for tartrazine and brilliant blue. Prolactin (PRL) showed an increase from the control at 100 mg/kg for tartrazine and brilliant blue at 100 mg/kg and 250 mg/kg. Testosterone (TEST) showed significant increase from the control at 250 mg/kg for tartrazine. Luteinizing Hormone (LH) levels were found to be elevated for tartrazine at 500 mg/kg, carmoisine at 250 mg/kg and 500 mg/kg, and brilliant blue at 500 mg/kg.

Conclusion: These dyes were found to have deleterious effects on reproductive hormones, with some effect on sperm viability. The use of appropriate concentrations of food dyes needs to be assessed and monitored to prevent any adverse effects on human health.

Key words: Food dyes, Tartrazine, Carmoisine, Brilliant blue, Albino rats, Hormones, Testes

Effets de colorants alimentaires sélectionnés sur le système reproducteur des rats albinos mâles

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RÉSUMÉ

Contexte: La consommation chronique de colorants alimentaires peut être nocive pour l'homme et peut être une cause d'infertilité chez l'homme.

Objectifs: L'étude visait à évaluer les effets du bleu brillant, de la carmoisine et de la tartrazine sur les indicateurs de reproduction chez les rats albinos mâles.

Méthodes: Les colorants ont été administrés individuellement à vingt rats albinos mâles (quatre groupes de cinq animaux chacun) par gavage oral pendant 28 jours.

Résultats: Le bleu brillant a montré une diminution de la motilité rapide progressive des spermatozoïdes (RPSC) à 250 mg/kg, aucune n'a été observée pour la tartrazine et la carmoisine. La tartrazine a montré une augmentation de la motilité non progressive des spermatozoïdes (NPSCM) à 500 mg/kg, et la motilité progressive des spermatozoïdes (PSCM) à 100 mg/kg était significativement différente du témoin à $p<0,005$. La tartrazine (500 mg/kg), la carmoisine (250 mg/kg) et le bleu brillant (250 mg/kg et 500 mg/kg) ont tous provoqué différents degrés d'atrophie et de nécrose dans les tubes séminifères des testicules. Aucun effet n'a été observé pour la carmoisine et le bleu brillant. L'hormone folliculo-stimulante (FSH) chez les rats mâles traités a montré une diminution significative par rapport au témoin à une dose de 100 mg/kg pour la tartrazine et le bleu brillant. Français La prolactine (PRL) a montré une augmentation par rapport au témoin à 100 mg/kg pour la tartrazine et le bleu brillant à 100 mg/kg et 250 mg/kg. La testostérone (TEST) a montré une augmentation significative par rapport au témoin à 250 mg/kg pour la tartrazine. Les niveaux d'hormone lutéinisante (LH) se sont avérés élevés pour la tartrazine à 500 mg/kg, la carmoisine à 250 mg/kg et 500 mg/kg et le bleu brillant à 500 mg/kg.

Conclusion: Ces colorants se sont révélés avoir des effets délétères sur les hormones de la reproduction, avec un certain effet sur la viabilité des spermatozoïdes. L'utilisation de concentrations appropriées de colorants alimentaires doit être évaluée et surveillée afin de prévenir tout effet néfaste sur la santé humaine.

Mots-clés: Colorants alimentaires, tartrazine, carmoisine, bleu brillant, rats albinos, hormones, testicules

INTRODUCTION

The colour of food is an important factor that affects acceptance and selection of the food.[1] Food dyes are a type of food additives that are added to food to provide colour, making the product more attractive and increasing its consumer acceptability.[2] A large amount of the foods we eat contain food dyes. Feeding is a constant and daily need for normal body function and yet we are at risk of exposure to various poisons. These dyes can be either natural or synthetic. Synthetic food dyes, which are derived from coal tar and petroleum, have been reported to be harmful to human health.[3]

The use of additives, especially colorants, in food and pharmaceutical industry is increasing dramatically.[4] In recent years, there has been controversy surrounding the use of synthetic food dyes due to their potential toxic effects. However, studies on the adverse health effects caused by artificial dyes, particularly those of the azo group, are insufficient and contradictory, and more research using various system tests such as mammals, plants, insects, and in vitro cell culture should be conducted to assess the true effects of these food additives at a cellular level.[5]

Toxicological testing is important to raise consumer awareness of these dyes, especially for vulnerable populations such as children, pregnant women, the elderly, and severely immunocompromised individuals.[6]

Male reproductive disorders can significantly affect a man's health status and overall quality of life. These disorders can develop at various stages, including fetal development, childhood, adolescence, or adulthood, and may result from various experiences and occurrences. While much is known about the male reproductive system and the causes of specific disorders, research on the mechanisms of action for certain pathologies is still limited. However, exposure to environmental contaminants has been suggested as a potential contributor to male reproductive disorders.[7] This study focuses on the colours tartrazine, carmoisine and brilliant blue, re-evaluating their effects on the male reproductive system. There is a need to re-evaluate them because of the crucial role they play in food consumption.

METHODS

Experimental materials

Rayner's Blue (Brilliant blue), Red (Carmoisine), and Yellow (Tartrazine) concentrated food dyes with batch numbers: VL15349, VL16047, and VL17214, respectively were used. These are common food dyes found in supermarkets in Jos, Plateau State, Nigeria and are produced by Healthy Food Brands Limited in West Sussex, England. The AccuBind ELISA Microwells test kit was obtained from Monobind Inc. (California, USA).

Experimental animals

Twenty two week old male adult albino rats (Wistar strain) weighing between 100 and 180 g were bred and housed in a germ-free environment at 25^{o}C with 33 % humidity and provided with ad libitum feed (Pellets)and water. The procedure was approved by an Animal Use and Ethics committee with number No. UJ/FPS/F17-00379.

Experimental design

Acute toxicity testing

The acute toxicity of tartrazine, carmoisine, and brilliant blue dyes were evaluated using Lorke's method. In phase 1, nine rats were divided into three groups of three rats per group, and administered different doses (10, 100, and 1000 mg/kg) of tartrazine dye orally. Observations were made for 24 hours for any signs of toxicity, such as dizziness, weakness, and difficulty breathing, as well as mortality. In phase 2, three animals were divided into three groups of one rat each, and the rats were administered higher doses (1600, 2900, and 5000 mg/kg) of tartrazine dye and then observed for 24 hours for any signs of toxicity, as in phase 1. The same procedure was repeated for carmoisine and brilliant blue dyes, and the doses that produced no mortality (D0) and the doses that produced mortality (D100) were noted.

Sub-acute toxicity testing

The sub-acute toxicity of tartrazine, carmoisine, and brilliant blue dyes was evaluated using the concentrate of the dyes. The rats were divided into four groups of five rats each. The first group received distilled water equivalent to 1 ml/100 g body weight as control. The second received 100 mg/kg, the third received 250 mg/kg, and the fourth group received 500 mg/kg of the tartrazine dye by the oral route. The same procedure was repeated for carmoisine and brilliant blue dyes respectively. Standard feed and water were given for 28 days.

Effect on sperm

At the end of 28 days, the rats were sacrificed by chloroform anaesthesia, and their scrotum was dissected to expose the testis and epididymis according to the

technique adopted by Lotfi *et al*.[8] A cut of approximately 2.0 mm in thickness was performed on the tail of the epididymis. The fragment obtained by cutting was incubated in a water bath in 5.0 mL of TALP (Tyrode's - Albumin - Lactate - Pyruvate) at 37^{o}C for 10 min. The supernatant was used for sperm analysis. 5.0 µL of supernatant containing the sperm was placed between the slide and cover slip and observed at 100x under the lowest light intensity. The evaluation of the movement of the sperm was held in three different fields and motility was expressed from the middle of the fields in percentage of motile sperm of the total sperm counted.

Histopathology

The testes were harvested, fixed in 10 % formalin, and embedded in paraffin wax for processing. Fine sections (7-9 mm thickness) were dewaxed in xylene, hydrated in decreasing percentage alcohol, and stained with hematoxylin and eosin as used by Wannang *et al*.[9] The stained sections were observed under a Leitz microscope, and their photomicrographs were taken at ×400 with a Canon PowerShot G2 Digital Camera.

Statistical analysis

Data was analyzed using one-way ANOVA, using the SPSS version 20 software for Windows. Differences between the values of various parameters for the control and treated animals were considered statistically significant at $p < 0.05$.

RESULTS

Semen analysis

Table 1 presents the results of the effect of food dyes on sperm cells' viability, specifically on their progressive motility, non-progressive motility, and rapid progressive motility. Tartrazine at 100 mg/kg and 500 mg/kg showed a significant increase in PSCM and NPSCM, respectively, compared to the control group. Brilliant Blue at 250 mg/kg showed a significant decrease in RPSCM compared to the control group. Carmoisine did not show any significant difference in any of the motility measures at any dose.

Table 1: Effect of food dyes on the viability of sperm cells in male albino rats

Treatment Group	RPSCM (%)	PSCM (%)	NPSCM (%)
Control	86.0 ± 0.00	10.0 ± 0.57	4.0 ± 0.60
Tartrazine 100 mg/kg	79.3 ± 0.33	14.0 ± 0.57*	6.67 ± 0.88
Tartrazine 250 mg/kg	75.3 ± 3.17	17.0 ± 1.73	7.67 ± 1.45
Tartrazine 500 mg/kg	65.3 ± 8.95	22.67 ± 7.23	12.0 ± 1.73*
Carmoisine 100 mg/kg	71.3 ± 9.52	20.0 ± 5.77	8.67 ± 3.76
Carmoisine 250 mg/kg	68.3 ± 4.91	21.0 ± 5.19	10.67 ± 0.33
Carmoisine 500 mg/kg	74.67 ± 3.71	20.67 ± 2.85	4.67 ± 0.88
Brilliant Blue 100 mg/kg	81.67 ± 0.88	14.0 ± 1.00	4.33 ± 0.33
Brilliant Blue 250 mg/kg	80.67 ± 1.20*	12.33 ± 0.67	7.00 ± 0.58
Brilliant Blue 500 mg/kg	82.67 ± 0.88	11.0 ± 0.57	6.67 ± 1.45

Values are mean ± SEM; * significantly different from the control at $p<0.05$; n = 5; RPSCM = Rapid Progressive Sperm Cell Motility; PSCM = Progressive Sperm Cell Motility; NPSCM = Non-progressive Sperm Cell Motility

Histopathological findings

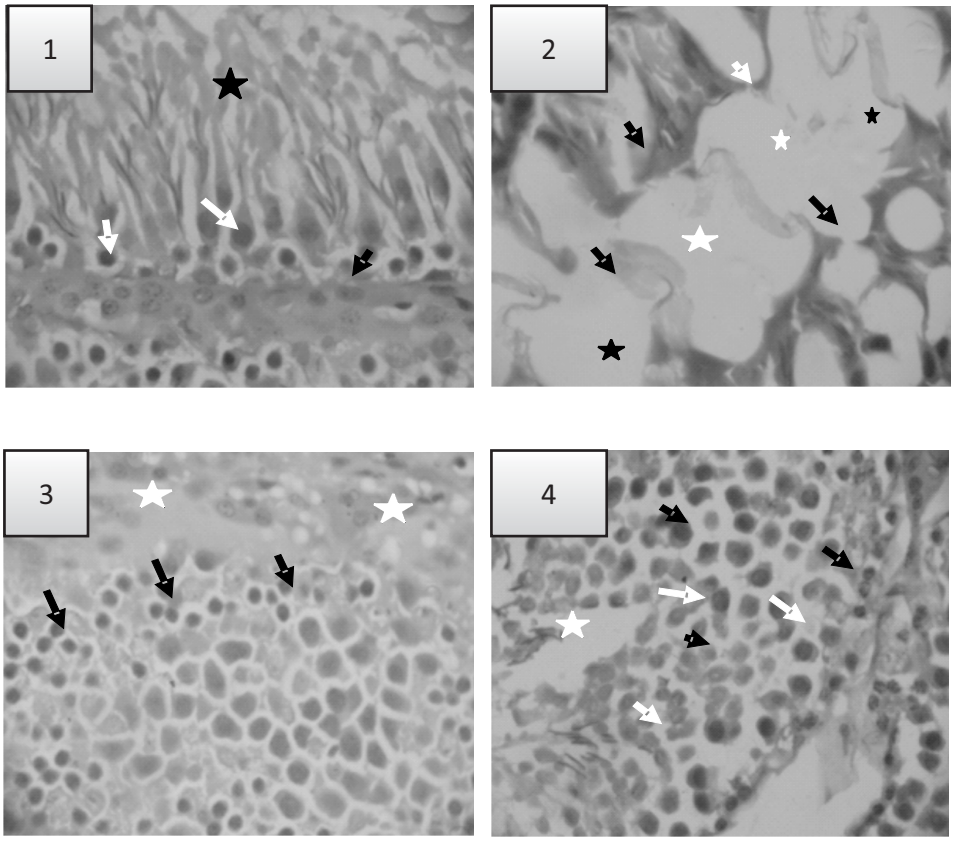

Figure 1: Histopathological micrographs of the testis of albino rats (Plates 1 - 4)

Plate 1: Testis of an albino rat from the control group presenting normal tissue architecture in seminiferous tubules, with different cells of the spermatogenic series at various stages of maturation. Spermatagonia are indicated by black arrows, primary spermatocytes by white arrows, spermatids by white arrowheads, and interstitial cells by black arrowheads. The lumen is represented by a black star. **Plate 2:** This plate depicts the testis of an albino rat administered 500 mg/kg body weight of Tartrazine. The plate shows severe seminiferous tubule atrophy, as evidenced by the massive reduction in tissue size, and severe necrosis, as seen by the loss of tissue components. In addition, there is a severe loss of interstitial cells (white arrows). Furthermore, the cells of the spermatogenic series are lost, and the lumen appears to be enlarged (black stars). **Plate 3:** Testis of an albino rat administered 250 mg/kg body weight of Carmoisine shows thickening of the interstitial tissue (white stars), severe distortion in spermatogenesis, and the complete absence of spermatids in the seminiferous tubules. The primary spermatocytes (black arrows) are the predominant cells in the seminiferous tubules. **Plate 4:** Testis of an albino rat administered 250 mg/kg body weight of Brilliant Blue, shows severe interstitial tissue atrophy resulting in the presentation of few remnants (white arrows) of the interstitial tissue. The white star indicates the seminiferous tubules lumen and the black arrows indicate primary spermatocytes. Although, few spermatids are seen within the seminiferous tubules (white arrowheads). H&E (X400)

Hormonal analysis

Table 2 shows the levels of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Prolactin (PRL), and Testosterone (TEST) in male albino rats treated with different doses of food dyes. Results show that Tartrazine and Brilliant blue at 100 mg/kg had a significant effect on FSH and PRL levels. Tartrazine at 250 mg/kg had a significant effect on TEST levels.

Table 2: Effect of food dyes on reproductive hormones in male albino rats

Treatment Group	FSH (mIU/ml)	LH (mIU/ml)	Prolactin (ng/ml)	Testosterone (ng/ml)
Control	18.37 ± 1.43	0.80 ± 0.40	5.17 ± 0.07	4.60 ± 1.22
TAT 100 mg/kg	5.80 ± 0.36*	5.70 ± 1.70	6.90 ± 0.42*	12.43 ± 5.18
TAT 250 mg/kg	24.90 ± 5.26	8.57 ± 5.99	5.73 ± 0.23	13.50 ± 0.71*
TAT 500 mg/kg	13.39 ± 4.27	13.87 ± 1.07*	5.83 ± 0.32	2.53 ± 0.29
CAM 100 mg/kg	13.05 ± 8.45	7.10 ± 2.40	5.40 ± 0.20	3.15 ± 2.05
CAM 250 mg/kg	23.63 ± 2.05	15.80 ± 6.04*	5.67 ± 0.57	38.73 ± 28.37
CAM 500 mg/kg	22.40 ± 5.06	10.47 ± 3.40*	10.90 ± 5.15	2.67 ± 0.26
BLUE 100 mg/kg	4.87 ± 0.72*	3.43 ± 1.82	6.17 ± 0.37*	7.67 ± 1.93
BLUE 250 mg/kg	6.00 ± 1.13	1.25 ± 1.25	6.65 ± 0.35*	5.50 ± 1.00
BLUE 500 mg/kg	14.37 ± 5.27	17.83 ± 2.22*	5.93 ± 0.24	8.32 ± 5.02

Values are mean ± SEM; * significantly different from the control at $p<0.05$; n = 5; FSH = Follicle Stimulating Hormone; LH = Luteinizing Hormone

DISCUSSION

The current study found that tartrazine at a dose of 100 mg/kg significantly affected Progressive Sperm Cell Motility (PSCM) compared to the control at $p<0.005$, while Tartrazine at a dose of 500 mg/kg significantly increased Non-progressive Sperm Cell Motility (NPSCM) compared to the control. These results are consistent with Mehedi *et al*., who observed decreased sperm count and increased sperm abnormalities in rats treated with 2.5 % Tartrazine.[10] Similar findings were reported by Abdel-Aziz *et al*. for sunset yellow and metanil yellow dyes.[11]

Results showed that the severe atrophy of seminiferous tubules observed in the photomicrographs of the testes, evident by a massive reduction in tissue size and severe necrosis, is consistent with previous findings by Montaser *et al*. that showed degenerative changes, atrophy, and necrosis in the majority of seminiferous tubules of testicular tissue of rats that received carmoisine.[12] These results are also supported by Visweswaran *et al*. and Ghonimi *et al*., who reported that carmoisine induces the production of free radicals that cause damage to the cellular compartment system of the rat testis.[13,14] Additionally, Mahmoud found that the administration of brilliant blue for 30 days induced significant histological changes in the testis of rats, where spermatocytes and spermatids were not distinguishable from each other, and necrotic cells were present.[15] The blood vessels in interstitial connective tissues were also congested and contained blood.

Brilliant blue had a decrease in Follicle Stimulating Hormone (FSH) levels at 100 mg/kg was, as well as an increase in Prolactin (PRL) and Luteinizing Hormone (LH) levels at 100 mg/kg and 250 mg/kg. These results are consistent with the findings of Mohamed *et al*. who observed a significant reduction in serum luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone levels accompanied by a significant increase in kidney function parameters due to Brilliant blue administration.[16] The hormonal indices in rats

administered with Tartrazine showed a significant decrease in Follicle Stimulating-Hormone (FSH), while Prolactin (PRL) showed a non-significant increase compared to the control group. Luteinizing Hormone (LH) and Testosterone (TEST) showed significant increases from the control group at doses of 500 mg/kg and 250 mg/kg, respectively. This contrasts with Elekima *et al*. who found a reduction in testosterone concentration in the acute study.[17] No significant difference was observed in the chronic study at an acceptable daily intake (ADI) dose of 7.5 milligram. Ghada *et al*. also demonstrated a reduction in the concentration of testosterone and interstitial cell-stimulating hormone (ICSH) in the blood serum of male rats treated with tartrazine, edicol erythrosine, and sunset yellow, affecting the balance of four hormones in the serum of male rats compared to the control group.[18]

The observed significant increase in Luteinizing Hormone (LH) for Carmoisine at 250 mg/kg, contrasts with the findings of Abbas and Al-hamadawi who reported a significant decrease in the levels of some sex hormones (testosterone, gonadotropin-releasing hormone, follicle stimulating hormone, and Luteinizing Hormone) in experimental animals' serum treated with 200 mg/kg and 400 mg/kg of chocolate brown, a similar azo dye.[19] Additionally, Amin *et al*. reported a decrease in LH, FSH, and progesterone and estrogen hormone levels in female albino rats treated with low, medium and high doses of Carmoisine, respectively.[20] However, variations in results could be due to biological variations. Testosterone concentrations in peripheral plasma of male rats have been reported to be highly variable, with marked transient oscillations of plasma testosterone in individual conscious rats demonstrated by Bartke *et al*.[21] Wong et al. reported changes in testosterone levels throughout the day, and there was little agreement between the patterns of diurnal variation from different laboratories.[22] Seasonal variations have also been described by Kinson and Liu, and Heywood reported variation in testosterone levels in male Wistar rats (250-300 g) bled under typical laboratory conditions. Plasma T in 114 rats ranged from 2-48 nmol l (0.801 ng/ml-19.23 ng/ml).[23, 24]

The variability in results may also be attributed to oxidative stress. According to Darbandi *et al*., several exogenous and endogenous factors can trigger excessive production of reactive oxygen species (ROS), leading to oxidative stress.[25] This stress can negatively affect male reproductive functions and cause infertility directly or indirectly by disrupting the signals between the hypothalamus-pituitary-gonadal (HPG) axis and other hormonal axes. The researchers further explained that the mechanism of ROS-induced disruption of male reproductive hormonal profiles could ultimately lead to male infertility. Therefore, further investigations should focus on preventing ROS-mediated hormonal imbalances.

CONCLUSION

The current study indicates that the intake of tartrazine, carmoisine, or brilliant blue has detrimental effects on certain hormonal parameters, and the histology of testes. It is crucial to raise consumer awareness about the harmful effects of these dyes and to advise caution in their use. Manufacturers should clearly state the risks of excessive use of synthetic food dyes. Regulatory agencies should also monitor and supervise the quantity of food dyes used by food vendors to ensure safe levels.

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